## **Souvenir cum Book of Abstracts**



## Asian Citrus Congress - 2023

Advancing Citriculture for Agro-economic Prosperity





In Association with



ICAR - Central Citrus Research Institute Nagpur, Maharashtra, India

28-30 October, 2023

Nagpur, Maharashtra, India

Indian Society of Citriculture Nagpur, Maharashtra, India



Asia-Pacific Association of Agricultural Research Institutions Bangkok, Thailand



Korean Society for Citrus and Subtropical Climate Fruits Jeju City, Republic of Korea





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## Asian Citrus Congress - 2023

## Souvenir cum Book of Abstracts



**Indian Society of Citriculture** 

https://accindia2023.iscindia.org.in



## Souvenir cum Book of Abstracts of Asian Citrus Congress - 2023

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**Organizing Committee** 







Message

I am happy to know that the Indian Society of Citriculture in association with ICAR-Central Citrus Research Institute Nagpur, Maharashtra is organizing the "Asian Citrus Congress-2023" on the theme "Advancing Citriculture for Agro-Economic Prosperity" from 28th to 30th October, 2023. A souvenir is also being published to mark the occasion.

Citrus farming is a source of livelihood for many people in India. I am confident that Asian Citrus Congress-2023 will be a remarkable confluence of global expertise and local wisdom in the field of citriculture. It will provide an opportunity for stakeholders to share their knowledge and practices at a common platform. I am confident that the ACC- 2023 will prove to be a fountain of new ideas, a platform for fruitful discussions and a catalyst for growth in this crucial sector.

I extend my warm greetings and felicitations to the organizers and participants. I wish the "Asian Citrus Congress-2023" and publication of souvenir all success.



New Delhi October 09, 2023 नितीन गडकरी NITIN GADKARI

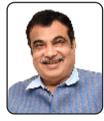




सड़क परिवहन एवं राजमार्ग भारत सरकार Minister Road Transport and Highways Government of India

मंत्री





Message

I am delighted to extend my warmest greetings on the momentous occasion of the Asian Citrus Congress – 2023, organized by the Indian Society of Citriculture in association with ICAR-Central Citrus Research Institute, Nagpur, India; Asia-Pacific Association of Agricultural Research Institutions, Bangkok, Thailand and Korean Society for Citrus and Subtropical Climate Fruits, Jeju City, Republic of Korea from the 28th to the 30th of October, 2023. It gives me immense happiness to learn that this esteemed event is taking place in the culturally rich Nagpur city in Maharashtra, India.

The Asian Citrus Congress – 2023 will prove to be a global platform for the exchange of cutting-edge knowledge, innovative research and best practices in the field of citrus cultivation. The theme of the Congress is "Advancing Citriculture for Agro-economic Prosperity" and calls upon distinguished foreign and national delegates, for deliberations on this topic

Citrus cultivation has long been an integral part of our nation's agricultural tapestry. The rich history and diversity of citrus fruits in India have not only contributed to our culinary heritage but also sustained the livelihoods of countless farmers across the nation. In this context, the Asian Citrus Congress – 2023 assumes a paramount importance as it brings together experts, researchers, and practitioners to deliberate on strategies that can further enhance the productivity, sustainability, and resilience of our citrus industry.

I applaud Dr. Dilip Ghosh, Director of ICAR-CCRI and Convenor of ACC-2023 as well as his efficient team for their dedication in organizing this event of immense significance. I welcome you all to Nagpur – The Orange City of India and wish the great success to this Congress.

Yours,

Nagpur 25 September, 2023

Radko (Nitin Gadkari)

नरेंद्र सिंह तोमर NARENDRA SINGH TOMAR



कृषि एवं किसान कल्याण मंत्री भारत सरकार कृषि भवन, नई दिल्ली MINISTER OF AGRICULTURE & FARMERS WELFARE GOVERNMENT OF INDIA KRISHI BHAWAN, NEW DELHI



Message

I am glad to hear that Indian Society of Citriculture (ISC) is organizing Asian Citrus Congress – 2023 during 28-30 October, 2023 in Nagpur, Maharashtra in collaboration with ICAR-Central Citrus Research Institute, Nagpur, India (ICAR-CCRI); Asia-Pacific Association of Agricultural Research Institutions, Bangkok, Thailand (APAARI) and Korean Society for Citrus and Subtropical Climate Fruits, Jeju City, Republic of Korea (KSCSCF).

India, being the third largest producer of citrus fruits in the world, has a big responsibility to raise a contingent of scientifically sound citrus growers and exporters who can take not only Indian citrus industry to greater heights, but also contribute to the Asian and global citrus industry at large. As we stand at the confluence of tradition and innovation, it is imperative that we address the challenges posed by changing climatic patterns, evolving consumer preferences, and the need for sustainable agricultural practices. I hope the congress will provide an ideal platform to explore these issues, share insights, and foster collaborations that can shape the future of citrus cultivation in India and beyond.

I congratulate the organizers led by President (ISC) and Convenor (ACC-2023) for thinking in this direction and conceiving the idea of Asian Citrus Congress – 2023 which focuses on empowering Asian citrus industry, fostering camaraderie, knowledge sharing, and innovative thinking among all participants. Let us collectively explore new horizons of citriculture and strengthen the bonds of collaboration as we work towards a brighter and more fruitful future for the global citrus community.

I extend my heartiest greetings to the organizers and participants of this mega event and wish the Asian Citrus Congress – 2023 a grand success.

1101

(Narendra Singh Tomar)



डॉ. हिमांशु पाठक Dr. HIMANSHU PATHAK सचिव (डेवर) एवं महानिदेखक (आईसीएआर) Secretary (DARE) & Director General (ICAR) कृषि अनुसंधान और शिक्षा विभाग एवं भारतीय कृषि अनुसंधान परिषव कषि एवं किसान कल्याण मंत्रालय, कषि भवन, नई दिल्ली—110 001

गारत सरह

GOVERNMENT OF INDIA DEPARTMENT OF AGRICULTURAL RESEARCH AND EDUCATION (DARE) AND INDIAN COUNCIL OF AGRICULTURAL RESEARCH (ICAR) MINISTRY OF AGRICULTURE AND FARMERS WELFARE Krishl Bhavan, New Delhi 110 001 Tel: 23382629 / 23386711 Fax: 91-11-23384773 E-mail: dg.icar@nic.in



Message

I am happy to know that Indian Society of Citriculture (ISC), in association with ICAR-Central Citrus Research Institute, India; Asia-Pacific Association of Agricultural Research Institutions, Thailand and Korean Society for Citrus and Subtropical Climate Fruits, South Korea is going to organize the "Asian Citrus Congress-2023" on Advancing Citriculture for Agro-economic Prosperity from 28th to 30th October, 2023 in Nagpur.

Citrus fruits have held a special place in our diets, cultures, and economies for centuries. The remarkable diversity of citrus species and their versatile applications have not only enriched our culinary experiences but also contributed significantly to the agricultural and economic landscapes of different countries.

In our quest to advance citriculture, it is imperative that we recognize the challenges posed by evolving climatic conditions, emerging pests and diseases, and shifting market dynamics. The participants will discuss to devise holistic strategies that not only ensure the resilience of citrus production systems but also enhance the livelihoods of the farming communities. This congress will provide a platform for researchers, practitioners, and stakeholders in the field of citriculture to exchange insights, share experiences, and collectively explore pathways that lead to agroeconomic prosperity.

I wish a fruitful and insightful congress.

(Himanshu Pathak)

New Delhi 25 September, 2023 डॉ. तिलक राज शर्मा उप महानिदेशक (बागवानी विज्ञान)

Dr. T. R. Sharma FNA, FNAAS, FNASC, FASC, JC Bose National Fellow Deputy Director General (Horticultural Science)



भारतीय कृषि अनुसंधान परिषद कृषि अनुसंधान भवन - ॥, पूसा, नई दिल्ली - 110 012 INDIAN COUNCIL OF AGRICULTURAL RESEARCH

KRISHI ANUSANDHAN BHAVANHI PUSA, NEW DELHI-110 012(INDIA)



Message

It is with great pleasure that I extend my warm greetings to all of you as we converge at the Asian Citrus Congress - 2023 under the theme, "Advancing Citriculture for Agroeconomic Prosperity." This congress serves as a remarkable platform for us to explore the intricacies of citriculture and collectively steer our efforts towards enhancing the agro-economic landscape.

The allure of citrus fruits transcends boundaries and cultures, making them an integral part of our lives. The congress theme aptly captures our shared goal of not only exploring the frontiers of scientific knowledge but also fostering economic growth through innovative practices in citriculture. Let us engage in meaningful dialogues that encompass disciplines and geographic boundaries, ultimately enriching our collective understanding and paving the way for transformative changes in the citriculture sector.

I congratulate Dr. Dilip Ghosh, Director, ICAR-CCRI and Convenor of the Congress and his entire team for convening the Asian Citrus Congress and to the researchers, scholars, and industry experts whose efforts have enriched the Souvenir.

I am sure that the discussions in the Congress will be insightful, interactions enriching and outcomes impactful.

I wish the Asian Citrus Congress-2023 a grand success.

New Delhi 25 September, 2023

(T. R. Sharma)



Dr. Dilip Ghosh DIRECTOR भा.कृ.अनु.प.- केन्द्रीय नीबूवर्गीय फल अनुसंधान संस्थान ICAR-Central Citrus Research Institute अमरावती रोड, नागपुर-४४००३३, महाराष्ट्र, भारत

Amravati Road, Nagpur – 440033, Maharashtra, India



Preface

It is with utmost pleasure that I welcome you all to the Asian Citrus Congress 2023 (ACC-2023), hosted in the vibrant city of Nagpur, famously known as the 'Orange City' of India. This unique mega event, dedicated to advancing the citrus industry in Asia, has brought together experts from across the globe to share their knowledge, experiences, and insights, for shaping future of citriculture and agro-economic prosperity.

We are honored to have Shri Natin Gadkari-Ji, Honorable Minister of Road Transport and Highways, Government of India, who not only ceremonially launched the ACC-2023 website and given his kind consent for inaugurating the congress, but also provided much needed mentorship, guidance and support. His involvement reflects the significance of this event and the government's commitment to the growth and development of the citrus industry. With nearly 300 delegates from 15 different countries including India, the ACC-2023 promises to be a platform for fruitful discussions, collaborations, and networking opportunities. We are delighted to have such a diverse and knowledgeable group of participants, whose expertise will undoubtedly contribute to the success of this event.

The Souvenir cum Book of Abstracts is a testament to our collective effort, featuring invited and contributory research abstracts. This publication would not have been possible without the contributions of our esteemed speakers, delegates, and reviewers. We extend our heartfelt gratitude to each one of them for their valuable inputs and efforts in making this Souvenir a valuable resource for all the participants. It's a tribute to the citrus community's dedication and a testament to our shared vision.

I would also like to express my sincere appreciation to the organizing committee members, volunteers, and the support staff for their tireless efforts in planning and executing this Congress. Their dedication and hard work have been instrumental in ensuring a smooth and successful organization. Lastly, I would like to thank our sponsors for their invaluable support. Their contributions have been crucial in making this congress a reality and providing a platform for the exchange of ideas and innovations in the citrus industry.

Once again, I extend a warm welcome to all the delegates and wish you a productive and enjoyable time at the Asian Citrus Congress-2023. May this event be a stepping stone towards a brighter and more prosperous future for the citrus industry.

Skguch

: director.ccri@i ; ccri.icar.gov.in

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Nagpur 25 October, 2023 (Dilip Ghosh) Convenor, Asian Citrus Congress - 2023 President, Indian Society of Citriculture Director, ICAR- Central Citrus Research Institute, Nagpur



## 2023 MARRINA About Asian Citrus Congress-2023

Citrus plants, one of the world's most important fruit crops grown in more than 150 countries, are regarded native to subtropical and tropical parts of Asia, Island southeast Asia, near Oceania and northeast Australia, inhabiting the planet earth well before the birth of human civilization. The history of citrus in Asia, one of the top three fruits in the continent, is highly fascinating and offers many opportunities for learning for citrus scholars. A genomic, phylogenic, and biogeographical analysis has established the southeast foothills of the Himalayas, which extend from eastern Assam, northern Myanmar, and western Yunnan, as the centre of origin for the genus Citrus, branching from a shared ancestor with the trifoliate orange *Poncirus trifoliata*. According to FAO, the production of various citrus fruits in Asian continent during 2021 was 83.59 million tones from a cultivated area of 5.42 million hectares, sharing 52% area and 53% production of citrus in the world. During last decade (2012 to 2021), the area and production of citrus in Asian countries has been increased 25.5% and 42.5%, respectively.

India, known for genetic diversity of citrus covering 27 native species, is third major producers of citrus in the world, contributing 10.76% area under citrus and 8.84% citrus production in the world. Among various citrus fruits grown in India, mandarins share the maximum area and production (both 42%), followed by lemons and limes (31% and 26%, respectively), sweet orange (19% and 27%, respectively) and other citrus fruits (8% and 5%, respectively) in 2022. Nagpur mandarin is the most famous cultivar in mandarin group in India. This cultivar is mainly cultivated in the Vidarbha region of Maharashtra state, where the ACC-2023 is being organized.

Given this wealth of citrus diversity, the ACC-2023 has been organized to reframe the priorities of Asian citrus industry and put forward a roadmap to combat the growing problems of citrus industry of Asia. The event brought together scientists, researchers, academicians, students, processors, exporters, citripreneurs, industry experts, and policymakers to discuss and exchange information on the latest developments in citriculture and to explore possible solutions to address the multipronged challenges faced by the citrus industry. It is an important event that provided a platform for knowledge exchange, addressing challenges, networking and promoting the citrus industry in Asia. ACC-2023 has given an opportunity to the researchers and industry experts to share their knowledge and expertise in the field of citrus improvement, production, protection, processing, and marketing. Participants could learn about the latest research findings on new technologies, innovative products, services and best practices which can lead to new research alliances, business opportunities and innovation. Overall, the ACC-2023 intented to promote the citrus industry in Asia by showcasing the latest developments, innovations and successes in the field of citriculture. This can help to shape the future of the citrus industry in Asia, attract investment, stimulate economic growth and support farmers and businesses. We also believe that by bringing together the best and brightest in the Asian citrus industry, we can unleash the full potential of this vital sector and drive growth, sustainability and prosperity across Asia.



## **Host Organization**

Indian Society of Citriculture (ISC) was established on 24<sup>th</sup> August, 1983 in New Delhi (Registration No. S/13760 of 1983). The broader aim and objectives of the Society is to advance the cause of Citriculture, encourage and promote citrus research, disseminate the knowledge and facilitate closer association among the stakeholders. The Headquarter of the Society was shifted from IARI, New Delhi to ICAR- Central Citrus Research Institute, Nagpur, Maharashtra (Formerly known as National Research Centre for Citrus) in



November 1997 to boost its activities and pursue the objectives more effectively, being placed in hub of citrus industry.

Since its inception, the ISC brings together researchers, academics, citrus growers, industry experts and policymakers from across India and works closely with the government and other stakeholders to address the challenges faced by the industry. Society also aims to disseminate information on the latest research, technology and best practices in citriculture and to encourage the exchange of ideas and experiences among its members. Overall, the ISC plays a crucial role in advancing the citrus industry in India and ensuring its long-term sustainability and profitability. Its efforts are helping to promote citrus cultivation as a significant contributor to India's horticultural sector and to the country's economy as a whole.

ISC has organized several National and International seminars, workshops and conferences on various aspects of citrus cultivation, such as production, protection, processing and marketing. Few landmark events organized by the society are National Symposium on Citriculture (17-19 Nov., 1997), International Symposium on Citriculture (23-17 Nov., 1999), National Symposium on Citriculture: A Road Map (22-24 Feb., 2006); National Symposium on Citriculture: Emerging Trends (24-26 July, 2008), National Seminar on Citrus Biodiversity for Livelihood and Nutritional Security (4-5 Oct., 2010), National Dialogue on Citrus Improvement, Production and Utilization (27-29 Feb., 2012), National Citrus Meet (12-13 Aug., 2013) and National Symposium on Citrus industry of India: Way forward (27-29 Nov., 2015). More details are available on ISC website (https://iscindia.org.in).

The ISC has organized thizs mega event in association with the following organizations.



ICAR-Central Citrus Research Institute (ICAR-CCRI) Nagpur, India



Asia-Pacific Association of Agricultural Research Institutions (APAARI) Bangkok, Thailand



Korean Society for Citrus and Subtropical Climate Fruits (KSCSCF) Jeju City, Republic of Korea



## Theme and Logo of ACC-2023

## Advancing Citriculture for Agro-economic Prosperity

Citrus is a major horticultural commodity in many Asian countries, providing a significant source of income and employment for farmers, processors, traders, and other actors in the value chain. To further unleash the potential of this industry, it is essential to connect innovators from different fields and expertise towards a common goal of advancing the sector. This can be achieved through our initiatives Asian Citrus Congress-2023, which provided a platform for researchers, extension personnel, policymakers, citripreneurs, and industry experts to share their ideas, innovations, and experiences.

By promoting innovation, exchanging knowledge, and collaboration among stakeholders, the Asian Citrus Congress-2023 aimed to act as a catalyst in enhancing the productivity, quality and sustainability of citrus production and trade in Asia, as well as in capturing the growing demand for citrus products in domestic and international markets. The congress also highlighted the importance of addressing the social, environmental, and economic challenges facing the citrus industry. The overarching theme of the ACC-2023 is to broaden the citrus industry in Asia in order to achieve inclusive and equitable development in citrus growing areas, support food and nutritional security, and contribute to the overall economic growth, sustainability and prosperity of all involved in the Asian citrus industry.



The near-round graphic on the left symbolizes citrus biodiversity in terms of size, shape and color; while Asian continent is represented by the white map at the centre. The typography represents the event title. The citrus fruit with green leaves at the upper right side of the logo reflects the freshness of the citrus industry and the power of mother nature. Overall, the logo uses a minimalistic approach to underline that the Asian Citrus Congress-2023 is an international event, while also suggesting a brighter future with its use of warm and vibrant citrus hues.



Asian Citrus Congress - 2023 Secretariat Conveys Heartfelt Appreciation to Our Distinguished Sponsors



## Plenary, Keynote, Lead and Invited Lectures of All Thematic Areas



**Plenary Lecture** 

Special Plenary Session

## **Evolution of citrus improvement in the 21<sup>st</sup> century: From conventional breeding to genome editing**

## **Manjul Dutt**

Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, USA.

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Citrus is affected by a plethora of abiotic and biotic issues. Currently citrus greening, also known as Huanglongbing (HLB), has emerged as the primary disease threatening the global citrus industry. This disease, caused by a bacterial pathogen that primarily affects the phloem, results in substantial economic losses across all commercial citrus varieties. HLB leads to rapid tree deterioration and the production of fruit that cannot be sold. While improved cultivation techniques can temporarily sustain infected trees, the most promising long-term approach for managing this disease involves using HLB-resistant trees that can thrive in areas where HLB is prevalent.

Traditional citrus breeding remains the most dependable method for enhancing citrus varieties. It has yielded numerous high-quality cultivars. However, most citrus varieties require a significant amount of time to reach maturity due to prolonged juvenility, and there is a considerable time lag from making the initial cross to the introduction of a new cultivar. With the decreasing costs associated with DNA and RNA sequencing, advanced plant breeding techniques such as genome-wide association studies (GWAS) and genomic selection have become more viable in citrus. These methods heavily rely on precise DNA data and can lead to the identification of molecular markers associated with specific traits of interest. Consequently, it becomes possible to efficiently assess large populations of seedlings for the desired trait in significantly less time and space compared to previous methods. By incorporating traits that reduce the juvenile phase, like those present in the Australian Finger lime (Citrus australasica), we have significantly shortened the time needed for turnover. Hybrid citrus plants with the Australian Finger lime as one of the parent exhibits early flowering within three years after hybrid seed germination, and this characteristic can be passed down to subsequent generations. Additionally, wild citrus species like Citrus latipes and Citrus indica, both native to Northeastern India, offer resistance to numerous environmental and biological challenges commonly encountered by cultivated citrus varieties. These wild species contribute essential genetic components that can lead to the development of more stress-tolerant cultivars. For instance, in our program, we have leveraged the genetics of Citrus latipes to create rootstocks highly tolerant to HLB. However, developing resistant scion varieties can be a lengthy endeavor. Because most wild species possess several undesirable traits, it will necessitate multiple generations of backcrossing to reintroduce the superior characteristics into the improved cultivar.

Genetic engineering techniques, notably transgenic technology, have been extensively utilized to introduce specific genes into citrus plant genomes. This approach aims to provide susceptible citrus cultivars with resistance to various environmental and biological stress factors while preserving their inherent traits. In our research program, we've explored the incorporation of different genes, including antimicrobial peptide gene constructs (AMPs) and genes that induce systemic acquired resistance (SAR), into commercial sweet orange cultivars,



yielding varying degrees of success. Through field trials conducted in regions affected by HLB and greenhouse experiments involving bacterial transmission by insect vectors, we've identified certain genes with the potential to confer tolerance to HLB. Initially, AMP genes showed promise, but their effectiveness waned over time. The most successful genes identified in our program have been SAR induction genes, namely NPR1 (from *Arabidopsis thaliana*) and SABP2 (from *Nicotiana tabacum*), which were over expressed in citrus using the constitutive d35S promoter. Independent transgenic lines carrying these genes consistently demonstrated tolerance to HLB under challenging field conditions. In addition to developing HLB-tolerant scions, we've also engineered transgenic rootstocks that express the same SAR induction genes. The rationale behind these engineered rootstocks is that they remain in a constant state of readiness, allowing for a rapid response to the disease. Currently, these rootstocks are undergoing testing to assess their ability to protect non-transgenic scion varieties. Another approach to managing HLB involves developing transgenic plants that are more acceptable to consumers using plant-based genetic constructs. We've established a transformation system that generates citrus plants without visible reporter gene expression. This system involves combining a citrus-derived gene responsible for anthocyanin production, a natural pigment, with an embryo-specific promoter to regenerate genetically modified plants in which the marker gene is deactivated.

Recently, the CRISPR-mediated genome editing approach has become increasingly popular as a precise tool for plant breeding. It allows for the specific targeting and modification of DNA sequences, typically resulting in frame shift mutations and the loss of gene function through DNA insertions and deletions. We have successfully developed protocols for CRISPR-mediated genome editing using Agro bacterium-mediated genetic transformation, employing the Cas9 protein to modify citrus cells. As a proof of concept, we disrupted the citrus phytoene desaturase (PDS) gene, which is involved in chlorophyll biosynthesis, leading to an albino phenotype. Editing the TAC1 gene resulted in trees with a narrower growth pattern, suitable for high-density planting. When we edited the CBF1 gene, it down regulated several cold tolerance-related transcription factors, increasing susceptibility to freezing in *Poncirus trifoliata*. This demonstrated that the CBF1 gene plays a crucial role in cold acclimation, freezing tolerance, and overall plant development in Poncirus. Furthermore, by editing negative regulators of the SAR process, such as NPR3 and NPR4, either individually or in combination, we observed enhanced expression of PR genes and improved tolerance to HLB. Finally, as an alternative to the Agrobacterium-mediated genetic transformation process, a novel technique utilizing cationic lipid nanoparticles has also been developed to deliver in vitro assembled Cas9/gRNA complex ribonucleoproteins (RNPs) into protoplasts to directly edit the Citrus genome.

Presently, traditional breeding methods, genetic modification as well as genome editing techniques require substantial long-term investments for the development, testing, and release (traditional breeding) or regulatory clearance (transgenics) of enhanced citrus cultivars. In the future, ongoing advancements in technology will considerably diminish the required time frame, enabling the swift creation of novel citrus varieties.



## **Plenary Lecture**

Special Plenary Session

## A comprehensive strategy for fast breeding in citrus

### **Kwan Jeong Song**

Horticultural Science Major, Jeju National University, Jeju 64243, Korea

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Worldwide, citrus is a very significant crop for both economic and human health reasons. Improving citrus fruit guality and reducing costs have been more crucial recently as competition among other fruits has increased. In order to achieve these goals as fast as feasible, a comprehensive breeding plan including gene pool growth, widespread hybridization, and effective selection is required. Despite the fact that cultivar improvement programs have been implemented in several countries with similar overarching goals, the methods employed differ depending on the accessibility of genetic resources and facilities as well as the expertise and experience of breeders. The most crucial factor is a gene pool expansion and evaluation for a greater availability of genetic resources, which is related to obtaining desired cross combinations. Hybrid population will be scaled up next, not progeny population. Achieving varied monoembryony parents and restoring pollen fertility will effectively accomplish this goal. Triploid selection, which results from crosses between tetraploid and diploids, will successfully produce seedlessness. Colchicine therapy and cell fusion are the main methods for producing two types of auto- and allo-tetraploids, respectively. Marker assisted selection (MAS) and single-stem training of seedlings grafted on dwarfing rootstock are required to shorten the breeding cycle. Following the successful application of MAS on hybrid populations, a number of genomic and transcriptomic techniques will be used to develop DNA markers linked to phenotypic traits. However, commercialization will ultimately determine whether new promising selections succeed or fail. To encourage early commercialization through evaluating genetic potential for horticultural performance, regional adaptation evaluation on various rootstocks and in various climatic situations against abiotic and biotic stress tolerance is an important component. Breeders, physiologists, entomologists, and pathologists should work together to operate all of these systems through systematic processes.



## **Plenary Lecture**

Special Plenary Session

## Citrus Huanglongbing research in Florida: Past, present and future prospects

### **Michael E. Rogers**

University of Florida, Institute of Food and Agricultural Sciences, Citrus Research & Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850 USA

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Citrus Huanglongbing (HLB) disease was first detected in Florida in 2005 (Halbert 2005).Following its discovery, surveys were immediately initiated in residential areas and commercial citrus groves to delimit the presence of HLB. Ultimately, HLB was found to be established throughout the entire commercial citrus growing areas of the state, making eradicating the disease unfeasible.

Compared to other parts of the world, citrus production costs per hectare are significantly higher in Florida (Muraro et al. 2001). Thus, the economic viability of citrus production in Florida depends on cultivating the highest quality fruit. Since eradicating HLB was deemed impossible, Florida citrus growers adopted a three-pronged approach to HLB management, based on the practices employed in other citrus growing regions with endemic HLB (Bove 2006). This approach included 1)managing insect vector populations, 2) removing trees with HLB symptoms and 3) growing all citrus nursery plants in certified disease-free greenhouses. These practices aimed to slow the disease's spread until new research-based HLB management strategies could be developed. The progression of HLB research over the past 20 years in Florida is briefly summarized below.

#### Past Research

Upon the initial detection of HLB in Florida, substantial research efforts focused on managing the citrus psyllid (*Diaphorina citri* Kuwayama), the insect responsible for spreading the HLB-causing pathogen *Candidatus* Liberibacter asiaticus (CLas) throughout Florida citrus groves. Research included pesticide efficacy trials, psyllid monitoring methods, investigations into psyllid movement and feeding patterns, and studies to understand the microbial interactions of CLas with its psyllid host (Grafton-Cardwell et al. 2013).As psyllid research advanced, management practices evolved accordingly. Florida growers transitioned from monthly pesticide sprays to coordinated area-wide psyllid management. In 2011,Florida established an area-wide psyllid management program, involving 48 "Citrus Health Management Areas" (CHMAs) spanning 194,249 hectares of commercial citrus(Rogers 2011).In each CHMA, growers synchronized pesticide applications within 1-2 weeks, resulting in an industry-wide reduction of psyllid populations by nearly 70%. However, the CHMA program was short-lived because, by its launch, most trees within commercial citrus groves already displayed HLB symptoms. The Florida citrus industry then shifted focus from intense vector management and tree removal to new production practices that extended the fruit-bearing life of diseased trees.

Early research in Florida also concentrated on developing methods for early detection of asymptomatic trees for subsequent removal, CLas bacterium culture methods, and understanding disease impacts on citrus trees. Early detection research faced challenges due to trees remaining asymptomatic for an extended period post-infection. The most reliable detection method is q-PCR, but it incurred significant costs and occasionally yielded false



negatives due to uneven pathogen distribution in the tree. As HLB became pervasive, early detection's relevance waned for Florida citrus growers. However, areas such as California (USA), where HLB is not widely established, retain this need.

Researchers made efforts to culture the CLas pathogen, reporting progress in scientific literature (Merfa et al. 2019). Yet, no pure CLas cultures have been sustained for more than a few generations in the lab. Attaining pure cultures of CLas remains a scientific community goal to facilitate research on the pathogen. The "Hairy-root assay" is currently the favored method to evaluate antimicrobial compounds' effects on CLas (Irigoyen et al. 2020).

One of the most important findings in the initial decade of HLB research in Florida was understanding CLas' impact on the citrus root system. Before canopy symptoms appear, it was discovered that up to 30% of the root system is destroyed by the pathogen(Johnson et al. 2014). Compromised root systems are less efficient in nutrient and water uptake, leading to nutrient deficiencies in above-ground portions of the tree due to reduced nutrient flow. Subsequent research aimed to improve root health, focusing on alleviating bicarbonate stress and managing root diseases like phytophthora.

### **Present Research**

Given growers' struggle to remain profitable, recent research focused on managing the health and productivity of diseased trees while long-term HLB solutions develop. Due to CLas' impact on root systems, research focused on new fertilizer and irrigation guidelines for HLB-diseased trees. Research-based fertilizer recommendations emphasized consistent macro- and micronutrient availability throughout active growth periods(Morgan and Kadyampakeni 2020). Proper nutrient levels improved canopy growth and fruit quality (Vashisth and Grosser 2018). Irrigation adjustments optimized water uptake by compromised roots, promoting better tree health and yield (Hamido et al. 2017).

Research focused on understanding how CLas affects tree health has shown that several of the naturally occurring plant hormones (i.e., giberellins, auxins and cytokinins) in citrus are reduced leading to oxidative stress within the trees(Tang and Vashisth 2020).Timely applications of hormone-containing products alleviated oxidative stress, enhancing tree growth, reducing fruit drop, and increasing yields. Guidelines for growers on use of these products are now available to improve the health of HLB-diseased trees.

Antimicrobial products are being evaluated as potential therapies to directly control the CLas bacterium. Past studies injecting compounds such as oxytetracycline did not lead to widespread adoption, likelydue to phytotoxicity caused by the injections(Van Vuuren 1977).Florida's recent oxytetracycline injection studies demonstrated improved tree health and yield, though phytotoxicity concerns require continued work (Archer and Albrecht 2023). Despite the potential phytotoxic risks, oxytetracycline trunk injections have been approved for use in commercial citrus groves in Florida. Many citrus growers have chosen to inject their trees to improve tree health in the short-term while longer term solutions are developed.

Currently, there are no known citrus varieties that are resistant to HLB, but there are some varieties that exhibit tolerance to HLB. The term 'tolerant' here refers to the ability of trees to continue to grow and produce fruit of suitable quality despite being infected with CLas, whereas 'resistant' refers to the inability of CLas to survive in the tree long-term. In general, mandarin and lemon varieties are considered more tolerant of HLB compared to sweet



orange and grapefruit. As HLB has spread throughout Florida, citrus breeders have been evaluating all the citrus germplasm in the field for evidence of tolerance to HLB. Several new mandarin, sweet orange, and various citrus hybrids from the University of Florida citrus breeding program have been identified as being more tolerant of HLB when compared to commercial varieties that have historically been grown in Florida (Grosser et al. 2023). These new sweet orange and mandarin varieties have been released by the university and are now beginning to be grown commercially in Florida. Citrus breeders are now making crosses with HLB tolerant lines to develop new varieties bred specifically for high HLB tolerance.

### **Future Research Goals**

Over the past two decades of HLB research in Florida, much has been learned about citrus and how CLas causes disease. Sequencing of multiple citrus genomes (Gmitter et al. 2020) along with a better understanding of those genes involved in disease expression have facilitated the use of biotech approaches to develop new citrus varieties that may prove resistant to HLB. Of the numerous transgenic citrus lines that have been developed and are being evaluated for HLB resistance in the field, the most promising lines include several sweet orange and grapefruit transgenic lines expressing the Arabidopsis NPR1 protein(Robertson et al. 2018). These lines have exhibited robust tolerance to HLB showing little or no symptoms after many years of being infected with CLas in the field. These promising transgenic lines are currently being reviewed by regulatory agencies in the U.S. for potential release for commercial production.

Researchers at the University of Florida have been successful in using gene-editing technology to develop nontransgenic citrus lines that are disease resistant. The first of these is a 'Hamlin' sweet orange which has been geneedited for resistance to bacterial citrus canker (Xanthomonas citri subsp. citri) disease(Su et al. 2023). Thus far, resistance has only been demonstrated in laboratory and greenhouse studies. These new lines are now under evaluation in the field to further confirm canker resistance and evaluate productivity of the trees under commercial grove conditions. Additionally, numerous citrus lines have been created using gene editing to modify genes which are believed to play a role in HLB disease expression. These new citrus lines are currently being mass propagated for field trials to evaluate against HLB. The use of biotechnology in new variety development will continue to be an important focus of citrus breeding programs at the University of Florida in the years to come.

During the time that HLB has been present in Florida, more than \$500million has been spent on HLB research. Over the past twenty years, the Florida citrus industry has gone from a situation where citrus was being overproduced to now struggling to produce enough fruit to maintain economically viable commercial citrus production. Current research-based production practices used by growers to maintain the health of diseased trees continue to prolong the life of the Florida industry. Ultimately, disease-resistant citrus varieties, developed through conventional breeding or novel biotech approaches, are the ultimate solution for the survival of the Florida citrus industry.

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**Plenary Lecture** 

**Special Plenary Session** 

## Nanotechnology for managing important citrus diseases

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Huanglongbing (HLB, also known a citrus greening) is one of the most devastating bacterial diseases of citrus caused by the bacterium, *Candidatus* Liberibacter asiaticus. HLB has affected majority of commercial citrus producing regions globally. It is spread by an insect, the Asian citrus psyllid. HLB affected trees produce fruits that are green, misshapen and bitter, unsuitable for sale as fresh fruit or for juice. Ironically, most infected trees die within a few years and currently there is no cure available. HLB has destroyed millions of acres of citrus crops throughout the United States and abroad. It has caused serious damage to Florida's \$9 billion citrus industry. Citrus production in Florida is down more than 90% since HLB onset in 2005. Moreover, HLB is now endemic in Texas and keeps California on high alert. As of 2019, the Jiangxi Province of China lost 25% of its groves. Brazil, where the disease was first spotted in 2004 has lost 52.6 million sweet orange trees, a 31% reduction. Citrus canker is another important bacterial disease that has seriously affected the growth and productivity of citrus around the world. Canker affects the leaves, stems, and fruit of citrus trees. It is caused by the bacterium *Xanthomonas citri* subsp. citri. Canker causes lesions on the plant parts, leading to defoliation, dieback, blemished fruit and premature fruit drop. It reduces the marketability of fresh fruit. Citrus canker is a surface/sub-surface restricted disease but the HLB is a vascular disease. Therefore, the management strategies for canker and HLB are uniquely different.

Foliar Copper (Cu) bactericides/fungicides are aggressively used globally on more than 300 crops including citrus. There is an increasing concern of Cu accumulation in field soil, Cu leaching potential into the surrounding ecosystem and development of bacterial resistance. Using nanotechnology, it is possible to improve Cu use efficiency and develop suitable Cu alternatives (such as nano-Zn, Nano-Mg and Nano-metal-nonmetal hybrid). This presentation will focus on laboratory, greenhouse and field efficacy outcome of several nanotechnology-enabled Cu and Cu– alternatives for citrus disease control, challenges towards developing industrially viable formulations and approaches to minimize regulatory challenges.



## **Plenary Lecture**

Special Plenary Session

## Climate change and opportunities for expansion of Mandarin Orange in India

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### I. INTRODUCTION

Mandarin Orange predominates fresh fruit market in Citrus.

Mandarin orange in Nagpur in the Vidharbha region of Maharashtra (central India) is under Tropical climate, growing mainly on rough lemon root stocks. Whereas Khasi Mandarin is grown in North eastern states under sub-tropical conditions. The entire Mandarin oranges (Khasi Mandarin) is grown under sub-tropical climatic conditions. Raised mainly on prior seedlings.

Kinnow Mandarin grown in Punjab, Haryana and low hills of Himachal Pradesh is grown in Sub-tropical climatic condition. Thus, Mandarin is already well spread and adapted in a variety of climatic conditions in India.

Indian horticulture (fruits, vegetables, flowers, spices, plantation crops, root & tubers, medicinal & aromatic plants and others) with an annual growth rate of 5.8 per cent (p.c) during 2000-2016 period proved that technological advancement, reasonable investment and enabling policy environment can influence in accelerating sustainable growth. In 2018-19, horticulture production in the country reached 314.67 million tonnes (M.T.), contributing 34.45 p.c. to agricultural GDP from just 15.08 p.c. cropped area.

Two central sector Missions, namely, I) HTM/TMNE for the 8 NE states (subsequently 3 more NW hill states included) and ii) NHM covering 18 major states & 3 UTs, have significantly impacted economic gains from horticulture farming. The National Horticulture Mission (NHM) with a target production of 300MT by 2012 (ended in 2014-15) has been rated as highly successful.

Three major concerns in Indian horticulture, namely, low productivity, quality assurance and risky produce marketing, are yet to be fully addressed and resolved for which governmental interventions are essential. Trade practices with perishable horticultural commodities are not always farmer friendly and distorted market signals often hurt farmers' interest. Ensuring cost competitiveness through higher productivity, supply of standard quality uniform primary produce and involvement of qualified private sector in export trade and agro-processing are the keys towards development and management. of horticultural value chains (Ghosh, 2018).

Value chains can be developed by encouraging aggregation of farmers, involvement of R & D institution (in suggesting GAP, export protocols), drawing support of agencies like APEDA, NAFED, etc. and investments from private sector.



#### **II. GOVERNMENTAL INITIATIVES TO PROMOTE INDIAN HORTICULTURE**

The Ministry of Agriculture, Government of India, through Central sector schemes implemented the following major Missions, which have created positive impacts towards sustainable development of Indian horticulture:

a) Establishment of National Horticulture Board (NHB) in 1984 for large scale adoption of modern package of practices, including value addition to horticultural produce.

b) Launching of Technology Mission on integrated development of horticulture in the North East (TMNE) in 1998 (later renamed as Horticulture Mission for North East and other Himalayan States) with a focus on end-to-end approach (production to consumption).

c) Launching of National Horticulture Mission (NHM) in 2005 with a focus on area based, regionally differentiated horticulture development in 18 major states and 3 UTs. A production target of 300 MT by the end of 2012 was envisaged.

#### **III. EMERGING OPPORTUNITIES IN CITRUS**

Loose skin Mandarin is gaining popularity in global market as fresh fruit and the NE Himalayan states including Nepal, Bhutan and other NER of India have good opportunities. The above three initiatives have sensitized the need of crop diversification towards high value horticultural crops for higher income to the farmers and better food and nutritional security in the country. For low rainfall, poorly irrigated and low fertilizer consumption areas (Low land productivity zones) conservation farming, precision farming and IPM technology will be more appropriate.

In the North East, in spite of liberal funding support and central assistance fast track development in many crops were not possible as basic R & D works could not be performed locally. Now when much improved infra structure, human resource and technologies are in place even last leg of commercial exploitation is possible in North East horticulture. (Hi-tech floriculture in Mizoram, combating citrus decline problem in NEH, high quality organic spices are some of success stories). Removal of certain land use regulations and improved small holder competitiveness due to technology adoption are positive signs to ponder with for accelerating horticulture promotion.

#### a) NEED FOR PRIORITY FIXATION and NEW INNOVATION BACKED PROGRAMMES

Based on information generated in recent field survey and keeping in view the projected target for area coverage by horticultural crops (as under) newer approach is suggested for faster growth in horticulture sector.

i) Field survey revealed that major problems faced by the horticultural farmers in the country are- finance(31%), marketing (30.5%), labour(18%), storage (15%) and others(5%) ( (Source: DFI, 2018)

ii) In the Report on Policies and Action Plan for Secure and Sustainable Agriculture, August 30, 2019, submitted by Dr. R.S.Paroda it has been shown that to ensure food and nutritional security, a total of about 30 million ha land needs to be brought under horticulture during next 5 years

The new approach of development focused mainly on a) availability of scalable innovations and the scope of technology commercialization, b) priority fixation in horticultural development areas of National importance and c) Farmers aggregation and MFI partnership:



#### b) AREA EXPANSION

Under MIDH Scheme of MoA &FW of Gol, there is good scope for area expansion by establishing new orchards/ garden of fruits, vegetables and flowers In area expansion design, small holder horticulture may get preference as crop diversification is already found to be more income generating (Source : NCAP studies showing that average land productivity is much higher with horticultural crops e.g. in 2011-12 av. Land productivity for food grains at 2004-05 prices was about INR 30 thousand per ha, while for the same period with horticultural crops it was INR 126 thousand per ha).

Commercialization of and management of horticultural value chain (Source: NAIP.2017) should enable in accelerating horticultural growth, ensuring higher income and better livelihood of even small-marginal farmers.

#### c) NICHE AREA DEVELOPMENT

With a focus on export promotion, APEDA of Govt. of India delineated Agri-export zones, vast majority (over 80%) of which have focus on horticultural crops. Presently, Fruits and Vegetables(F&V) are exported mainly to nearby countries (Bangladesh, Malaysia, Pakistan, Saudi Arabia, UAE, Qatar Sri Lanka and some European countries) contributing only 14% of export. Export items of F & V are collected randomly from many places and often quality of the produce does not meet the demands of advanced markets. International fresh fruit market has a large demand for loose skin Mandarins/Tangerines.

Innovative Technologies for North East, easter Himalaya states. Citrus Development in the North East with Export promotion focus and Immediate Action Points are :

Mid hills of eight NE states, totaling about 50 districts, have sizable area with favourable soil – climate condition to produce high quality mandarin orange (Khasi mandarin, Sikkim orange,). NE region (NER) is considered as one of the primary gene centres of citrus fruits and wild species like *Citrus indicia* was found growing well in natural condition in NER. The Khasi mandarin (*Citrus reticulata*), with loose skinned excellent quality fruits enjoys high marked demand for table purpose for use in the entire Eastern Himalayan states including Nepal , Bhutan and the NER of India. In the international market citrus juice from sweet orange (*C. sinensis*) is the most important product and of late mandarins/ Tangerines are gaining popularity as fresh fruit with remunerative prices. The mandarins in the entire eastern Himalayan range are raised from seedlings (not as grafts raised on rootstocks in other places, including in Nagpur orange) and experiencing severe decline due to infection of diseases like Greening and Phytophthora, which have devastated mandarins in Nepal and Bhutan as well. Production of disease free planting material on standard root stocks through adoption of'' Bud wood certification' technique, is now possible for large scale area expansion / replanting programme in the NER

Development of Bud Wood Certification protocol by the ICAR-CICR, Nagpur is the major innovation towards combating citrus decline and rebuilding a sound technology backed citrus industry in the North East. Establishment of disease free Scion banks in protected insect proof poly houses, raising of large number of nucellar seedlings of standard rootstocks (Rough lemon, Rangpur lime, Carrizo citrange and others) in controlled condition, preferably in net houses; collection of certified bud sticks from the scion banks and budding/ grafting by trained grafters on 10-12 month old rootstock seedlings are the major activities under the technology package. Nursery manuals, budding/ grafting timings, nutrition & plant protection measures and others developed by ICAR-CICR, Nagpur and ICAR-RC, Umiyam, Meghalaya can be followed with definite advantage.



The new research station of ICAR-CICR, Nagpur located at the Biswanath Chariali campus of AAU, Jorhat and the Central Institute of Horticulture (CIH) of Ministry of Agriculture & FW, GoI at Medzip hema, Nagaland as the base centres can go ahead with infra structure development for nursery production programme and trainings. Earlier under the "DBT Mission on Quality Planting Material Production in NER" some initiatives were taken at the Regional centre of "The Energy Resource Institutes (TERI), Guwahati for production of grafted Khasi mandarin in large number. Similarly, some initiatives for quality planting material production of Citrus, Khasi mandarin in particular, were taken by Government of Mizoram (in collaboration of Israel) and Government of Nagaland (under TMNE/HTM). Thus, with certain limited support it should be possible to organize production of sufficient number of certified, healthy grafted planting material of citrus in NER itself, enabling expansion of about 50 thousand ha by Khasi mandarin and another10 thousand ha with sweet orange (Valencia orange), Assam Lemon and Kachai Lemon (of Manipur) by next 3 years time.

The famous Nagpur mandarin belt of Maharashtra and Khasi mandarin of NER will be able to meet the demands of both domestic as well as export market of mandarins. Due to climate change threat, shifting from use of seedling plants to grafted plants on hardy rootstocks is desirable and bud wood certification will ensure freedom of deadly diseases like Greening and others The DBT of GoI is in advance stage of launching a Citrus research Net project involving ICAR, SAU, DBT and a few central Govt. institutions working in NER.

The FAO of U.N., under a TCP, has provided technical support to Nepal to develop 'Bud Wood Certification' system for Nepali orange. Infrastructure for mass production of quality planting material free from deadly diseases once established in the North East India, the unique Citrus industry of Eastern Himalayan region will have a new look with good economic impact.



## **Plenary Lecture**

Special Plenary Session

## Potential management strategies to combat HLB disease of citrus plants

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Citrus Huanglongbing (HLB) or citrus greening, the most destructive disease of citrus plants worldwide, causes extensive economic losses to the citrus industry by shortening the life span of infected tree. It is caused by an unculturable phloem-restricted Gram-negative bacterium, *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus, and *Candidatus* Liberibacter americanus. Among the three species, *Candidatus* asiaticus (CLas) is the most widespread and most virulent strain which is transmitted by the vector Asian citrus psyllid, Diaphorina citri. The natural host range of CLA includes species in the Rutaceae family with severe symptoms mainly in sweet oranges, mandarins, tangelos, and grapefruit followed by lemon, rough lemon, and sour orange (Bove, 2006). The ornamental Rutaceous species, orange jasmine Murraya paniculata and Murraya koenigiiare commonly grown in citrus production areas and have been shown to be hosts of the Asian citrus psyllid.

HLB is an old bacterial disease probably more than a century whose origination was China and most probably in Indo-Pak subcontinent. The recent estimate suggests that over 60 million citrus trees have been destroyed by HLB worldwide. HLB infects nearly all major citrus cultivars and causes substantial losses to the citrus industry by not only affecting the marketability of infected fruit but also shortening the lifespan of infected trees. There are no effective control measures to manage the disease. HLB-causing bacteria are at present largely unculturable which limits ability to examine carefully the bacteria's biology, making it difficult to develop effective, specific control strategies. The current management strategy of HLB is to chemically and biologically control citrus psyllids and remove infected trees, which have not been able to stop the spread of HLB.

The new management strategies need to be devised for the management of HLB. Some of the potential strategies to combat HLB disease could be following:

i) Characterization of proteins critical for survival of bacterium for developing effective potential inhibitor molecules

Availability of genome sequence of CLA makes it easier to select key proteins critical for survival of the bacteria and then screening, designing and testing the potential molecules against these proteins/enzymes which can be developed as antimicrobial compounds for controlling this serious disease. The proteome analysis reveals a lot of potential drug targets including, but not limited to, periplasmic amino acid binding proteins, ATIC enzyme of de-novo pathway of nucleotide metabolism, transcriptional regulators etc. The potential inhibitor molecules can be tested for their efficacies on HLB infected citrus plants under controlled conditions.



ii) Use of plant defense protein as effective antimicrobial agents

The effectiveness of plant defense proteins in controlling the disease can be evaluated. The plant defense genes would include antimicrobial proteins, proteinase inhibitors, 2S albumins etc. The protease inhibitors are known to have insecticidal activity and can be deleterious towards the Asian Citrus Psyllid (*Diaphorina citri* Kuwayama), the insect host of CLA which feeds on phloem of plants.

iii) To develop transgenic varieties expressing antimicrobial proteins. The ongoing research work including in planta and field studies will pave the way in the management of Citrus HLB.

The approach would involve the development and testing of transgenic variants of model citrus plant by introduction of a single or more than one known gene involved in plant defense from other related sources. Also, the use of plant origin genes in developing transgenic variants would help in addressing the ethical and related issues. The genes can be expressed under two different type of promoters i.e constitutive and phloem-specific promoter. The effectiveness of transgenic variants of model citrus plant to resist the CLA infection can be examined.

iv) The use of new breeding technologies such as CRISPR based genome editing of key gene(s) in the host plant utilized by bacteria for establishment of infection.

In the context of bacterial infections in plants, CRISPR technology could be employed to edit the genes that bacteria exploit to establish infections. The approach of modifying these genes is to make the host plants less susceptible to infections or to enhance its natural defense mechanism. Compared to traditional breeding methods, CRISPR technology allows for highly specific modification to be made to the plant's genome. Also, CRISPR editing can be done relatively fast accelerating the development of resistant plant varieties. Developing such a plant variety that are resistant to bacterial infections could contribute to more sustainable and environment friendly agricultural practices.

The ongoing research work including in planta and field studies will pave the way in finding an ultimate approach to control this most destructive disease and help the citrus growers to overcome HLB problem.



**Plenary Lecture** 

**Special Plenary Session** 

## Navigating the citrus landscape in India: Growth avenues, potential roadblocks and action points

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The Indian citrus industry is diverse, with various citrus species cultivated in different regions across the country. Commercially important citrus species cultivated in India are mandarins (*santra*), sweet oranges (*mosambi*, *sathgudi*), acid limes (*nimbu*), lemons, grapefruits and pummelos. Amongst these mandarins (*Citrus reticulata* Blanco) are produced in highest quantity in India occupying nearly 40% of the total area under citrus cultivation and these fruits secure a special place in the dietary platter of Indians mostly as fresh fruit. Different varieties of mandarins are found to exist throughout the length and breadth of the country. Nagpur mandarin is widely cultivated in Vidarbha region (Nagpur and Amravati Divisions) of Maharashtra and also some parts of Madhya Pradesh. Kinnow mandarin is grown in Abohar (Punjab), Sri Ganganagar (Rajasthan) and parts of Haryana. In Karnataka, Coorg mandarin is popular while Khasi mandarin dominates in North East India. Apart from this, Sweet Oranges (*Citrus sinensis* Osbeck) with varieties likeMosambi, Sathgudi, Blood red, Malta, Hamlin, Pineapple, Valencia, and Jaffa; Acid Lime (*Citrus aurantifolia* Swingle) with varieties like Kagzi lime, Vikram, Pramalini, PKM-I, Baramasi and Kagzi seedless lime; Lemon (*Citrus limon* Burm.) with varieties like Baramasi, Assam lemon, Pant lemon, Gandhraj, Italian lemon and Eureka lemon; Grapefruit (*Citrus paradisi* Mad.) with varieties like Duncan and Marsh seedless Ruby and Pummelo (*Citrus grandis* Osbeck) with varieties like Chakotra and local selections are also cultivated.

The North-eastern region of India is considered a treasure house of citrus germplasm, with 23 species, one subspecies and 68 varieties reported from there. All citrus cultivars have their own features and even challenges. The Khasi mandarin cultivation in the North-Eastern region is of seedling origin and faces decline due to lack of proper cultivation practices. The region's challenges include water scarcity for pesticide spraying, steep slopes, and proximity to thick forests. Coorg mandarin in Karnataka is under threat due to severe greening disease infection. This region uses citrus plants as shade/cover crops for coffee plantations. In northwestern India, Kinnow mandarin is a successful commercial fruit crop, with advanced cultivation practices like drip irrigation and the use of rough lemon rootstock. Export to neighboring countries has been successful.

Vidarbha region of Maharashtra has a large concentration of Nagpur mandarin plantations. ICAR-Central Citrus Research Institute, Nagpur, has identified a seedless Nagpur mandarin variety and recommended alternative rootstocks like Rangpur lime and Alemow for improved sustainability. Sweet orange cultivation has faced decline in Punjab, but Mosambi in Maharashtra and Sathgudi in Andhra Pradesh are cultivated commercially. Several high-yielding acid lime varieties/ promising clones like Parmalini, Vikram, Jai Devi, Sai Sarbati, Acid lime-7 and Acid lime-8 have been released. ICAR-CCRI, Nagpur, has released new varieties of grapefruit, pummelo and sweet



orange, offering improved characteristics and yield potential.

The demand for citrus fruits is expected to increase significantly due to population growth and changing lifestyles. Achieving a minimum annual growth rate of 5% is necessary to meet domestic demand and explore export markets. Sustainable practices, including disease-free planting material, rootstock development, water management, and climate change mitigation, are vital for the industry's future.

The citrus industry of India is facing numerous challenges. Disease management remains a significant challenge, with greening disease (HLB), *Phythophthora* induced diseases and canker posing serious threats. Developing resistant varieties and implementing integrated pest management (IPM) are essential. Post-harvest management practices, including handling, packaging, and storage, need further development to reduce losses and maintain fruit quality. Mechanization and automation should be adopted for irrigation, pruning, spraying, and harvesting to enhance efficiency and reduce labor costs. Soil health, water resource management, and climate change adaptation strategies are crucial for long-term sustainability. Promoting citrus for its health benefits and organic cultivation can tap into the growing demand for healthy and safe fruit. Research on rootstocks, cultural practices and insect pest management should continue to improve citrus cultivation. 'On the other hand, only 1.72% of the countrys production is exported. This low export rate can be attributed to several factors including lack of quality control, inadequate post-harvest management and logistical challenges.

ICAR-CCRI, Nagpur, in this aspect, has made significant contributions to the citrus industry, including disease-free planting material production, developing molecular disease diagnostic tools, eco-friendly integrated management of insect pests and diseases, identifying maturity standards, weed control, mechanized handling technologies etc. These advancements have reduced post-harvest losses and improved fruit quality. Sustainable practices, soil health maintenance and climate change adaptation strategies should be a focus for the future. The Indian citrus industry faces challenges but also holds great potential. The use of cutting-edge technologies such as Artificial Intelligence (AI), Internet of Things (IoT), drones and robotics can revolutionize the citrus industry. Alpowered drones can help farmers increase output and expedite the production of crops. IoT can enhance warehousing and logistics while AI can enhance quality traceability. Citrus processing waste represents a significant resource that can be valorized for various applications. For instance, these wastes can be used as raw materials for manufacturing biofuels, enzymes, vitamins, antioxidants, animal feed, antibiotics, etc., through solid state fermentation (SSF) processes. With a focus on sustainable practices, research and technological advancements and increased awareness of citrus's health benefits, the industry can meet growing demand and thrive in the years to come.

Indian citrus industry can work hand in hand with other citrus industries of other countries. Strengthening the Asian citrus industry through collaborations, scientist/student exchange programs and new trade policies is a commendable goal that can lead to economic growth and improved agricultural practices across the continent. Establishing a Asian Citrus Council with participation of major citrus-producing countries of Asia can serve as a platform for information sharing, policy development and collaboration on research and development. Exchange Programs for Scientists and Students across Asian countries will facilitate the sharing of knowledge, research findings and best practices in citrus cultivation, disease management and post-harvest handling. Collaborative Research Projects need to be undertaken to address common challenges faced by the citrus industry such as pest and disease management, climate change adaptation and varietal improvement. Technology Transfer by establishing mechanisms for the transfer of agricultural technologies and best practices between countries can be



facilitated through exchange programs of agricultural extension or advisory services personnel of each country. Developing and adopting common standards for citrus production, quality control, and certification across the region will facilitate trade and ensure product quality, making Asian citrus products more competitive in international markets. Formulating new trade policies and agreements among Asian countries will help to reduce trade barriers, tariffs and non-tariff barriers related to citrus products. Investment should be made in transportation and logistics infrastructure to improve the supply chain for citrus products. Collaboration should be done on marketing campaigns to promote Asian citrus products both domestically and internationally. Capacitybuilding programs should be supported for citrus growers, processors and exporters. Collaboration on environmentally sustainable citrus production practices can minimize the industry's ecological footprint. Joint risk management strategies should be developed to deal with common challenges like climate-related risks, pest outbreaks and market fluctuations. Collaboration should be encouraged between government agencies and the private sector within each country to ensure a coordinated effort in implementing these strategies. Organizing regular citrus industry summits and conferences can help bring together stakeholders, share progress and set future goals for the development of the Asian citrus industry. By implementing these strategies and fostering a spirit of cooperation and mutual benefit, Asian countries can strengthen their citrus industry, enhance food security and contribute to the economic development of the region.

Given its wealth of biodiversity, the citrus sector in India has a great deal of potential to grow and make a substantial contribution to the agricultural and economic development of the nation. However, it faces production challenges and trade barriers that needs for coordinated efforts from researchers, policymakers, and industry players. The Indian citrus sector may overcome its challenges and emerge as a world leader in citrus export and production by introducing new citrus varieties, advancing post-harvest management techniques, and incorporating cutting-edge technologies. To revitalize and transform the Indian citrus industry into a competitive and sustainable player on the world arena, a comprehensive strategy and holistic approach must be implemented.



## **Plenary Lecture**

Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

# How the specificities of citrus reproductive biology drive the strategies for the selection of seedless varieties and the development of environmental adapted rootstocks.

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The high interspecific heterozygosity of some of the most important horticultural groups of citrus such as sweet oranges, grapefruits or lemons resulting from natural inter specific crosses do not allow the use of sexual recombination within groups for their improvement. Indeed, it would generate too much phenotypic diversity and the loss of the initial ideotype. In general, selection for these ideotypesis based on spontaneous or directed mutation to obtain phenotypic variants. In the future, genome editing could also be used to modify a particular trait. Infra specific crosses are mainly used for varietal diversification in the mandarin group but with a limited number of recombination events. However, whatever the species or the variety the juvenile phase is long (4-8 years) and then limits the possibility to develop a genealogic selection with multiple generations. Varieties that have lost their juvenile phase are sought for successive backcrossing to introgress genes of agronomic interest from wild species into cultivated species. The gametophytic self-incompatibility and apomixis are reproductive traits that hamper citrus breeding. The fresh fruit market requires the production of citrus seedless fruit. Sterility or gametophytic self-incompatibility (e.g. clementine) associated with parthenocarpy makes it possible to achieve this objective. The sterility can results from spontaneous or induced mutations (e.g. grapefruits, sweet oranges), cytoplasmic male sterility (e.g. Satsuma mandarin) or polyploidy (e.g. Tahitian lime a spontaneous triploid genotype). This presentation gives an overview of the strategies used by different laboratories at the international level to obtain sterile varieties, and more adapted rootstocks taking into account the particularities of citrus reproduction and adapting the breeding method to the genetic status of the crop.



## **Plenary Lecture**

Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Good horticultural practices are critical for maintaining productivity of HLBaffected sweet orange trees

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Huanglongbing (HLB; Citrus greening) is a bacterial disease that threatens citrus production worldwide. Florida citrus growers are currently producing the state's most iconic commodity at the mercy of Huanglongbing's onslaught of the industry, with numbers reaching record lows every year; in past 15 years Florida citrus production has decreased by more than 75%.

Once CLas is transmitted to a citrus tree, the sieve pores are plugged and phloem disruption is observed. The disruption of vascular function, loss of root mass, and altered mineral nutrition in CLas-infected trees lead to arrested tree and fruit growth, increased fruit drop, a decline in production, and could eventually lead to tree death. Visible symptoms of HLB typically include yellowing of leaves, blotchy mottle, chlorotic patterns of leaves resembling those induced by Zn and Fe deficiencies, shoot dieback, and reduced tree height. Currently, there is no cure or resistant germplasm for HLB, therefore, often growers find themselves in difficult situation in managing diseased orchards. Thus, current strategy of citrus growers in Florida is to maintain tree growth with basic horticultural techniques such as use intensive nutrition management and plant growth regulators.

Recently findings demonstrate that the basal cause of poor growth and decline in HLB-affected trees is due to a deficit of growth-promoting hormones such as auxin, gibberellins (GA), and cytokinins, and over-accumulation of defense-related hormones such as salicylic acid(SA) and abscisic acid (ABA).As a result of hormonal imbalance upon CLas-infection, HLB-affected trees lag in bud emergence along with growth which contributes to compromised leaf and shoot development as well as higher bud dieback resulting in thinner canopies and reduced life span with HLB progression. During leaf development phases, higher rations of defense related hormone to growth promoting hormone suggests tradeoff of resources on defense over growth in HLB-affected trees. Hence the strategies to exogenously apply growth-promoting hormones, such as Gibberellic Acid (GA) and 2,4-D (a synthetic auxin), have been shown to improve yield, canopy density, and reduce fruit drop in HLB-affected sweet oranges. Multi-year field trial with GA has shown that repeated application of GA (Sep-Jan) can suppress the return bloom and improve the fruit and leaf growth by improving source to sink ratio and helps in stomatal oscillation thus improving transpiration, and water uptake within the plant body, and photosynthetic activity and carbohydrate synthesis through increasing sink activity (improved vegetative growth), thus better fruit retention on the tree and higher fruit yield. As a result of these finding, Florida growers have adopted use of plant growth regulators to enhance citrus production in Florida.



Mineral nutrients are essential for the growth and development of plants and microorganisms and are important factors in plant-disease interactions. How each nutrient affects a plant's response to disease, positively or negatively, is unique to each plant-disease complex. In general, nutrient-pathogen interactions are not well understood due to their complex nature and dependence on a number of external factors. Plant nutrient deficiency or toxicity may affect disease susceptibility through plant metabolic changes thereby creating a more favorable environment for disease development. When a pathogen infects a plant, it alters the plant's physiology directly or indirectly, particularly with regard to mineral nutrient uptake, assimilation, translocation, and utilization. Pathogens may immobilize nutrients in the soil or in infected plant tissues. They may also interfere with translocation or utilization of nutrients, inducing nutrient deficiencies or toxicities. Although disease resistance is genetically controlled, it is considerably influenced by environmental factors. Some disease resistance genes in plants are only activated by specific environmental stimuli. Mineral nutrition is an environmental factor that can be easily controlled in agricultural systems, the effects of which can be substantial. In the last decade, several scientific reports have demonstrated that good fertilization improves the health and productivity of HLB-affected trees. HLB-affected roots are functional and efficient in nutrient uptake; however, reduced biomass limits the nutrient uptake capacity of trees as a whole. To compensate for the reduced root-to-shoot ratio, the existing roots undergo anatomic and transcriptomic changes to improve nutrient uptake efficiency. It is possible that higher input of energy in nutrient uptake possibly results in reducing the root longevity of HLB-affected trees. Moreover, Therefore, it is suggested that HLB-affected trees should be supplied with constant nutrients at higher rates than what is recommended for healthy trees. Such fertilization practices possibly mitigate the abiotic stress in HLBaffected trees. The availability of nutrients is also critical for both healthy and HLB-affected trees to carry out normal tree function, growth, development, and tree defense response. In nutrient-deficient conditions, many metabolic processes are presumably suppressed to conserve energy, which reduces growth. It is also possible that HLB-affected trees, which are in a constant state of elevated tree defense response and oxidative stress, require these nutrients at higher rates than healthy citrus. Thus, good nutrition management practices are critical for the productivity of HLB-affected trees as the availability and uptake of nutrients allow HLB-affected trees to respond to abiotic and biotic stresses.

Altogether, good nutrition management along with use of PGRs such as GA are good alternatives for citrus growers in absence of any cure for HLB to promote vegetative growth and thus improve source to sink ratio in HLB-affected and therefore, improve fruit growth and yield.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# The effects of new tools for citrus (*Citrus* spp.) plantings on citrus diseases in a multi-year comparison

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Huanglongbing (HLB) is a destructive citrus disease that has ravaged the Florida citrus industry in the last fifteen years. Much of the disease management has focused on the insect vector Diaphorina citri. As new tools for D. citri management like exclusion netting on trees, reflective ground cover, and red kaolin clay sprays are being explored for psyllid management, it is important to monitor HLB. While HLB is the main concern in new groves, other diseases may be affected in unexpected ways by the new tools. Over a 3-year period, we monitored for HLB symptoms, Candidatus Liberibacter asiaticus (C Las) titer, greasy spot (Zasmidium citri-griseum), citrus canker (Xanthomonas citri subsp. citri), sooty mold (Capnodium citri), and phytophthora root rot (Phytophthora nicotianae). We found the exclusion netting was best to prevent HLB symptoms and keep the CLas titer low (P< 0.0001). Reflective mulch slowed the infection rate by two months compared to the kaolin clay and standard insecticide management. The exclusion netting also suppressed canker incidence and severity significantly (P< 0.0001) compared to all other treatments, with nearly no canker observed. Additionally, canker severity was reduced by half compared to mulch treatments and standard insecticide management from the kaolin clay. Greasy spot affected all trees in the first season and severity was higher within the netting by the third year. Sooty mold was infrequently observed in most treatments but was problematic under the exclusion nets, where nearly every tree was affected when there were populations of honeydew forming insects. None of the trees had phytophthora root rot symptomsbut propagules were measured at damaging levels (20 CFU/cm<sup>3</sup> soil) in the reflective mulch and exclusion netting treatments. In conclusion, exclusion netting is the most promising treatment to avoid HLB and citrus canker for young trees, but that greasy spot, sooty mold, and phytophthora will need to be managed.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Past and present of pest management in Jejucitrus, and threatening pests in the future

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Citrus is an important industry in the Jeju area, Korea, and since 1960, it has been the center of the local economy, and thus pest control has been treated as primary important for the safe production of citrus. In this presentation, I will review the past and present of citrus pests and the management strategy in Jeju, and discuss future threats related to climate change. As in other countries, in the 1980s, the intensification of insecticide resistance was evident in Jeju citrus due to the indiscriminate use of pesticides. In other words, citrus pests were simplified to citrus red mite (CRM), aphid species, and citrus leaf miner (CLM). Since the 1990s, as integrated pest management (IPM) has been adopted, some species such as CRM have been effectively managed. After that, from the mid-2000s, chillithrips, suspected of being the cause of climate change, suddenly began to cause damage, and several species of pests, such as scale insects, became a problem unlike before. On the other hand, since 2000, as the area of vinyl greenhouse cultivation with late mature citrus has rapidly increased, thrips, CLM, aphids, and CRM have become more important pests. We are facing the problem of serious insecticide-resistant pests that we experienced in the open field citrus again. As a solution to this, various control strategies such as the alternating spraying system of chemicals with different modes of action and the use of microbial agents have been introduced. Currently, due to climate change probably, there are concerns about the invasion of Oriental fruit flies and Asian citrus psylla, known as the vector of green diseases, and monitoring is being strengthened. In addition, I would like to introduce research results on citrus pest control and monitoring technology in Jeju.



Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus

# Beyond vitamin C: A deep dive into the health-promoting components of citrus fruits

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Citrus fruits have great taste, texture, and numerous health-promoting phytochemicals, including the well-known antioxidant vitamin C, carotenoids, organic acids, and different sugars. Although vitamin C may be the best-known citrus bioactive, recent evidence has highlighted the beneficial effects of many other bioactive compounds found in citrus fruits, including coumarins, phytosterols, and various aglycone and glucoside derivatives of phenolic acids, flavonoids, and limonoids. The levels of these metabolites are affected by the type of citrus variety, environmental factors, and physiochemical dynamics in the fruit and post-harvest processes. These phytochemicals have diverse chemical and structural properties resulting in antioxidant, anti-inflammatory, and anti-cancer activities. Although most of the research on health-beneficial compounds has focused on their direct effects through digestive absorption, recently efforts have also aimed to understand how they affect gut health. Further, this research has the potential to understand the cause of various chronic diseases, and thus prevent or alleviate these diseases. Moreover, semi-modification of these complex natural compounds is helping us to understand their biological effects in present applications and devise future uses.

#### Introduction

The citrus fruit is a unique berry called a hesperidium that is covered in a tough skin and contains seeds under the rind's surface in segments packed with pulp vesicles. These fruits are cultivated in subtropical and tropical regions worldwide, boasting an annual harvest of roughly 121 million tons globally, and are consumed in various forms, such as fresh fruit, juice, pickles, dried fruits and incorporated into a diverse range of foods. The majority of commercial citrus species arose through hybridization and selective breeding, resulting in some uncertainty about their ancestry. The chemical composition of the rind and pulp vary substantially in different citrus fruits, providing a wealth of diverse phytochemicals for researchers to explore and a plethora of unique, varied flavors and nutritional benefits for consumers.

The healthy aspects of citrus fruits come from phenolic acids, flavonoids, limonoids, and vitamins. Recent advances in bioactive purification, analytics, and instrumentation from our research group and others have given additional



insight into their unique health-promoting characteristics. Here, we briefly examine the health-promoting properties of three key classes of bioactives from citrus.

#### **Biological Significance of Citrus Bioactives**

#### Phenolic acids

Phenolic acids contain an aromatic ring and a carboxylic acid moiety; in plants, phenolic acids such as caffeic acid are precursors of lignin and other phenolic acids function in plant immunity. In the human diet, phenolic acids act as antioxidants, with potential effects on inflammation-related chronic diseases. Among the diverse phenolic acids, caffeic acid, ferulic acid, and coumaric acid are the most abundant in citrus fruits. Caffeic acid is a potent antioxidant and anti-inflammatory agent and can be extracted from oranges and lemons in significant amounts. Similarly, ferulic acid has anti-cancer effects and is found at high levels in grapefruits and limes. A high level of coumaric acid was also detected in oranges and lemons; this phenolic acid has antioxidant properties and may reduce the risk of chronic illnesses. Cinnamic acid and ellagic acid are also detected in various citrus varieties and these effective antioxidants may affect cancer cells proliferation, diabetes, and heart problems.

Although researchers have made substantial progress in identifying and characterizing citrus phenolic acids, we lack knowledge on their in vivo functions and factors that impact their bioactivity, such as bio-availability and absorption. Further investigation is necessary to explore sustainable sourcing, stability, and bioavailability to expand their potential applications. Additionally, well-designed human studies are necessary to establish the therapeutic effectiveness of citrus polyphenols. Although how they affect the human microbiome, and the effects of different phenolic acids in combination, remain unclear, recent research has explained the possible mechanisms of support of gut health by citrus bio-actives through chemical neutralization, increase in microbiome colonies and inhibition of pathogenic organisms mainly by flavonoids and other bioactive principles.

#### Flavonoids

Flavonoids contain aromatic rings in a 15-carbon structure, and a ketone group. In plants, flavonoids function as pigments and as signaling molecules in plant–rhizobia symbiosis. Flavonoids are abundant in oranges, grapefruits, and lemons and include hesperidin, naringin, quercetin, polymethoxyflavones, tangeretin, and nobiletin. Hesperidin is abundant in oranges and lemons, has anti-inflammatory and antioxidant qualities, and can help to lessen the risk of chronic illnesses including heart disease and cancer. Naringin is abundant in grapefruits and also has anti-inflammatory and antioxidant properties, along with the potential to decrease the risk of chronic illnesses such as diabetes and cancer. Similarly, quercetin has anti-inflammatory and antioxidant properties, and lowers the long-term risks of diseases including cancer and heart disease.

Studies on plant flavonoids have illuminated their diverse functions and effects on human biology. In one of our studies, we examined eight flavonoids (apigenin, scutellarein, kaempferol, rutin trihydrate, neohesperidin, neoeriocitrin, naringenin, and naringin) and a coumarin (bergapten) and identified their antioxidant potential. We also investigated citrus flavonoids for various biological activities such as the ability to inhibit quorum sensing in Vibrio harveyi, antineoplastic activity, drug-induced programmed and unprogrammed cell death, and induction of phase-II drug metabolizing enzymes. Another study on effect of structurally similar related flavonoids on colon cancer cells inhibition, results suggest out of eight flavonoids used, apigenin and quercetagetin are most effective in inhibiting SW480 cells as measured by both gene and protein expressions.



Further investigation is needed to explore various aspects of citrus flavonoids such as their stability, and bioavailability, to expand the potential applications of flavonoids derived from citrus fruits. Integrating experimental data and artificial intelligence methods in studying citrus flavonoids could aid in the discovery and development of new molecules for targeting various health conditions. Future research on flavonoids may also extend the list of their health-promoting effects and identify individual and combinatorial effects of specific flavonoids.

## Limonoids

Limonoids are triterpenoids that are unique to citrus fruits and are characterized by their bitter taste. Some of the most common limonoids found in citrus fruits are limonin, nomilin, and obacunone. Limonoids have potential anti-inflammatory properties and substantial research has focused on their anticancer properties. These studies have indicated that citrus limonids may inhibit the growth of colon, breast, prostate and pancreatic cancer cells and induce cell death, thereby potentially reducing the risk of certain types of cancer. Obacunone has been identified as a potent inhibitor of proliferation, an activity attributed to its ability to induce apoptosis in cancer cells and activate phase II enzymes in cancer-induced mice. However, the levels of obacunone and obacunone glucoside in citrus are relatively low, ranging from 7.2 to 60 ppm in different parts of the fruit, primarily in the peel and seeds, rather than in the juicy pulp. Further,obacunone has been found to trigger apoptosis and suppress inflammatory markers in colon and prostate cancer cells, inhibiting their proliferation. This was achieved through activating intrinsic apoptosis, with down-regulation of the androgen receptor and prostate-specific antigen. Further research is needed to fully understand the mechanisms and potential therapeutic applications of citrus limonoids in cancer prevention and treatment.

Another active area of research focuses on citrus bioactives and gut health. Indeed, flavonoids, phenolic acids, and limonoids have been shown to have potential benefits for gut health by helping promote the growth of beneficial gut bacteria, inhibiting pathogen bacteria, reduce inflammation in the gut, and improve overall digestive function. As emerging research explores the connection between gut health and overall health, new studies will reveal how citrus bioactives affect this connection. Animal and human studies have shown that flavonoids found in citrus such as hesperidin, supplementing citrus fruits, juice have shown increased content of faecal short-chain fatty acids and increased counts of *Lactobacillus* spp., *Bifidobacterium* spp., and *Clostridium* spp. in intestine samples along with increasing the population of fecal *Bifidobacterium* spp. And *Lactobacillus* spp. This intriguing evidence for promotion of gut health by citrus and its constituents is likely to spark follow-up studies identifying the mechanisms of effect for the various citrus bioactives, particularly limonoids.

#### New and modified citrus bioactives

The detection of new citrus bioactives and the study of modified bioactives from citrus has revealed their potential as antiproliferative and tumour inhibiting agents and opened to a new area of research focused on exploring the structure–activity relationship of citrus bioactives. In our previous findings, we discovered that citrus limonoids inhibit p38 MAP kinase activity. We also found that the presence of a seven-membered A ring, together with an acetoxy group (as seen in nomilin), are crucial factors determining the p38 MAP kinase inhibitory activity. Similarly, we developed furan-less limonin and nomilin derivatives, which also inhibited glutathione S-transferase enzyme activity. In the new limonin derivative limonin 7-methoxime, modification at C7 seems to increase the potency against E. coli K12 biofilms. These modified structures help us to change the bioactivity by modifying functional



groups, rings, and adding or deleting sugar molecules. Structure–activity properties also affect the bioavailability of these bioactives and significantly contribute towards their various effects on health related to chronic syndromes, gut health, and inflammatory responses. Using modifications to tailor the activity of citrus bioactives holds substantial promise for future research.

#### **Future intervention studies**

Human trials provide valuable insights into the potential benefits and risks associated with consuming specific plant species. Citrus consumption is a prime example of this, as it is endorsed as a significant component of the human diet due to its potential health benefits. Clinical studies on understanding the benefit of dietary components pose challenges like obtaining baseline data for each nutrient, multiple nutrients and phytochemicals, food interactions, and stability of nutrients. Therefore, regular use of specific ingredients and products are often tested for 4–10 weeks. Citrus has been used as in many observational studies. This includes study to understand the correlation between risk of oral premalignant lesions and consumption of fruits and vegetables in men and understand the benefit of fruit and vegetable consumption in preventing pancreatic cancer. These studies have indicated that citrus and its constituents have potential benefits in preventing cancer.

Studies on controlled diet supplemented with citrus fruits or products in healthy volunteers may provide some insight on bioavailability and efficacy of major constituents like flavonoids and limonoids. Our research, along with other sources, has revealed that including citrus in one's diet can enhance cardiovascular well-being and prevent cancer. Further investigation into fruits and vegetables, even commonly consumed ones, will help elucidate their specific health benefits and risks. For example, recent inquiries into the potential correlation between grapefruit consumption and increased breast cancer risk, as well as a possible link between citrus consumption and higher malignant melanoma risk, highlight the need for thorough scrutiny before making conclusive inferences about any negative effects of these fruits. This emphasizes the critical need to continue researching the potential positive and negative effects of commonly consumed and underutilized crops. Indeed, some underutilized crops are only partially domesticated, similar to many primitive potato cultivars, and may contain toxins that can be reduced through traditional culinary or processing methods. Carefully conducted trials will tease apart the effects of citrus fruit bioactives to help consumers avoid potential negative effects and enhance the many positive effects.

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Latest Developments in Technology Outreach, Citriprineurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector

# Navigating the future of citrus agriculture in the Philippines: An examination of enhanced production management

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The citrus industry in the Philippines is a vital component of the country's agricultural landscape, contributing significantly to its economy and providing livelihoods for many. The Philippine citrus industry has experienced substantial growth over recent years, with an increasing demand for citrus fruits both domestically and internationally. Citrus production is predominantly found in the Calabarzon, Central Luzon, and Northern Mindanao regions. The industry largely comprises small-scale farmers, but commercial orchards are also on the rise, adopting modern cultivation techniques and technologies. In 2020, the total land area dedicated to citrus production in the Philippines was estimated to be around 33,000 hectares. Common citrus fruits grown in the country include calamansi (Philippine lime), pomelo, oranges, and mandarins. In 2019, the total citrus production in the Philippines was approximately 1.2 million metric tons. This abstract provides an up-to-date snapshot of the citrus industry, focusing on various key aspects including production systems, plant genetic resources, cultural management practices, pest and disease management, and soil, water, and fertilizer management.

The Philippines is rich in citrus genetic diversity, harboring a wide range of citrus species and varieties. Local and exotic varieties, including calamansi, pomelo, and mandarins, are extensively cultivated. Ongoing efforts in germplasm collection, conservation, and breeding programs are essential for preserving genetic diversity and improving citrus cultivars for disease resistance and fruit quality. Citrus Resource Information System (CitRIS) serves as a comprehensive repository of invaluable plant genetic resources within the Philippines, offering a meticulously curated collection that spans regions nationwide. This initiative not only characterizes and accurately identifies these diverse citrus varieties but also strategically maps their suitability across various geographical locations within the country, providing a vital resource for the sustainable advancement of citrus production systems

To meet the increasing demand for citrus, Filipino farmers are adopting improved cultural management practices. These include high-density planting, proper pruning techniques, and the use of quality planting materials. Sustainable practices such as integrated pest management (IPM) and organic farming methods are also gaining



popularity among growers. Citrus orchards in the Philippines face various pest and disease challenges. Citrus greening disease (HLB), twig blight disease, brown spot, greasy spot, canker, scab and melanose poses a significant threat to citrus production. Integrated pest management strategies, including the use of biological control agents, beneficial insects and organic pesticides, are being implemented to mitigate these issues. In addition, effective implementation of plant quarantine regulatory laws and the accreditation of tissue culture and nursery facilities are of paramount importance. These measures not only safeguard the citrus industry against the introduction and spread of diseases and pests but also ensure the production of high-quality planting materials, further bolstering the resilience and sustainability of the Philippine citrus sector. Soil testing and analysis are becoming more common to determine nutrient requirements. Precision irrigation systems are also being adopted to optimize water use efficiency. The use of organic and slow-release fertilizers is on the rise to improve soil health and reduce environmental impact.

The citrus industry in the Philippines stands as a dynamic and vital force within the nation's agricultural sector. Its substantial contribution to the economy and its role in providing livelihoods for many cannot be overstated. Over recent years, this industry has demonstrated remarkable growth, buoyed by the escalating demand for citrus fruits both domestically and abroad. Spanning regions like Calabarzon, Central Luzon, and Northern Mindanao, it boasts a landscape that blends small-scale farming traditions with the adoption of modern cultivation practices. The Philippine citrus industry exemplifies resilience, adaptability, and growth. With an unwavering commitment to preserving genetic diversity and adopting sustainable practices, it is poised to meet the evolving demands of the future while remaining a cornerstone of the nation's agricultural success.



# **Keynote Lecture**

Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Towards development of multi-stress resilient Citrus

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Fruits are essential part of the human diet as lack of fruits in the diet leads to inadequate intake of micro- and macronutrients, which causes different forms of malnutrition. The world population is projected to exceed 10 billion by the end of 2050 and fruit production has consistently been inadequate to meet the demand. Citrus is one of the most important fruit crops with worldwide cultivation in more than 140 countries with an annual production of around 160 Mt. Besides a rich source of vitamin C and refreshing taste, Citrus also provides several other micro- and macro nutrients including dietary fibres, sugars, flavonoids, carotenoids, etc. It is also known to have antioxidant, anti-inflammatory, anticancer, antimicrobial and anti-insect activities and also effective against several diseases including cardiovascular disorders, diabetes, obesity, cancer, etc.

Changes in the climatic/environmental conditions have negatively impacted citrus fruit crop production and quality. Citrus spp. is susceptible to both, abiotic stresses including drought, salinity, alkalinity, flooding, extreme temperatures, oxidative stress and nutrient deficiency and biotic stresses such as insect vectors, sap feeders, mites, bacteria, herbivores, viruses and fungi. Under natural conditions, citrus experiences several stresses simultaneously, damaging both quality and yield.

Due to its long juvenile period, conventional breeding in citrus takes time for trait improvement. This led the scientific community to find newer and faster ways to develop citrus cultivars with inbuilt stress tolerance. Agrobacterium-mediated transformation of *in vitro* germinated epicotyl segments is one such possibility,. As, Citrus is a non-host to Agrobacterium, commercial species including *Citrus aurantifolia* and *Citrus sinensis* are highly recalcitrant to genetic transformation with Agrobacterium. By using callus-inducing treatments to the epicotyls during pre-culture and co-cultivation by supplementing auxin and cytokinin rich media, we developed highly efficient and reproducible Agrobacterium-mediated transformation of *C. aurantifolia* and *C. sinensis*. Moreover, we modified the protocol for *in vitro* rooting in place of *in vitro* micrografting to combat the loss of transgenic lines. Using this protocol, the whole transgenic plants of *C. aurantifolia* and *C. sinensis* can be developed in about ~4 months with transformation efficiency of 30% and 22 % for respective species.

Further, we constitutively over expressed a novel dual activity purine nucleoside phosphorylase from *Putranjiva roxburghii* (PRpnp), a PNP family protein with trypsin inhibitory activity, in *C. aurantifolia* Cv. Pramalini and *C. sinensis* Cv. Mosambi. The protein showed nuclear-cytoplasmic localization upon transient over expression, suggesting that it could regulate various important biological functions by maintaining the intracellular pool of purine. Overexpression of PRpnp significantly enhanced tolerance to multiple stresses such as drought, salt, oxidative stress, alkaline pH, and two pests, *Papilio demoleus* and *Scirtothrips citri* in transgenic plants. Thus, it



provided much needed multi-stress tolerance. To reveal the underlying mechanism of multiple stress resistance, we performed global gene expression studies which showed up-regulation of genes, related to hormone biosynthesis and signalling, plant defense, growth and development. The transcriptome results were confirmed via LC-MS/MS analysis, which showed increased endogenous accumulation of plant hormones such as ABA and JA in transgenic plants, which together provided multiple stress resistance by synergistic actions. Moreover, as PRpnp also inhibits trypsin proteases in the insect gut, it also enhanced stress tolerance towards insect pests in transgenic plants. Additionally, being a purine salvage enzyme, we anticipated the role of PRpnp in cytokine (CK) metabolism as well. LC-MS/MS analysis showed enhanced endogenous levels of CK-free bases in transgenic plants, thus confirming the role of PRpnp in CK metabolism. In conclusion, we found over expression of PRpnp enhanced overall plant vigour and enhanced multiple stress resistance in transgenic *C. aurantifolia*.



# **Keynote Lecture**

Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Citrus huanglongbing: Strategies to develop disease-resistant varieties through breeding.

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Citrus is a very important fruit crop in many tropical sub-tropical countries. In the United States of America, majority of the citrus cultivation is limited to the states of California, Florida, and Texas. Since 2005, the threat of citrus huanglongbing or, citrus greening has concerned the citrus industries in the USA; the disease is associated with an uncultivable, alpha-proteobacterium, Candidatus Liberibacter asiaticus, and spread by the Asian citrus psyllid (Diaphorinacitri). In the USA, the state of Florida was first affected by HLB and the disease spread throughout the state in a short time through the movement of infected citrus, vector dispersal through ornamental plants, transport of psyllid-infested citrus fruit in open trucks, and other incorrect practices (Halbert et al., 2010 and 2012; Manjunath et al, 2008). The financial losses because of HLB are significant in Florida; citrus production reduced from 16 million tons in 2004 (before HLB) to 7 million tons in 2022. Because of the nature of the pathosystem, detection of diseased trees is inefficient during the initial stages of infection; the Asian citrus psyllid reproduces profusely, and effective vector control of an insect that has established well in an area is extremely challenging. Current methods of HLB management are considered economically unsustainable, and ecologically undesirable. Some of the promising approaches towards HLB mitigation arethe use of CUPS (citrus under protective structures; exclusion of the insect vector is effective in keeping the trees disease-free; Schumann et al., 2017) and treatment with antibiotics like oxytetracycline (to eliminate the HLB pathogen from infected trees). While CUPS maybe an option for research facilities interested in protecting valuable germplasm, applicability for commercial groves involves substantial initial investment and logistic challenges. Antibiotic delivery, as it is presently administered, involves drilling a hole in the stem and injecting the antibiotic (Archer et al., 2023); the long-term effects of the use of antibiotics on the tree and for the environment need to be studied.

The availability of disease-resistant cultivars will facilitate long-term citrus cultivation since it will be financially affordable and, environmentally friendly. HLB-resistant cultivars are needed for sustainable citrus cultivation in the presence of the pathogen and the psyllid vector that are already present in the citrus-growing areas and cannot be eliminated. In the cultivated citrus types, there is no documented disease resistance to the HLB pathogen. Certain cultivars are known to be more tolerant than others and research is underway exploiting all possible avenues for disease mitigation. To identify HLB-resistant germplasm in the taxonomic group, we conducted field trials using 100 accessions belonging to the citrus sub-family, Aurantioideae. Field testing of the test plants for six years in an HLB-endemic region of Florida helped identify resistant taxa in the subgroup (Ramadugu et al., 2016). The Australian limes, *Eremocitrus glauca*, and *Microcitrus* species (the Australian desert lime, finger lime, and related species are currently classified under the genus citrus) were identified as valuable in breeding resistance since they have high field tolerance/resistance to HLB and are sexually compatible with certain citrus types. Using multiple citrus cultivars and three types of Australian limes, we demonstrated that resistance to HLB is heritable and certain hybrids of *Microcitrus* generated in the breeding program had stable, broad-spectrum resistance to the HLB pathogen in the field conditions (Ramadugu et al., 2019). To introgress the resistance traits into citrus varieties, we generated advanced hybrids (second generation) with further crosses. Molecular studies including genome



sequencing of four Australian limes, identification of structural variants (copy number variations, presence or absence of genomic regions, single nucleotide polymorphisms, insertions, deletions, translocations) in resistant and susceptible parents and phenotyped, selected, representative hybrids are in progress (Singh et al., 2023). A project-specific pan-genome will facilitate the recognition of Microcitrus-derived genomic fragments correlated with HLB-resistance traits. Identification of quantitative trait loci associated with the HLB resistance trait will greatly facilitate the selection of promising hybrids in our ongoing breeding program aimed at developing disease resistance in citrus.

Citrus breeding is a time-consuming process; it is a tree crop with a generation time of 4-12 years. Biological complexities associated with citrus involve nucellar embryony, male and female sterility, sexual incompatibilities, juvenility associated with delayed flowering and fruiting, sexually imperfect cultivars propagated by grafting, and, most importantly, the delay in confirming HLB resistance/tolerance when exposed to different pathogen isolates in different climatic conditions. To prevent the spread of citrus diseases, it is essential to test bud wood from novel hybrids before the transport of germplasm across state borders in the USA. To facilitate this process, we have developed novel pathogen testing arrays and conducted greenhouse and field testing in Florida and Texas. The molecular data generated in the project facilitates the development of molecular markers essential for accelerating genome-assisted breeding.

Since HLB resistance traits are derived from citrus relatives that constitute crop relatives with fruit traits that may not be acceptable as substitute citrus varieties, it is essential to select advanced hybrids with organoleptic properties similar to commercial cultivars. We conduct taste panels to assess public acceptability and metabolomics analysis to identify compounds in the novel hybrid types that approximate sweet orange, mandarin, grapefruit, pummelo, and lemon. The availability of disease-resistant hybrids with citrus-like flavor profiles will be needed to replace current cultivars that maybe inappropriate for cultivation in an HLB atmosphere.

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# **Keynote Lecture**

Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## An overview of the Turkish citrus industry and existing challenges

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Türkiye ranks as a significant global citrus producer, renowned for its thriving citrus industry encompassing a wide array of citrus fruits, including oranges, mandarins, lemons, and grapefruits. While citrus cultivation is widespread across various regions within the country, the primary centers for citrus production are the Mediterranean and Aegean regions. In the 2021-2022 season, Türkiye achieved an impressive milestone, producing 1.75 million metric tons (MMT) of oranges, marking a substantial 35 percent increase compared to the preceding year (1.3 MMT in 2020-2021). Nonetheless, expectations for the forthcoming 2022-2023 season point to a potential decline in production, attributed to unanticipated cold weather conditions experienced in March 2022. Over the past three years, the industry has witnessed a diversification of orchard varieties, yet this positive trend has been counterbalanced by a reduction in the number of orchards. This decline can be attributed to the rising costs of agrochemicals, fuel, labor, and the volatile nature of global demand. In an effort to bolster the industry, the government provides partial financial support to farmers to alleviate the burden of production costs. Moreover, the government has implemented the use of pheromone traps as part of its strategy to combat the Mediterranean fruit fly, a significant factor contributing to losses in fruit quality and quantity. Additionally, the escalating prevalence of viral, fungal, and bacterial pathogens underscores the critical importance of implementing integrated pest management strategies. Growers have also expressed apprehensions regarding the sustainability of effective irrigation practices for their orchards, given the dwindling water levels in the region's reservoirs and the overall paucity of rainfall in the southern regions of the country. Furthermore, shifts in trade regulations, global demand, and various economic considerations may exert an influence on the future landscape of Türkiye's citrus industry. Therefore, to ensure the longevity of the industry and meet the evolving demands of consumers, it is paramount to adopt proactive measures and exhibit adaptability.

## 1. Introduction

Türkiye is positioned at the northernmost limit of the world's citrus production regions. The introduction of citrus fruits to Türkiye dates back to the 1930s, marking the inception of a strategically important sector that has long been a leading contributor to the country's exports of fresh fruits and vegetables. Due to favorable ecological conditions, citrus cultivation has experienced rapid growth, resulting in a notable increase in citrus tree numbers in recent years. While the production seasons for different citrus species and varieties in Türkiye vary, they typically commence in September and extend through May. The citrus industry has evolved into a sector that supports more than 5 million jobs within Türkiye, and it holds a significant role in the functionality of the Turkish economy. Predominantly concentrated in the Aegean and Mediterranean regions, key citrus cultivation centers are found in provinces such as Adana, Mersin, Antalya, Hatay, Muğla, and Osmaniye.



In 2021, according to citrus report, Türkiye's citrus fruit production reached around 5.4 million tons. The Mediterranean Region played a dominant role in this output, contributing 84% of oranges, 92% of tangerines, 93% of lemons, and 97% of grapefruits. Tangerines held the largest share in the citrus production at 34%, followed by oranges at 32%, lemons at 29%, and grapefruits at 5%. These figures translate to 1,742,000 tons of oranges, 1,819,000 tons of tangerines, 1,550,000 tons of lemons, 249,000 tons of grapefruits, and 2,615 tons of bitter oranges. Remarkably, there was a substantial 23% overall increase in citrus production compared to the previous year, with the most notable surges seen in orange production, up by 31%, and lemon production, up by 30%. When analyzing citrus production from 2017 to 2021, there was a noteworthy 12% rise in total citrus production compared to 2017. Among the individual citrus varieties, lemons exhibited the most significant growth at 54%, while the Washington cultivar of oranges experienced an 18% decrease (Eda AYGÖREN, 2021).

Türkiye's role in global citrus production is significant, attaining substantial levels of output. Data from the United States Department of Agriculture (USDA) for the 2021/22 season reveals that Türkiye ranks seventh worldwide in orange production, contributing 1.8 million tons (Sinem Duyum, 2022). In tangerine production, it holds the third position with a yield of 1.8 million tons. Moreover, Türkiye occupies the fourth spot in lemon production, generating 1.4 million tons, and is ranked fifth in grapefruit production, with an output of 250,000 tons. Analyzing global citrus exports, Türkiye stands as the fifth-largest exporter of oranges (6%), leading in tangerine exports (28%), taking second place in lemon exports (30%), and securing the third position in grapefruit exports (18%).

## 2. Production Trends

## 2.1 Leading Citrus Producer

In Türkiye, the area dedicated to citrus orchards expanded to encompass 1.7 million decares in 2021. When compared to the situation five years ago, there has been a 19% increase in the total acreage of these orchards. More specifically, lemon orchards experienced a significant surge of 61%, while tangerine orchards saw growth by 20%. Conversely, there were reductions in the sizes of orange orchards by 6% and grapefruit orchards by 7%. During the same year, tangerine orchards constituted 37% of the overall citrus orchard area, lemon orchards comprised 31%, orange orchards accounted for 29%, and grapefruit orchards represented 3% (TÜİK, 2023).

#### **2.2 Export-Oriented Production**

The export of citrus fruits plays a significant role in Türkiye's fresh produce exports. Based on data from the Mediterranean Exporter Associations (AKİB) as of March 2022, citrus exports constitute a substantial portion, accounting for 43% of Türkiye's total fresh fruit and vegetable exports. Among these exports, fresh vegetables make up 37%, while fresh fruits contribute 19% (AKIB, 2023)

In the year 2021, Türkiye exported a total of 1.9 million tons of citrus products. Within this export volume, tangerines comprised 45%, lemons 33%, oranges 14%, and grapefruits 8%. When examining data from the past five years, there is a noticeable 29% increase in total citrus exports. Tangerines showed remarkable growth, increasing by 84%, lemons by 31%, and grapefruits by 14%. Conversely, orange exports declined by 34%.

Regarding citrus imports in 2021, the total stood at 72,000 tons, with oranges dominating imports at 60%. Following oranges, tangerines accounted for 33%, lemons for 5%, and grapefruits for 2%. Analyzing data from the past five years, there has been an 18% increase in total citrus imports since 2017. Notably, grapefruit imports witnessed a substantial decrease of 59%, while tangerine imports experienced a remarkable increase, soaring by 322% (Eda AYGÖREN, 2022).



#### 2.3 Domestic Consumption

In the 2020/21 season, Türkiye's citrus consumption reached around 2 million tons. Oranges accounted for 41% of the total citrus consumption, tangerines made up 32%, lemons contributed 24%, and grapefruits represented 3%. While there hasn't been a significant shift in overall citrus consumption over the past five years, lemon consumption experienced the most substantial increase at 47%, while orange consumption showed the most significant decline at 40%.

During the same season, per capita consumption was as follows: the average person consumed 24.7 kg of citrus in total, with oranges making up 9.8 kg, tangerines at 7.7 kg, lemons at 5.8 kg, and grapefruits at 0.8 kg. Over the last five seasons, per capita citrus consumption decreased by 7% (TÜİK 2023).

#### 3. Factors affecting citrus Productin in Türkiye

#### **3.1 Weather and Climate Factors**

The climatic conditions in these regions are of paramount importance for citrus cultivation. Citrus trees exhibit a somewhat dichotomous preference when it comes to temperature: they rely on cool winters to induce a period of dormancy and ensure robust bud growth in the subsequent season, but they are also vulnerable to damage from severe frost. The Mediterranean and Aegean regions strike an optimal balance due to their mild winters. Additionally, the precipitation patterns in these areas further underscore their suitability for citrus farming(Khalid et al., 2022). Citrus plants typically require an annual rainfall of 1000-1200 mm, while the Mediterranean and Aegean regions receive average annual rainfall of approximately 600 mm and 1200 mm, respectively. To compensate for potential water deficits, particularly during the hotter months, irrigation systems are often utilized to maintain soil moisture at around 50-70% of its field capacity. Furthermore, the coastal location of these regions provides an added advantage

#### 3.2 Government Support

To support the citrus industry, the government offers partial financial assistance to farmers to help defray their production expenses. Additionally, the government deploys pheromone traps as a measure to combat the Mediterranean fruit fly, a significant factor contributing to both quality and quantity losses. Moreover, the increasing prevalence of viral, fungal, and bacterial pathogens underscores the critical need for integrated pest management strategies. Growers also express apprehensions regarding the future of effective irrigation for their orchards due to declining water levels in the region's reservoirs and the overall limited rainfall in the southern part of the country. Alterations in trade regulations, global demand, and other economic factors may also impact the future trajectory of Türkiye's citrus industry. Consequently, to ensure the industry's longevity and meet the changing needs of consumers, proactive measures and adaptability are of paramount importance.

#### 3.3 Diseases

As the citrus cultivation area has significantly expanded, there has been a notable rise in both native pest populations and the introduction of foreign pests. Multiple surveys conducted in Turkish citrus orchards have identified a total of 89 pests, 34 diseases, 16 nematodes, and 155 weed species that exert detrimental effects on citrus farming. Among these, 17 pests, 8 diseases, 1 nematode, and 10 weed species hold economic importance and must be regarded as primary pests. The remaining species are currently considered potential pests. Over the past two decades, unintentional introductions into Türkiye have included Polyphagotarsonemusl atus(Banks), Parabemisiamyricae (Kuwana), Aleurothrixus floccosus (Maskell), Paraleyrodesminei laccarina, and



Phyllocnistiscitrella Stainton. These introductions resulted in substantial damage during their initial establishment periods.(Uygun & Satar, 2008)

## 4. Conclusion

In conclusion, the Turkish citrus industry has exhibited remarkable growth and prominence on the global stage, cementing its position as a key player in citrus production and exports. The favorable ecological conditions, diverse citrus varieties, and strategic geographic location have all contributed to the industry's success. Türkiye's citrus production and export numbers underscore its significance, with impressive figures in oranges, tangerines, lemons, and grapefruits.

However, the Turkish citrus industry is not without its challenges. The sector faces an array of issues, including pest and disease pressures, climate change impacts, water scarcity concerns, and evolving market dynamics. The introduction of new pests and diseases poses a continuous threat, necessitating vigilant monitoring and innovative pest management strategies. Additionally, water resources must be managed efficiently to address the issue of declining water levels in dams and erratic rainfall patterns in the southern regions of the country.

Furthermore, global trade regulations and economic factors can impact the industry's future trajectory, necessitating adaptability and proactive measures by growers and stakeholders. Sustainability initiatives, research collaborations, and government support are pivotal in addressing these challenges.

In light of these challenges, the Turkish citrus industry's resilience and capacity for innovation should not be underestimated. Through ongoing research, sustainable practices, and collaborative efforts, the industry can navigate these hurdles and continue to thrive, delivering high-quality citrus products to both domestic and international markets. With its rich history and promising future, the Turkish citrus industry remains a vital contributor to the nation's agricultural and economic landscape.

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# **Keynote Lecture**

Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Battling against citrus canker and huanglongbing in the São Paulo citrus belt, Brazil

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The São Paulo state citrus belt

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Brazil leads the world in orange production and orange juice processing. The São Paulo citrus belt (SPCB) is the main region of citrus production in Brazil, covering 336 municipalities in the state of São Paulo and the West-Southwest area of Minas Gerais (Figure 1). This region accounts for ~70% of the planted area and ~84% of the orange production in the country. The 2023/2024 season is projected to produce 309.3 million 40.8-kg boxes (12.6 million tons) of oranges from 208.9 million trees grown in 399.4 thousand ha. The production is expected to remain steady, with a slight dip of 1.6% from the previous season and a marginal increase of 1.0% over the last decade. The sweet orange-growing area accounts for 86% of the SPCB. The rest of the belt is divided between acid limes (51.8 thousand ha) and tangerines (12.6 thousand ha), adding up to 461.9 thousand ha in total (Figure 1).

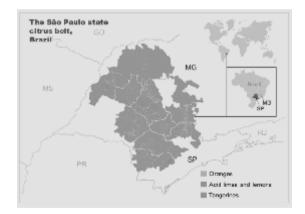


Figure 1. Distribution of citrus production in the São Paulo citrus belt in Brazil, composed of citrus-growing areas in São Paulo (SP) state and west-southwest Minas Gerais (MG) state. Neighboring states: GO, Goiás; MS, Mato Grosso do Sul; PR, Paraná; RJ, Rio de Janeiro. Source: Fundecitrus.

The SPCB is one of the citrus-growing areas in the world where the most significant number of economically important citrus diseases and pests occur. The favorable climate and the spatial, temporal, and genetic continuity of the citrus-growing areas make the citrus industry prone to a high risk of known and emerging phytosanitary threats like citrus canker (*Xanthomonas citri* subsp. citri) and huanglongbing – HLB (*Candidatus* Liberibacter asiaticus).

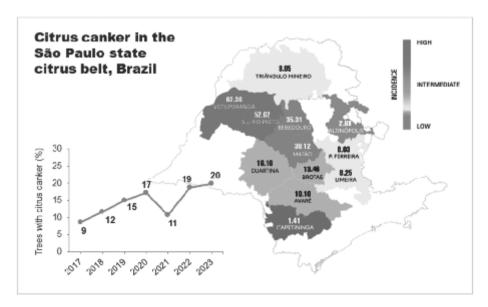


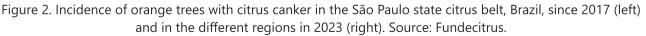
## Citrus canker

Citrus canker, also known as Asiatic citrus canker, was successfully contained for 60 years by a rigorous eradication program since it was first detected in the SPCBin 1957. However, in 2017 a new federal regulation came into effect allowing each state to define its status and approach for managing the disease: area with no occurrence/canker-free area, area under eradication, and area under risk mitigation. The SPCB has adopted the latter and the incidence of affected trees reached nearly 20% in 2023 with a heterogenous distribution throughout the belt (Figure 2).

Even though the disease has become more widespread, the premature fruit drop rate caused by citrus canker has remained stable, with minor fluctuations in recent years. In 2022/2023, the disease accounted for 0.17% (out of 21.3%) of fruit drop before harvest, a slight decrease from the previous season. This happened because the drop rate due to canker in a given year reflects the infections in the spring-summer of the previous season. The rain regime was close to normal in the last two seasons, making the incidence of affected trees grow again, but at a lower speed. However, in these years, the rain only intensified at the end of spring or the beginning of summer, when most fruits were no longer susceptible to infections. This scenario not only slowed down the epidemics but also kept the drop rate stable.

With approximately a fifth of the orchards affected in the citrus belt, citrus canker has grown below initial projections. The year 2017 marked not only the change in legislation that regulates the control of citrus canker in SPCB, suspending the eradication program and allowing the management of the disease, but also the beginning of a period with successive years of low rainfall. This coincidence prevented the disease from spreading as anticipated. This was due to the fact that the spread of citrus canker, within and between orchards, depends heavily on the occurrence of rain accompanied by winds. The water promotes the release of bacteria from older lesions and the wind carries rain splash to neighboring trees or orchards.







Advancing Citriculture for Agro-economic Prosperity

A more drastic increase of citrus canker is expected for the next season. The sustained onset of the rainy season in September-October until the end of summer in March sets up an ideal scenario for the disease. The forecast of an "El Niño" regime in the SPCB for the next season, with temperatures and rainfall above average until the first half of 2024, reinforces the possibility of continued growth of the incidence of affected trees and the need for more attention to the control measures.

Currently, citrus canker is being successfully managed with copper sprays, windbreaks, and control of the citrus leaf miner. Copper is the main measure of control and is applied in the rainy months, during spring and summer every 14 to 21 days, using 0.7 to 1.0 of metallic copper/ha/application, totaling 8 kg metallic copper/ha/season on average. The use of windbreaks is particularly important for the production of canker-free fresh fruit. Planting arboreal barriers in the perimeters of the orchards, especially in association with copper sprays, significantly reduces the incidence of harvested fruit with symptoms and increases pack out. Citrus leaf miner control is performed with insecticides during the spring and summer months to avoid the penetration of the canker bacterium through wounds.

#### Huanglongbing

The situation with HLB is different. All efforts to control HLB since its first report in Brazil in 2004 helped to slow down the disease progress. For 18 years, the incidence of affected trees never exceeded a quarter of the orange belt. However, the reluctance of growers to remove diseased trees and the recent detection of resistance of psyllids to neonicotinoids and pyrethroids have contributed to a dramatic increase in 2023, when the incidence of diseased trees reached 38%, a 56% increase in comparison to the previous year (Figure 3).In 2022, the population of psyllids monitored by the Phytosanitary Alert of Fundecitrus was twice the population verified in 2021, which, in turn, had already been the largest ever recorded since the beginning of the historical series, in 2009. This record increase in the psyllid population, associated with the maintenance of diseased trees in the orchards, culminated, in the alarming increase of the disease in 2023.

In some areas, the incidence of affected trees exceeds 70%. By contrast, in others, such as Votuporanga and Triângulo Mineiro, the incidence is lower than 2% (Figure 3). Historically, these regions have rainfall more concentrated in late spring and summer and high temperatures, conditions that generate fewer sprouts in the citrus trees. Because these sprouts mature more quickly due to the warmer temperatures, there is less time for the psyllids to reproduce and infect the trees. In this climate, the bacteria also have more difficulty moving in the phloem. Therefore, the combination of fewer infectious psyllids, a shorter window of vulnerability of trees, and as lower spread of the bacteria within the tree explain the lowest incidence of HLB in these areas, even if they are only a few hundred kilometers away from the most affected areas.

Fruit drop due to HLB in 2023 reached 5.48% (out of 21.3%). In some regions, where the incidence of the disease is higher, HLB-related crop loss exceeded 10%. The impact of HLBon the production of the SPCB is expected to continue to increase in the coming seasons following the rise in the number of affected trees.

Currently, the recommendation, particularly in areas with low incidence, is to continue planting healthy nursery trees, scouting and eliminating inoculum sources inside (required by federal law for citrus trees up to 8 years old in commercial orchards) and around the farm (required by federal law for any HLB symptomatic citrus trees in noncommercial, backyard orchards), and controlling the vector at a regional level to prevent its introduction and spread in the orchards. Additionally, the establishment of new orchards in regions with low or no incidence of HLB



is becoming a key strategy for the sustainability of the citrus industry. The selection of areas that are five to ten kilometers distant from other commercial citrus plantings and the removal of inoculum in backyards and pastures within the buffer zone around the farm are decisive factors for the success of the citrus industry against HLB.

Long-lasting and sustainable solutions against HLB are being researched by Fundecitrus in collaboration with national and international partner institutes. Fundecitrus plays a leadership role for the SPCB in carrying out research and providing technical assistance to citrus growers.



Figure 3. Incidence of orange trees with huanglongbing – HLB in the São Paulo state citrus belt, Brazil, since 2008 (left) and in the different regions in 2023 (right). Source: Fundecitrus.



# **Keynote Lecture**

Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus

# Advances in handling and marketing of fresh citrus fruit

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Asian countries contribute to around 40% (nearly 60 million tonnes) of the world citrus production (143.75 million tonnes). Importance of fresh citrus fruit consumption in human diet is more because of bio-availability of antioxidants, vitamins, fibre, pectin and flavonoids as compared to processed products. Most Asian countries (China, India, Japan, Pakistan) are the leading mandarin (Citrus reticulata Blanco) type citrus producers as compared to major sweet orange production in Europe and America. China is the largest citrus producer in Asia and also tops in the world with 37.73 million tonnes. In Chinese citrus production, mandarins constitute more than 20 million tonnes while in India mandarin production is 4.5 million tonnes. Indian citrus production touched a new high of 14 million tonnes of citrus from 1.08 million hectare area in 2020-21 thus India becoming the third largest citrus producer in the world. Iran, Pakistan and Japan are other major citrus producing countries in Asian continent. During last 3-4 decades, scientific developments, technological advancements, financial support from government institutions and private sector participation have transformed citrus fruit handling scenario in many countries. Across north-west India, now 80-85% Kinnow fruit is cleaned, wax coated and graded on packing-lines, packed in printed cartons and transported to distant corners of the country as against conventional manual handling and packing in jute gunny sacks. Considerable guantity of Nagpur mandarin is also now being handled on packing lines for sorting, wax coating, grading and packing in vented plastic crates(around 25%) as compared to manual handling and packing in wooden boxes. From central India, most of the wax coated fruit is exported to Bangladesh. India exported 91,700 tonnes of citrus fruit in 2019 valued at Indian Rs. 322 crore. The share of Asian countries in global export and import of fresh citrus is very less because of local domestic consumption of fruit in those countries.

Although, Imazalil and Thiabendazole are still being used as fungicides to control post-harvest rots, new molecules such as Pyrimethanil, Azoxystrobin and combinations like Azoxistrobin with Difenoconazole, Fludiaxonil with propiconazole and Imazalil with pyrimethanil are also available.

Growers have started using refracto-meters to measure fruit TSS in the field. Refrigerated vans and warehouses dedicated to citrus fruit are being used. Alternatives to fungicides such as antagonistic microbes, hot water, GRAS (Generally Recognised As Safe) chemicals and edible coatings are emerging as acceptable and effective treatments in post-harvest handling.

Physical methods of fruit disinfection to reduce decay during handling include treatment with Ultraviolet rays, Ozone and Corona discharge treatment, Non-thermal gas plasma or cold plasma, anolyte or electrolysed water, high temperature dry and moist air and hot water treatment. These emerging areas of research has potential to be utilized as commercial treatments on large scale in the future.



Keeping in view concerns of the consumers, the alternatives for the fungicides are being investigated worlwide. GRAS chemicals such as sodium carbonate and bicarbonate, borax, peroxyacetic acid, sorbic acid and chlorine are being used for dis-infection and reduce pathogen load. The commercial products are also available based on these GRAS chemicals for use in packing houses.

Microbial antagonists are now commercially available for control of post-harvest diseases. Products named as Biosave TM, Shemer TM, Pantovital and Yield Plusare used to control Botrytis, Rhizopus and Penicllium rots.

Plants are rich in wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids which have antimicrobial activity. Antifungal edible coatings are increasingly being evaluated commercially to replace traditional wax coatings. Edible bilayer coating such as Chitosan and polymer like carboxymethyl cellulose have been found to be effective in extending shelf life of citrus fruit.

Electronic vision-based graders using deep learning based AI models, near–Infra-red sensors, e-nose, biodegradable plastic films & smart packages, telematics and internet of things (IoT) for reefer containers, non-destructive fruit quality analysis in orchard for crop maturity mapping using GPS are some of the advancements involving precision that are defining citrus fruit handling presently.

Good Agricultural Practices (GAP) at pre-harvest level, organic post-harvest treatments for protecting environment and fruit quality and safety management are the key issues for success in future citrus handling. In organic management of post-harvest diseases, the approach could be integrated – including cultural practices to minimize infection and injuries in the field, followed by two or more eco-friendly, less polluting, safe and less risky practices.

In value chain and marketing, grower, pre-harvest contractor, commission agent, wholesaler and retailer are the important functionaries. The major share of price paid by the consumer is taken by retailer (50%) followed by grower (34%), wholesaler (10%) and pre-harvest contractor (6%). In the past, very few grower's groups / producer organizations existed that could assert their share in the prices. In India, many farmer producer organizations (FPOs) or farmer producer companies are formed during last 7-8 years and growers interests are being safeguarded in value-chain with their share in consumer price increasing up to 70%. Citrus fruit producers' cooperative societies are also engaged in marketing of produce and ensuring better returns to the growers. Block chain technology is emerging as a new tool for safety, traceability and reliability of the produce quality being marketed and for satisfaction and information to all the functionaries in the value chain. It increases efficiency of the value chain and tracking and monitoring of all steps of marketing is possible. Telematics has increased efficiency in logistics, marketing and export of citrus fruit.



Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Aspirations in new commercial citrus cultivars for Indian context: Opportunities

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Believed to be originated in North – East India, citrus has not only expanded world-wide but expressed in different cultivars. A very wide range cultivars havaing size, colour, shape, taste, medicinal and nutraceutical properties, harvesting season harnessed through germplasm material makes citrus unique for crop improvement scientists. The complex unresolved phylogeny attributed to perennial, highly heterozygous, nucellar polyembryonic, sometimes self – incompatible parameters badly restrict the available resources and efforts to get desired output. As a result citrus where is originated has still remained in nascent stage. In India citrus is cultivated in 10.86 lakh hectares with an annual production of 142.62 lakh tonnes and contribute about Rs. 26000 to 28000 crore in GDP every year from raw fruit and processed products. Indian citriculture commercially dependent on mandarin, sweet orange, acid lime, grapefruit and pummelo. Ironically the huge varietal base exists I global markets for all kinds of citrus cultivars is not available in India. Nonetheless India exports citrus fruits to many countries of the world. Although India tops in acid lime production, mandarins are the most widely cultivated cultivar in India and in 2021-22 mandarins worth 54.17 million USD was exported by India to 13 countries. Nagpur mandarins which are a specialty of the Vidharbha region of Maharashtra are one of the finest mandarins of the world and have a huge export potential. Nagpur and Amravati districts of Maharashtra together produce 7 lakh MT of Nagpur mandarins on 1.26 lakh hectares.

Mandarin (*Citrus reticulata* Blanco) with 42% and sweet orange (*Citrus sinensis* Osbeck) with 23% share are important table fruits followed by acid lime (*Citrus aurantifolia* Swingle) with 20% and others with 13% share. India is the largest producer of lime in the world. The average annual growth rate in production during 1961 to 2012 was 20.3% in terms of area and 20.9% in terms of production. Further, Maximum productivity of limes has been recorded in Karnataka, Madhya Pradesh and Andhra Pradesh. In case of easy peeler mandarin, maximum production has been recorded in Punjab and Rajasthan. Highest productivity in case of sweet oranges has been recorded in Karnataka followed by Andhra Pradesh and Punjab. In India, there are 26 states engaged in citrus production but 9 states cover more than 70% of area accounting for 89% of total production.

Although, India has experienced 10-fold increase in production during the last five decades, the productivity has not witnessed any phenomenal change. The seediness, undesirably high limonin content (14-16 ppm), which act as a precursor for bitterness, poor fruit quality, low acidity ( > 0.25%) and colourless juice without flavour in mandarin and sweet orange are hindering the processing and export of Indian citrus cultivars. Lack of suitable rootstock having adequate tolerance to biotic and abiotic stresses also augment the worries of Indian citriculture. There are several non-edible Citrus species growing in wild and semi-wild state in the North-Eastern region like *C*.



*indica, C. ichangensis, C. macroptera, C. latipes, C. assamensis,* sour pummelo (*C. megaloxycarpa*), which are yet to be exploited for commercial citrus improvement programmes. The pharmaceutical and nutraceutical potential of these wild species also left unravelled. Systematic research approach is necessary to evaluate these accessions for identifying important traits concerning biotic and abiotic tolerance / resistance. The different varietal improvement programmes in India are invariably dominated by clonal selection only. Number of improved varieties has also been released by SAUs. So far in India, promising/ released varieties of citrus during last 5-6 decades include 13 of acid lime, 1 interspecific hybrid of acid lime x lemon, 2 of sweet orange, 3 of mandarin, 1 rootstock and 4 of lemon. Several introduced varieties have also been released for cultivation in mandarin, sweet orange and grapefruit. ICAR – Central Citrus Research Institute has more than 600 accessions to its National Active Germplasm Site including exotic collections. However, conventional breeding programme along with mutation breeding and biotechnological interventions such as development of triploids, somaclonal variants, cybrids etc. through tissue culture in India still is at a nascent stage. The breeding programmes for scion and rootstock varieties at ICAR – CCRI is focused on:

#### **For scion**

- 1. Seedlessness in all commercial citrus groups
- 2. Better flesh colour, higher TSS, acidity and juice contents
- 3. Low limonin and other bitter phenolic contents
- 4. Early and late maturity to widen the window of harvest
- 5. Regular and cauliflorous bearing habit
- 6. Tolerance to biotic stresses such as greening, viruses, bacterial diseases etc.

#### For rootstocks

- 1. Adaptability to all commercial scions
- 2. Tolerance to biotic stresses especially Phytophthora, nemotodes, borers etc.
- 3. Tolerance to abiotic stresses such as salinity, drought, inundation etc.
- 4. High adaptability to wide agro-climatic zones
- 5. Higher efficiency for nutrient uptake and water use efficiency
- 6. Dwarfness

The heterozygosity and long gestation period of the crop improvement discourages the researchers. The research programmes, therefore, must address to these problems through various improvement methodologies. The different hindrances in citrus breeding are as follows.

• Citrus breeding is a very long duration affair due to prolonged seed- seed cycle i.e. juvenility in field (5-10 years). Therefore, it is cost and land intensive.



- Studies on inheritance of characters are very difficult due to highly heterozygous nature of citrus species. Also, show single gene inheritance of traits makes it difficult to study characters F₁ hybrids.
- Difficult to have intergeneric crosses with viable and ample seeds.
- Most of the times few hybrid progenies become available for selection due to abnormal recombinants and culminate into poor survival.
- Possibilities that the hybrids may not flower or may have very long juvenility or may be completely ovule or pollen sterile cannot be overruled.
- It is quite possible the undesirable traits of the relatives may be introgressed into the progeny by hybridization resulting in expression of inferior traits.
- Absence of phenotypic markers along with nucellar polyembryony makes selection of zygotic seedling difficult. Although, this situation is improving through the use of DNA based molecular markers.

In the present attempt the present status of Indian citriculture in pondered to find opportunities for developing new commercial cultivar for sustainable growth.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Doubling the Farmers Income through Indo- Israel Citrus Production Technology

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Nagpur Mandarin is an important and major fruit crop grown in Central India and area under this crop is 1.47 lakh hectares. The average productivity of the Citrus in India is around 11.08 t ha<sup>-1</sup>. The major component of limiting the productivity of citrus in India viz. quality planting materials, planting on raised bed, high density planting, use of pruning, use of drip irrigation and fertigation. For the standardization of high density planting four spacing viz, 6mx6m, 6mx4m, 6mx3m, and 6mx2 m spacing were tried and at the same time seven model demonstration farm were developed in high density planting system along with traditional planting system. Among the all spacing 6mx3mx spacing was found most suitable for Nagpur mandarin in Vidarbha region. Pruning of Nagpur mandarin crop is an essential for canopy management and for improving the guality production. Mechanical citrus pruning machine is used first time in Central India for pruning of Nagpur mandarin orchards. For standardization of pruning time and severity of pruning, tractor drawn pruning machine was used during December-January for Ambia bahar on five year old high density (6m X 3m spacing) Nagpur mandarin orchard. Ambia bahar fruits of Nagpur mandarin are harvested up to last week of November. December and January is a rest period and again ambia bahar flowering are emerge during February. For the standardization of time and severity of pruning, three pruning time (First week of December, third week of December and first week of January) and four pruning levels (8ft from ground level, 10 ft from ground level, 12 ft from ground level and no pruning)was tested during 2013-14. From the result it is revealed that flowering was observed in all treatments. Significantly higher fruit yield (28.91 t ha<sup>-1</sup>) was recorded in treatment pruning performed at 10 ft height from ground level. Time of pruning also had significant result in case on fruit yield and fruit size. Pruning performed during third week of December resulted significantly maximum yield per ha(25.33t ha<sup>-1</sup>). As regards the fruit size, significantly maximum fruit weight was recorded when pruning was performed during third week of December, However, significantly maximum fruit diameter was recorded in first week of December. Two to three fold increase in fruit yield and two to two and half fold increase in income to the citrus growers. In addition to the fruit yield and guality, number of branches and leaves were increased in pruned plants as compared to the un-pruned plants. Staking with bamboo is required in un-pruned plants whereas no staking is required in pruned plants. Fruit development was observed inside the canopy and hence it was uniform and bigger size and fruits are protected from sun burn in pruned plants as compared to the un-pruned plants.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Citrus clean plant programme: A way forward

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Citrus is considered to be one of the most remunerative fruit crops of India, having a lasting niche in the international trade and world finance. India, known for genetic diversity of citrus covering 27 native species, is third major producers of citrus in the world. Among various citrus fruits grown in India, the production of mandarins is 42%, followed by lemons and limes 26%, sweet orange 27% and other citrus fruits5%. In India diseases caused by virus and virus-like pathogens remain to be one of the most potential limitations, due to which, the average yield of orange in India is very low (11.6 Mt/ha) as compared to other countries like USA (32.6 Mt/ha), Brazil (24.7 Mt/ha) and China (13.7 Mt/ha).

Planting material plays an important role in the production of fruit crops. Inadequate availability of quality planting material is one of the important deterring factors in development of a sound fruit industry. At present 30-40% demand for planting material is being met by the existing infrastructure. Farmers do not have access to certified disease-free material as a result of which production; productivity and quality of the produces uffers. Much of the dependence is on the unregulated and unmonitored private sector in most of the states. The existing nurseries lack modern infrastructure such as greenhouses, mist chambers, efficient nursery tools and gadgets, implements and machinery and sell plant material of unknown pedigree. Of the many other constraints, inferior quality seed being procured, un-availability of standardized root stocks, unavailability of improved tools, non adoption of plant protection measures, non-existence of virus indexing, use of diseased scion woods, procurement through open quotations and non-maintenance of healthy stocks of elite varieties are worth mentioning.

The expansion of fruit production and markets into new geographic areas provides novel opportunities and challenges for the agricultural and marketing industries. In today's competitive global market environment, producers need access to the best plant material available in terms of genetics and health if they are to maintain a competitive advantage in the market. An ever-increasing amount of plant material in the form of produce, nursery plants, and breeding stock moves vast distances, and this has resulted in an increased risk of pest and disease introductions into new areas. One of the primary concerns of the global fruit industry is a group of systemic pathogens for which there are no effective remedies once plants are infected. Approximately, sixteen pathogens are known to infect citrus in India, among which, citrus tristeza virus (CTV), Indian citrus ring spot virus (ICRSV), citrus yellow mosaic virus (CiYMV), citrus yellow vein clearing virus (CYVCV), citrus variegation virus (CVV), citrus exocortis viroid and citrus greening bacterium are of serious concern. These pathogens and diseases require expensive management and control procedures at nurseries and by producers locally and nationally. Here, we





review (i) the characteristics of some of these pathogens, (ii) the history and economic consequences of some notable disease epidemics caused by these pathogens, (iii) the changes in agricultural trade that have exacerbated the risk of pathogen introduction, (iv) the path to production of healthy plants through the National Clean Plant Centers and state certification programs, and (vi) current efforts to develop and harmonize effective nursery certification programs within India as well as with global trading partners.

Viruses and virus-like agents are often difficult to detect in propagation material, such as rootstocks and bud wood, and as a consequence it is easy to inadvertently bring these damaging pathogens to a field where they weren't present before. Once these diseases are established, they are incurable and it is often very difficult to prevent them from spreading. The best and primary tool for avoiding diseases caused by virus and virus-like pathogens is prevention by using clean propagation materials.

To meet this, Certification programs are important for the long-term sustainability of citrus and other crops which are vegetatively propagated. Safeguarding and supporting the fruit production in India, the Atmanirbhar Clean Plant Program'(ACPP) was initiated by Ministry of Agriculture and Farmers welfare (MoAFW) funded by Asian Development Bank (ADB) in association with National Horticultural Board (NHB) and Indian Council of Agricultural Research (ICAR). The clean plant centres (CPC) will focus on diagnostic and pathogen elimination services, Production of clean propagative plant material and maintenance of foundation blocks of pathogen-tested plant materials. The Clean plant material will be available to states for state certification programs and to private nurseries and farmers. The components of a certification program include a quarantine program, a clean stock program, and a certification program to provide for distribution of the high quality, virus testes propagating material into the citrus industry for the benefit of the citrus industry as a whole. Sustainability of a certification program and other considerations are summarized.

The CPC quality initiative will also support centres in fulfilling their roles in diagnostics, therapy, maintenance, and distribution of plant material that has tested free of targeted pathogens. The CPC Quality Initiative was also to develop centres to meet comparable standards of quality for laboratory management, facilities, equipment, and trained personnel for both non-regulatory and regulatory objectives as outlined in their facility's quality Procedures. The quality initiative aims at engaging leaders of the respective industries by not only receiving pathogen-free propagative material, but also ensuring that the quality of distribution, propagative strength/ viability, selection, and availability of cultivars be guaranteed.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Characterization and detection of emerging viruses in citrus using modern tools

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Virus and virus like diseases are one of the major constraints to achieve the potential yield in citrus. As these diseases are graft transmissible, accurate detection is the major strategy to prevent their introduction and spread to new areas or new susceptible varieties. The identification and detection viral agents have always been a challenge in citrus crop particularly when the disease is caused by a new virus or its variant. Yellow ring spots and yellow vein clearing are two common symptoms often observed in different citrus cultivars in North West India and were shown to be caused by mandriviruses which have emerged during last two decades in several citrus cultivars.

In the initial studies Indian citrus ring spot virus (ICRSV) was identified to cause the ring spot disease but subsequently another mandarivirus citrus yellow vein clearing virus (CYVCV) was also found associated with disease either alone or in mixed infection with ICRSV. Mandariviruses are emerging as new viral pathogens for citrus growers not only in India but also in China, Turkey and Pakistan. The genome of first mandarivirus i.e. ICRSV was characterized by complex procedure of virus purification, RNA isolation from purified virus but the second mandarivirus i.e. CYVCV was characterized by use of high throughput sequencing (HTS). The complete genome sequence of four isolates of CYVCV associated with different citrus species was determined by sequencing of genome fragments using multiple overlapping primers and 5' & 3' ends by RACE profiling. Genome of all the four isolates of CYVCV were 7531 nucleotide long excluding the 3'poly (A) tail and comprises of six open reading frames namely RdRp (RNA dependent RNA polymerase), TGB (triple gene block proteins), CP (capsid protein) and NB (Nucleic acid binding protein). Based on the amplification and sequencing of coat protein gene, new isolates or species under the genus Mandarivirus has been observed. As, HTS and bioinformatics based post sequencing unassembled sequence reads (SRA) analysis has become a preferred approach for robust virome profiling in a targeted host for detection of targeted and non-targeted virus detection and characterization, it was employed for characterization of viruses associated with yellow clearing, yellow mottling and mosaic disease in kinnow mandarin. HTS identified another new species of mandarivirus i.e. citrus yellow mottle virus (CiYMV) in addition to CYVCV. The presence of CiYMV in kinnow mandarin is being reported for the first time from India. The presence of CiYMV as well as CYVC virus was also confirmed through RT-PCR amplification of coat protein gene followed by sanger sequencing. A RT-PCR assay was earlier developed for detection of two mandariviruses viz., ICRSV and using degenerate primer which also detected the presence of CiYMV. As in the present study CYVCV and CiYMV were commonly present, a duplex RT-PCR was developed for their specific detection in kinnow mandarin plants. The duplex RT-PCR study indicated that CYVCV was more widespread compared to CiYMV. This study will be very useful for production of clean planting material in citrus and bud wood certification programme.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Control and management of citrus graft-transmissible pathogens in Tunisia

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Citriculture represents a strategic sector in Tunisia that covers around 27,000 ha and housing 7 to 9 million trees. The annual yield is approximately 300,000 tons of fruits(DGPA 2022). Similar to other crops, citrus varieties suffer from numerous diseases particularly tograft-transmissiblediseases (such as psorosis, Tristeza, etc.) which consistently impact not only on production, but also the fruits quality and the trees longevity. Given the absence of curative measures to control suchplant health issues, the main way approach involves utilizing disease-free propagation materials. The Tunisian citrus certification program for sanitary improvement and production of healthy plants free from virus and virus like diseases started in 1994. It has following objectives: a) virus sanitation of the local varieties using shoot-tip grafting *in vitro*, thermotherapy and recently somatic embryogenesis (STG); b) the use of new rootstocks tolerant to Tristeza such as Citrus volkameriana, Citrumelo swingle and Citrange carrizo. The organization and the steps of this program were established according to the Tunisian law of citrus plant certification. This law has put the rules of sanitation controls that guarantee production of virus free certified plants mainly from Citrus tristeza virus, Citrus psorosis virus, virus like diseases (Impietratura, concave gum, blind pocket), Citrus stubborn disease caused by spiroplasma citri and viroids (mainly Citrus exocortis viroid and Cahexia citrus viroid). Healthy mother plants are conserved under screen-house in the Citrus Technical Center (CTA) and multiplied in order to obtain pre-basic and basic materiel that is delivered to nurseries to create their own healthy mother blocks. Since its establishment, this certification program allowed the sanitation of more than 18 local varieties and 15 imported varieties. Nurseries are annually assisted and supplied with about 3500 basic plants grafted on tristeza tolerant rootstocks for the production of certified materiel.



Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus

# Citrus fruits: A treasure trove of phytochemicals and antioxidants for nutritional security

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The genus Citrus belonging to the Rutaceae family is cultivated worldwide and widely acknowledged for nutritional content as well as therapeutic properties. Citrus is one among the topmost fruit well- known for its peculiar flavor and aroma; has an increasing demand among the consumers. In India, citrus fruit crop is grown in 1091 thousand hectares with production of 14150 thousand tonnes with an average productivity of 11.97 t/ha. The North-East hill region of India lies between 21.5° N to 29.5° N latitude and 85.5° N to 97.5°E longitude is rich in citrus biodiversity and home of many wild and underutilized citrus species having medicinal importance. These citrus species acts as a predominant source of ascorbic acid, pectin, folic acid, flavonoids, carotenoids, total phenols, limonoids, antioxidants exhibiting promising health benefits. The study was undertaken to screen citrus varieties like Kachai lemon, Soh-narengi, Hill mandarin, Citron and Jora Tenga of North East hill region of India for phytochemicals mainly limonin, ascorbic acid, flavonoids and total phenols. The antioxidant potential was adjudged with ABTS, DPPH and FRAP assays. The content of ascorbic acid ranged from 20.715 to 44.597 mg/100ml while limonin content varies from 9.258 to 20.509 ppm respectively. The hesperidin a flavonoid within the flavonone subclass was found highest in Hill mandarin (64.854 ppm), Soh-narengi (49.794 ppm) and Citron (42.069 ppm). Soh-narengi was found to contain highest total phenol content i.e. 18.370 mg GAE L<sup>-1</sup>. The antioxidant activity measured by the ABTS method ranged from 2.337 to 4.979 mM L<sup>-1</sup>Trolox. Concerning the DPPH assay, the values ranged from 5.886 to 9.637 mM L<sup>-1</sup> Trolox. The FRAP values varied from 0.688 mM L<sup>-1</sup> Trolox (Jora Tenga) to 2.113 mM L<sup>-1</sup> Trolox (Soh-narengi). The North-eastern citrus germplasm assessed were found to be potential source of phytochemicals and antioxidants and can offer an enormous scope of contribution to human health and nutrition



Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus

## Strategies to mitigate chilling injury in citrus fruits

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Low non-freezing temperature is an important critical parameter in maintaining postharvest quality of stored citrus produce. It can help to reduce disease incidence, seize insect pests, and modify ripening processes. However, it is important to be mindful of the potential for chilling injury (CI) that can occur when these commodities are exposed to excessively low temperatures. Different horticultural commodities have specific recommended safe storage temperatures to avoid CI. For instance, lemons, limes, and oranges have recommended minimum storage temperatures of 10°C, 9°C, and 2°C, respectively. These temperatures are determined based on the sensitivity of each commodity to chilling stress. When exposed to chilling stress, chilling sensitive species, undergo various physiological and biochemical alterations, as well as cellular dysfunctions. These changes can include alterations in cellular structure, increased metabolic alterations, and loss of membrane integrity. If fruits and vegetables are stored at temperatures below their recommended minimum storage temperatures below their recommended minimum storage temperatures below their recommended minimum storage temperatures below their recommended minimum storage temperatures in cellular structure, increased metabolic alterations, and loss of membrane integrity. If fruits and vegetables are stored at temperatures below their recommended minimum storage temperature, they may exhibit a range of CI symptoms. These symptoms can include brown pitting of the rind, watery breakdown of internal and external tissues, fermented odor, and red blotch in citrus fruits.

Exposing chilling-sensitive crops, to non-lethal high temperatures can indeed help lower the subsequent injury caused by holding them at cold temperatures. This is also applicable to many varieties of citrus fruits, with 'Fortune' mandarin being a particularly useful model system for studying the metabolic events underlying CI. Researchers have made significant efforts to understand the physiological and biochemical basis of chilling tolerance in citrus fruits at various maturity stages. This includes investigating the impact of plant hormones, changes in lipid and carbohydrate components, alterations in phenylpropanoid metabolism, and processes associated with oxidation. Despite these efforts, there is still a lack of comprehensive information on the mechanisms controlling CI in citrus fruits.

To mitigate CI in citrus fruits, various strategies are being employed. One such strategy involves temperature treatments, such as short-term high-temperature treatments. These treatments can take the form of hot water dips, hot water brushing, curing, or conditioning with hot humid air. Studies have shown that these treatments can effectively lower the incidence of CI in citrus fruits. These heat treatments can act as a kind of stress that safe guards the fruit from other stresses, particularly cold stress and CI. By subjecting the fruits to non-lethal temperature before chilling storage, they undergo physiological and biochemical changes that enhance their tolerance to cold temperatures and reduce their severity of CI.

Heat treatments have been found to induce protective mechanisms in citrus fruits, including the formation of heat shock proteins and the activation of additional defense mechanisms in the citrus albedo. The effectiveness of different curing and heat treatment conditions can vary depending on the type of fruit and the specific treatment



applied. One consistent finding is that conditioning citrus fruits with hot air at 37°C for 3 days has been effective in increasing their tolerance to chilling without causing heat damage. This has been studied in fruits harvested at different maturity stages and across different citrus seasons, despite variations in fruit susceptibility throughout the season.

Other heat treatments such as rinsing and brushing, hot water dips, and intermittent warming, at temperatures around 60°C for a few seconds, have also been found to be successful in mitigating CI in citrus fruits. For example, hot water dips at 53°C for 2-3 minutes have been shown to increase the levels of putrescine, a naturally occurring compound that can help mitigate CI in various citrus fruits including grapefruit, lemon, oroblanco, and kumquat. However, the specific conditions need to be optimized for different citrus varieties and fruit maturity stages to achieve the desired effect.

The application of novel generally recognized as safe (GRAS) chemicals has shown promise in mitigating CI in citrus fruits. These chemicals not only help in reducing CI but also aid in preserving the health-promoting compounds and aesthetic value of the fruits. Various chemicals and plant growth regulators, such as 0.1mM methyl jasmonate (MeJA), 0.5mM salicylic (SA), and jasmonic acid (JA) have been studied for their impact on CI in citrus fruits, especially 'Fino' lemon. SA, JA, and their metabolites also lower the incidence of CI in citrus, potentially viaincreased antioxidant activities, increased total phenolics, and suppression of membrane lipid peroxidation and reactive oxygen species (ROS)formation. Additionally, the application of spermidine and putrescine at concentrations of 1 and 1.5 mM, respectively, has been shown to reduce various CI-related symptoms in oranges. The application of 10 µM MeJA or 2mM SA has been found to alleviate CI in 'Eureka' lemons stored at -5°C for 42 days. Similarly, a dose of 0.5mM MeJA when applied to 'Kinnow' mandarin has been reported to result in zero CI after a storage period of 40 days. This treatment was found to delay the loss in fruit weight, maintain firmness, and preserve higher levels of total soluble solids, total phenol content, ascorbic acid, and antioxidant activity (AOX), while also reducing titratable acidity content.

Thiabendazole, a common postharvest fungicide used to control decay in citrus fruits, has been found to not only reduce postharvest decay but also decrease the incidence of CI. Additionally, when thiabendazole is applied as a warm dip at 40°C, it further decreases the expression of chilling symptoms. Waxes are commonly applied to citrus fruits as a postharvest treatment to replace the natural waxes or cuticles that may be removed during the brushing, handling, and packing process. These waxes decrease the water loss from the fruit by mitigating CI. However, the effectiveness of different waxes can vary depending on the type of wax and citrus fruit being treated.

Modified atmospheric packaging (MAP) is a technique that is not widely used commercially but has shown potential in reducing CI in citrus fruits. The high relative humidity maintained within the storage carton in MAP helps reduce water loss and decrease CI symptoms. The use of molecular technologies has provided a popular tool for studying the expression of genes in response to cold stress cues. Although the understanding of molecular events underlying chilling tolerance in crops is still in its early stages, studies in citrus fruits have revealed the complexity of molecular mechanisms involved. One study in cold-stored 'Fortune' fruit found that heat-conditioning for a longer duration for chilling tolerance is an active process that involves the stress-related proteins, expression of transcription factors, and activation of secondary metabolism. Additionally, the induction of heat shock proteins was observed in the early stage as an acclimatation of chilling stress.

While these findings provide important insights into the molecular mechanisms underlying chilling stress in citrus fruits, further research is needed to understand the precise functions of the identified chilling-associated responses. However, access to new molecular strategies has provided excellent tools for continuing the study of the mechanisms underlying cold stress. Continued research in this area will contribute to a better understanding of the molecular basis of chilling tolerance in citrus fruits. This knowledge can ultimately be used to develop strategies for enhancing chilling tolerance and improving postharvest storage and quality of these crops.



Latest Developments in Technology Outreach, Citriprineurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector

## Citriculture in Nepal: Status, prospects, constraints and strategies

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Mid hills of Nepal ranging from 800 to 1400 masl altitude all across the country are considered favorable for all types of citrus fruits cultivation. However pumelo, acid lime and lemon can be cultivated successfully in the upland condition of terai, inner-terai, foothills and river basin areas of Nepal. Citrus crops cover about 28.29% of the total area under fruit cultivation in Nepal. Citrus crops are potential exportable commodities, particularly to India, Bangladesh and China. However, the domestic production meets only fewer percentage of national demand during main season that fresh as well as processed citrus worth hundred million rupees is being imported every year. The value of imports of citrus fruit totalled 20 million USD in year 2021. India, Egypt, South Africa and Uganda are major exporting country to Nepal. Among them India accounts for 97% export, Egypt 2.33%, and rest from South Africa and Uganda in year 2021. At present, there are 46 citrus producing districts among 77 districts of Nepal having more than 500 ha area under cultivation. Recently, there is a dramatic increase in acid lime production in Terai area with the recommendation of two suitable offseason varieties namely; Sunkagati 1 and Sunkagati 2 from the National Citrus Research Program, Dhankuta. Further, there are three mandarin varieties (Khoku, Banskhark, Paripatle Agute Suntala-1) released for commercial cultivation in mid-hills of Nepal. Mandarin is top most cultivated citrus covering 54% area followed by lime (18%) and sweet orange (13%) and rest of the area is covered by other citrus species. The citrus industry is still at a traditional level that needs to be transformed into commercial production. Most farmers have no access to the certified planting materials free of diseases including *Phytophthora* root rot, citrus greening, canker and Tristeza virus. Chinese fruit fly, scale insects and fruit sucking moths are becoming more problematic insects in Nepali citrus orchards. Similarly, there is a lack of varietal diversity for extending the production season at farmer's field. Therefore, the production of existing varieties is limited to very short period during the normal season. Despite several issues of citrus cultivation the area is expanding quickly using poor quality seedlings/saplings. Hence the national productivity is not comparable with other countries. Citrus research is primarily focusing on varietal development, nursery management, disease management and post-harvest handling of fresh fruits. Small to medium scale storage structures are mostly storing mandarin and sweet oranges at 8-12°C with more than 85% RH and farmers are facing storage loss upto 30% due to blue and green mold disease. The citrus research and development should focus on the production and distribution of quality planting materials along with the adoption/dissemination of good agriculture practices. Therefore, future strategies should focus on addressing the problems of short production periods for existing varieties, low productivity and production, inferior fruit quality, and citrus decline due to disease and pests, including management factors. Similarly, problems in institutional mechanisms and coordination for orchard establishment, management, marketing and entrepreneurship for this crop should be adequately dealt with by research and development.



## Lead Lecture

Latest Developments in Technology Outreach, Citriprineurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector

## Reinforcing the citrus sector in North-eastern region of India – Prospects, challenges and strategies

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The Northeastern Himalayan region is considered the center of origin of several *Citrus* species, as evidenced by the natural undisturbed populations of the Citrus gene pool, which contains 23 species, 1 subspecies, 7 probable natural hybrids, and 68 varieties of citrus (Bhattacharya and Dutta, 1956; Sharma et al., 2004). However, only two types of citrus are commercially grown in the region: mandarin and lemon. Among the mandarin groups, Khasi mandarin covers the largest area in the region due to its premium quality, and Assam lemon is in the lemon group. In addition, sweet orange cv. Valencia, pomelo, lime, etc. are also grown in the region but restricted to home yards only. Today, there are mainly four ecotypes of mandarin observed in the region, viz., Khasi mandarin, Sikkim mandarin, or Darjeeling mandarin, Hill mandarin, and Kinnow mandarin. Among the various indigenous citrus species, Khasi mandarin, Tamenglong orange, Arunachal orange, Kachai lemon, Memong Narang and Kaji nemu have already been brought under geographical indication registry.

The major citrus growing belts of the region are Assam (Tinsukia, Dibrugarh), Arunachal Pradesh (Basar, Pasighat, Along), Meghalaya (Khasi hills, Jaintia hills, Ri-Bhoi, and Garo Hills), Manipur (Tamenglong, Ziribom), Mizoram (Kolasib, Tawitaw, Aizal, Thingdawal), Nagaland (Wokha, Mokokchung, Tuensang), Tripura (Jampui hills), and Sikkim (East Sikkim). Citrus occupies an area of about 136.82 thousand hectares with an annual production of 803.69 thousand tons and a productivity of 5.8 t/ha (NHB 2013). The productivity in the region is very low compared to the national average of 12.5 t/ha, indicating a good prospect for technological intervention to improve the productivity of citrus in the region.

#### Challenges

The citrus industry in the region is lagging as compared to other parts of the country, despite having a good prospect for cultivation. The existing growing orchards are not up to par due to poor management and communication bottlenecks, as well as a lack of market-oriented fruit processing industries. The main challenges of the citrus industry are the decline of citrus orchards, which has led to dissatisfaction among the citrus growers, and ignorance of the orchards, which has caused a setback in the industry. The citrus decline is complex and may be attributed to several factors. The challenges encountered by the citrus industry in the region include

1. Lack of disease-free quality planting material for establishing a viable and productive citrus industry. Majority of the citrus orchards in the region are of seedling origin due to limited use of vegetatively propagated plants on resistant rootstock.



- 2. Heavy and long-spell of rainfall causes heavy soil erosion and nutrient loss, and it also resulted the attack of many insect pests and diseases during the rainy season. On contrary, moisture stress during the long dry spell from December to March affects the performance of the orchards. Climatic uncertainties like a long spell of drought and strong hailstorms during flowering and fruiting cause blossom and fruit drops in citrus.
- 3. Most of the citrus plantations are on higher slopes, leading to heavy erosion of the fertile topsoil and a deficiency of nutrients in the soil, ultimately affecting the yield. The soil of almost 90% area in the region is acidic. However, there is limited use of soil amendments in the citrus orchards. There is also complex problems of soil nutrient availability, including micronutrients, due to the non-application of nutrients by farmers.
- 4. General neglect and non-adoption of scientific cultivation practices such as faulty planting systems, nonadoption of pruning, inadequate weeding, and excessive intercropping with nutrients and water exhaustive crops.
- 5. Non-adoption of soil and moisture conservation practices.
- 6. The prevalence of insects-pests, and diseases such as CTV, Huanglongbing, and trunk borer is aggravated by the non-adoption of plant protection measures. There is also heavy infestation of phanerogamic parasites, lichens, and moss.
- 7. Poor transport-storage-processing infrastructure resulting in high cost for bringing the harvest to the market from scattered production pockets and high post-harvest spoilage.

#### Strategies for boosting productivity

Considering the excellent climatic conditions, abundant rainfall, and fertile soil (high organic content) in the region, the productivity of citrus crops is quite low compared to national productivity, but citrus has a bright future in the region, and the productivity level in the region could be enhanced by adopting the appropriate technologies of citrus cultivation. There are two strategies for reinforcing citrus in the Northeastern region.

#### Strategy I: Short-term plan

The immediate strategy for reinforcing the citrus industry in the region is to rejuvenate the citrus orchards. In old and senile orchards, heading back, and framework of citrus is one of the steps to reinvigorate the productivity of the tree. However, in the unproductive orchards of the young population, removal of disease-infected parts is necessary to avoid the source of infection for other trees. In both cases, the adoption of the calendar of operations is primary in successful citrus cultivation.

### Strategy II: Long-term plan

Citrus is infected by numerous viruses; thus, the long-term strategy is very important for the future survival of the citrus industry. The following are the steps in adopting this strategy;

### I) Production of quality clonally planting materials (QCPM)

The first step in controlling viral diseases is the exclusion of the disease and the entry of the infected propagation



scion. The major transmission method among citrus viral diseases is grafting infected buds. Therefore, the production of quality clonally planting materials (QCPM) is the most important aspect of citrus production.

#### ii) Establishment of Virus indexing facilities

Indexing is an indispensable procedure to produce and diagnose disease-free plants. Different techniques or combinations of techniques have been applied in this regard, and the effectiveness of each depends upon the facilities available. Generally, indexing can be divided into two types.

**Field indexing;** also known as biological indexing includes the mechanical inoculation through direct contact or vegetative propagation and/or through insect transmission.

Laboratory indexing; also known as quick indexing includes serological, molecular, and chemical assays.

Commonly used indexing methods are tissue grafting, budding, and insect transmission for biological indexing and enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) for quick indexing strategies. Although all viruses and virus-like pathogens can be detected through PCR and its derivatives, polyacrylamide gel electrophoresis (PAGE) is commonly used for the detection of viroids.

### **Action Points**

The following general strategies may also be effective in reinforcing the citrus industry;

- 1. **Germplasm conservation:** A rapid loss of species in its natural habitat in the region has been reported. As per the IUCN norms, seven Indian Citrus species, viz., *C. indica* Tanaka, *C. assamensis, C. ichangensis* Swingle, *C. latipes* Tanaka, *C. macroptera* Mont, *C. rugulosa*, and *C. megaloxycarpa* are endangered as indicated by threat perception analysis (Singh and Singh, 2003).
- 2. **Production of quality planting material:** The mass production of disease-free quality planting materials through conventional budding, micro-budding and shoot tip grafting, etc. on rough lemon or Rangpur lime rootstock is the most important aspect.
- **3. Proper site selection for orchards:** Citrus cultivation can be done in plains as well as in hilly areas with good drainage and fertile soil. To get sufficient sunlight, hill planting should be done in a southward direction.
- **4. Planting system:** Adoption of proper planting system such as proper layout and system of planting, adoption of contour bunds, half-moon terraces, etc.
- **5. Cropping system:** Adoption of suitable intercropping such as French bean, rice bean, black gram, cowpea, and other vegetable crops in mandarin orchards during the pre-bearing stage (1-5 years). Cultivation of leguminous crops as intercrops in mandarin can also be taken in bearing orchards provided leaving the basin area free.
- 6. Orchard management: Application of proper nutrition depends on the age of the tree and the soil fertility status of the orchards. Proper training of trees for well-spaced branches and proper pruning for the removal of undesirable growth and dead twigs is important. Lifesaving irrigation at 15-20 day intervals during dry periods (December to March) is highly essential. The orchard should be kept free from weeds. Recommended measures should adopted for minimizing fruit drop.



- **7. Plant protection:** Integrated management of insect-pests and diseases of citrus is the most important aspect not only in Northeastern region, but also for entire world.
- 8. Natural resource management: Adoption of soil and moisture conservation techniques like making contour bunds, bench terraces, and half-moon terraces may minimize soil erosion during the rainy season. In addition, mulching, trenching, etc. reduced soil erosion and also helped in the conservation of moisture during the winter season.

Citrus cluster should be promoted through farmers producers companies. Mass dissemination of rejuvenation technologies in participatory mode is crucial for reviving the citrus sector. Organized and better market linkage for growers of remote locations will facilitate the availability of quality produce. Harnessing the power of GI tag can fetch premium prices for the growers. Establishment of cluster-based storage-processing-packaging unit will also minimize the post-harvest spoilage and provide a good price for the surplus produce during the glut season. In addition to commercial citrus fruits, the underutilized citrus species should be promoted through bioprospecting and value-addition.

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Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Diversity of citrus in north eastern region of India

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The North Eastern region of India has lot of climatic variations because of its unique position in the Indian subcontinent. It has been blessed by nature with one of the richest flora and fauna on the earth and regarded as one of the 'Biodiversity Hot Spot' areas in the world. Its unique phytogeographical positions, topography and high degree of precipitation are some of the important factors which are mainly responsible for its enormous biological diversity. In a naturally cross pollinated genus like the citrus, nature has eventually created different forms of citrus and the region has the conducive environment, suitable soil and topography for perpetuation of these various forms. It can be regarded as 'a live museum of Citrus'. The wide adaptability of citrus fruits of Arunachal Pradesh is reflected in its general distribution of topographical situations. Out of various species of citrus, Khasi Mandarin, Valencia and Assam lemon, Kachai lemon are some of the commercially traded variety of citrus in the region. The Khasi Mandarin of Arunachal Pradesh is unique in its quality. However the same fruits are under different names in different provinces. Even within the same district a variety may often be known by different names. Actually farmers' fields, forest of Arunachal Pradesh are the treasures of citrus species. Other than the commercial species, some of other species of citrus namely Rough lemon, Kamala Australia, Samphola, Citron, Singkin, various Limes and Lemons, Pummelos, Grapefruit etc. are available in various types either in homestead or in forests. In a recent study conducted, different types of citrus were found under different citrus species. Considering this diversity, there is an urgent need to conserve them either in situ or in collective forms. The present paper will discuss about the citrus biodiversity of the region

#### Introduction.

The unique phyto- geographical positions, topography and high degree of precipitation are some of the important factors which are mainly responsible for enormous biological diversity of citrus in north eastern region.. The region has been growing numerous forms of citrus from a hoary past and it also abounds in its wild forms. The wild adaptability of citrus is reflected in their general distribution. No other province in India probably has such a richer natural array of diverse forms of citrus and the state has the conducive environment, suitable soil and topography for perpetuation of these various forms. It can be regarded as 'a live museum of Citrus'. Where the diversity of forms is a great the room for confusion in regards to identity, no other important genus has probably been more liable to confusion than citrus.

#### **Materials and Method**

An extensive survey was carried out to explore the citrus resources of north eastern region during recent few years for documenting them which are available in the farmers field as well as in the forests in wild form. These



germplasms were collected, conserved in the college farm from time to time. Futher, the College of Horticulture and Forestry, Central Agricultural University, Pasighat also organizes Arunachal Citrus Show and competition among citrus growers so that large number of citrus germplasm are collected. The morphological characters and physico-chemical properties of the fruits viz., fruit weight, circumference of fruit, volume of fruit, rind weight, rind thickness, seed weight, seed number, seed length, seed breath , juice content, total soluble solids etc. of the collected fruits were analysed. The dates were collected from five random samples of each groups and presented.

### **Results and Discussion**

The results of the variability of citrus resources are presented as Table 1. A perusal of Table- 1 indicate that there is variations not only in various species but also within the species. The observations indicates that largest mean fruit weight up to 1706.6 kg was found in Pummelo while lowest fruit weight up to 41.9 gm was found in lime. The Mandarin which is called loose skinned orange have four distinct groups. Among these khasi mandarin is gaining commercial importance in Arunachal Pradesh. Samphola, a hardy plants with robust in nature bears a lot of large size fruit ranging from 620.6g to 883.9g which are having more juice content(207.0-320ml/fruit). Rough lemon fruits are having rough skin with 20-30 seeds per fruit. Rough lemon plants are highly adaptable to wide range of soil and mosly recommend as rootstock for mandarin. Among large sized citrus fruits, grapefruit borne in cluster and fruits are smooth skinned while pummeloes are borne singly. Juice content of grapefruit ranges from 160ml/fruit to 250ml/fruit while in case of pummels juice content varies from 120ml/fruit to 200ml/fruit. Rind of pummeloes are thicker than the grapefruit. Lime fruits are small fruit with fruit weight ranges from 38g to 50,6g per fruit with rind thickness of 1mm. Among sweet orange, Valencia and Mosambi are found common in Arunachal Pradesh as commercial fruit crop. Valencia and Mosambi are comparatively having more self life and seed content of these fruits are less (2-4 seeds/fruit). Kamla Australia is very hardy plant with large number of fruits in the

Type of fruit	Sample	No. Fruit weight (g)	Circum- ference (cm)	Volume of fruit (cc)	Fruit weight after 10 days of harvest (g)	Rind weight (g)	Rind thickness (mm)	Seed No.	Seed Length (cm)	Seed Breath (cm)	Juice content (ml)	TSS	Seed weight (g)
1) KM	1	174.6	25.0	190.	155.2	29.9	1.5	18	0.9	0.3	50.0	11.0	3.6
	2	158.5	24.0	192.	144.3	28.1	1.5	17	0.9	0.3	51.2	11.0	3.4
	3	190.0	25.0	185.	137.2	27.0	1.5	18	0.9	0.3	52.2	11.0	2.4
	4	231.3	26.0	178.	145.8	26.2	1.5	18	0.9	0.3	53.0	11.0	2.3
	5	159.6	24.0	140.	144.7	27.1	1.5	18	0.9	0.3	53.0	11.0	2.5
Mean		182.8	24.8	177.1	145.4	27.8	1.5	17.0	0.9	0.3	51.9	11.0	2.8
2) Samphola	1	863.7	39.0	920.0	809.4	269.8	8.0	42	1.4	0.8	320.0	9.8	11.3
	2	883.9	40.0	1020.0	834.6	313.4	10.0	30	1.0	0.7	310.0	10.0	7.9
	3	620.6	35.0	600.0	535.5	185.6	10.0	26	1.4	0.8	207.0	9.0	5.8
	4	698.4	35.0	740.0	653.6	214.3	12.0	36	1.5	0.9	295.0	8.7	9.8
	5	695.5	34.0	750.0	652.3	184.5	9.0	32	1.2	0.8	255.0	10.0	10.2
Mean		752.4	36.6	806.0	697.1	233.5	9.8	33.2	1.3	0.8	277.4	9.5	9.0

#### Table1: Variability of citrus fruits



Type of fruit	Sample	No. Fruit weight (g)	Circum- ference (cm)	Volume of fruit (cc)	Fruit weight after 10 days of harvest (g)	Rind weight (g)	Rind thickness (mm)	Seed No.	Seed Length (cm)	Seed Breath (cm)	Juice content (ml)	TSS	Seed weight (g)
3) Rough Lemon:	1	156.7	20.5	120.0	131.1	26.1	3.0	27	1.1	0.5	50.0	8.0	4.3
_	2	142.8	20.0	120.0	121.3	24.2	3.0	21	1.3	0.5	50.0	8.0	3.4
	3	158.2	21.5	140.0	137.9	35.7	5.0	20	1.0	0.5	60.0	8.8	3.4
	4	192.4	22.5	140.0	158.2	43.4	6.0	30	1.1	0.5	40.0	9.8	5.5
	5	130.0	16.9	104.0	109.7	25.9	3.4	19.6	0.9	0.4	40.0	6.9	3.3
Mean		156.0	20.3	124.0	131.6	31.1	4.1	23.5	1.1	0.5	48.0	8.3	4.0
4) Sinkin	1	340.2	27.5	340.0	301.0	156.9	10.0	14	1.0	0.6	40.0	6.0	1.6
	2	266.1	24.5	260.0	224.4	102.2	9.0	7	1.0	0.6	50.0	7.0	0.9
	3	305.1	26.5	300.0	270.5	105.8	9.0	6	1.0	0.6	50.0	7.0	1.2
	4	271.9	25.0	300.0	243.5	119.0	10.0	6	1.0	0.6	35.0	7.0	0.9
N 4	5	242.8	24.0	240.0	212.0	94.3	10.0	5	1.0	0.6	40.0	8.0	0.8
Mean		285.2	25.5	288.0	250.3	115.6	9.6	7.6	1.0	0.6	43.0	7.0	1.1
5) Grape Fruit	1	654.0	42.0	690.0	618.9	110.8	7.0	56	1.5	0.7	250.0	9.0	17.1
	2	473.0	31.0	450.0	444.9	85.1	6.0	44	1.6	0.8	250.0	9.0	15.8
	3	409.4	30.0	390.0	367.3	48.7	3.0	45	1.5	1.0	160.0	11.0	15.0
	4	478.8	32.0	400.0	429.5	47.6	3.0	37	1.5	1.0	210.0	10.0	20.1
	5	365.6	39.5	320.0	331.1	34.8	3.0	28	1.4	1.0	250.0	11.5	11.9
Mean		476.2	34.9	450.0	438.3	65.4	4.4	42.0	1.5	0.9	224.0	10.1	16.0
6) Citron	1	2030.4	36.5	1900.0	1766.9	1197.4	20.0	31	1.0	0.6	90.0	8.0	8.1
	2	1046.4	31.0	920.0	901.2	674.6	20.0	4	1.0	0.6	20.0	6.0	0.8
	3	948.8 1081.8	28.5 25.5	780.0	805.5 904.8	561.9 665.7	15.0 15.0	35 32	1.0 1.0	0.6 0.6	15.0 15.0	8.0 9.0	5.7 5.5
	5	1118.1	23.5	910.0	904.8	748.4	15.0	31	1.0	0.6	10.0	9.0 7.0	6.9
Mean	-	1245.1	29.2	1082.0	1056.0	769.6	17.0	26.6	1.0	0.6	30.0	7.6	5.4
7) Assam Lemon	1	232.0	19.0	240.0	205.4	69.8	3.0				40.0	8.0	
	2	242.3	20.0	260.0	213.9	79.7	3.0				35.0	8.0	
	3	283.3	23.0	300.0	253.1	96.7	4.0				30.0	7.0	
	4	246.0	21.0	230.0	218.9	64.0	4.0				50.0	7.2	
	5	257.3	22.0	260.0	225.4	70.0	4.0				55.0	7.1	
Mean		252.2	21.0	258.0	223.3	76.0	3.6	-	-	-	42.0	7.5	-
8) Pummelo	1	2125.3	55.0	3200.0	1991.0	481.0	10.0	1	2.0	1.0	200.0	9.0	0.4
	2	1866.9	52.0	2440.0	1743.3	454.5	10.0	15	1.7	1.2	120.0	9.0	5.0
	3	1767.3	51.0	2270.0	1183.5	354.8	10.0	1	1.7	1.3	150.0	10.0	0.6
	4	1330.2 1443.1	50.5 49.0	1630.0 1680.0	1641.2 1331.7	318.5 388.2	10.0 10.0	1	1.6 1.8	1.2 1.2	150.0 125.0	11.0 11.0	0.8 0.8
	5	1-4-1-3.1	I 40.0	1 1000.0	1.1.2.1.7	1 500.2	1 10.0	<u> </u>	1.0	1.4	123.0	11.0	L 0.0



Type of fruit	Sample	No. Fruit weight (g)	Circum- ference (cm)	Volume of fruit (cc)	Fruit weight after 10 days of harvest (g)	Rind weight (g)	Rind thickness (mm)	Seed No.	Seed Length (cm)	Seed Breath (cm)	Juice content (ml)	TSS	Seed weight (g)
9) Lime	1	39.2	13.0	40.0	35.1	13.7	1.0	5	0.8	0.4	25.0	10.0	0.2
	2	38.3	13.0	40.0	34.7	15.7	1.0	5	0.7	0.6	15.0	10.0	0.5
	3	50.6	14.0	50.0	44.4	17.5	1.0	9	0.8	0.5	20.0	10.0	1.0
	4	38.0	13.0	50.0	34.6	12.4	1.0	9	0.7	0.6	20.0	10.0	1.0
	5	43.3	13.5	40.0	34.6	11.4	1.0	2	0.7	0.6	20.0	10.0	0.1
Mean		41.9	13.3	44.0	36.7	14.1	1.0	6.0	0.7	0.5	20.0	10.0	0.6
10) Sinkin	1	70.0	16.0	60.0	66.9	15.7	1.0	19	1.0	0.6	45.0	9.0	2.0
	2	60.6	15.0	60.0	55.0	16.3	1.0	21	1.0	0.7	35.0	9.0	2.2
	3	59.7	15.0	50.0	53.1	15.1	1.0	13	0.8	0.6	25.0	9.0	1.1
	4	90.3	18.0	80.0	80.0	25.2	1.0	31	0.8	0.5	35.0	9.0	3.0
	5	73.5	17.0	70.0	67.1	25.0	1.0	11	0.7	0.5	30.0	9.0	2.1
Mean		70.8	16.2	64.0	64.4	19.5	1.0	19.0	0.9	0.6	34.0	9.0	2.1
11) Valencia	1	164.0	21.0	180.0	162.0	25.8	1.0	2	1.1	1.0	70.0	10.0	0.6
	2	147.5	22.0	200.0	183.4	31.1	3.0	4	1.1	1.0	80.0	10.0	1.0
	3	155.1	22.0	180.0	174.5	37.8	4.0	1	1.2	0.8	75.0	10.0	0.1
	4	143.5	20.0	160.0	136.4	37.1	4.0	4	1.2	1.0	72.0	10.0	0.2
	5	152.7	21.0	170.0	147.8	27.2	2.0	3	1.2	1.0	73.0	10.0	0.1
Mean		152.6	21.2	178.0	160.8	31.8	2.8	2.8	1.2	1.0	74.0	10.0	0.4
12) Mousambi	1	171.8	21.0	170.0	156.5	32.8	3.0	4	1.2	0.8	80.0	9.0	1.1
	2	195.5	20.0	160.0	140.3	23.5	2.0	4	1.2	0.8	70.0	9.0	0.7
	3	183.4	21.0	160.0	147.2	30.5	2.0	4	1.2	0.8	70.0	9.0	1.1
	4	143.1	21.0	150.0	136.0	25.3	2.0	4	1.2	0.8	70.0	9.0	1.1
	5	163.2	21.0	150.0	142.3	25.0	2.0	4	1.2	0.8	72.0	9.0	1.1
Mean		171.4	20.8	158.0	144.5	27.4	2.2	4.0	1.2	0.8	72.4	9.0	1.0
13) Kamala	1	438.4	30.	440.	404.9	189	10.	12	1.0	0.7	90.	7.5	2.7
	2	485.1	32.0	480.0	448.8	248.4	13.0	28	1.1	0.7	80.0	7.5	6.7
	3	637.0	36.0	850.0	578.7	191.0	12.0	27	1.1	0.7	90.0	7.0	4.2
	4	519.8	33.0	520.0	469.8	269.9	12.0	20	1.1	0.6	90.0	7.5	5.2
	5	423.1	32.0	488	421	182.2	12	23	1.1	0.6	90	7.5	3.8
Mean		500.7	26.2	555.6	464.6	216.2	11.8	22.0	1.1	0.7	88.0	7.4	4.5



plants. Fruits are having tapering end . Fruit weight ranging from 423.1g to 637g per fruit. Rind weight varies from 11.4 gm to 13882 gm in different citrus species while juice content of fruit also varies from 20 ml to 256 ml. Highest juice content was observed in Samphola. Fruits are seedless as in some types of Assam lemon and Cleopetra Mandarin while

grapefruit contain seeds up to 56.0 number per fruit. Taste ranges sweet to sour. Rind color varies from yellow, orange, green, skin smooth to rough.

Thus the wide adaptaptabity of citrus fruits of Arunachal Pradesh is reflected in its general distribution of topographical situations. Out of various species of citrus, Khasi Mandarin, Valencia and Assam lemon are some of the commercially traded variety of citrus in Arunachal Pradesh. The Khasi mandarin of Arunachal Pradesh is unique in its quality. However the same fruits are under different names in different provinces. Even within the same district a variety may often be known by different names. Other than the commercial species, some of other species of citrus namely Rough lemon, Kamala Australia, Samphola, Citron, Singkin, various Limes and Lemons, Pummelos, Grapefruit etc. are available in various types either in homestead and sometimes in forests in wild form

#### Conclusion

Considering this diversity, there is an urgent need to conserve them either in *situ* or in collective forms. Most of the farmers do not realize the importance of these citrus species as some of the species are not having market demand. Therefore, many citrus species are in the verge of extinction. To protect these valuable resources, proper attention is required. The traditional village councils of the region are efficient to manage the natural resources within their jurisdiction in sustainable manner. The traditional village councils are still revered by the villagers and can serve as important vehicles for conservation of these resources. Hence, they should be trained for this direction. Concerted efforts are now high time from all the concerned agencies to conserve these citrus resources for further exploitation

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Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Breeding of citrus for nutraceutical properties and environmental stress tolerance: Challenges and prospects

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Citrus genera are known for their richness in nutraceutical bioactive primary and secondary metabolites, including ascorbic acid (vitamin C), β-carotene (precursor of vitamin A), tocopherols (vitamin E), organic acids, amino acids, fatty acids, flavonoids, alkaloids, terpenoids, limonoids, anthocyanins and polyphenols. Different bioactive compounds have a well-established role in human health. For example, vitamin C acts as an overall health booster, especially for the immune system. Flavonoids like hesperidin and naringin function as antioxidants and have antiinflammatory properties. Polyphenolic compounds such as guercetin and catechins, also act as antioxidants and may have potential anti-cancer properties. Antioxidant compounds present in citrus fruits help in prevention or delay in oxidation process. Studies have shown that common traits like vitamin C, flavonoids, carotenoids, polyphenols, etc. are crucial for improved nutraceutical properties and play a vital role as antioxidants in plant defences against biotic and abiotic stresses. The prevalence of citrus rootstocks namely, rough lemon, Karna khatta, sour orange, Rangpur lime, Cleopatra mandarin etc. in India is presently threatened by the spread of phytophthora rot, Citrus Tristeza Virus (CTV) combined with abiotic constraints such as drought, salinity and alkalinity. Besides, huanglongbing disease is also a serious threat to commercially grown mandarin and sweet orange scion varieties. Hence, for sustainable growth of the citrus industry, attention is required to develop highly prized scion varieties with tolerance to huanglongbing and multi-stress tolerant rootstock hybrids. Breeding citrus varieties with improved nutraceutical and stress tolerance traits is essential to address changing human health issues and threats to the citrus industry due to climate change, given the economic significance of citrus crops. These traits could be combined in citrus scion breeding by using a range of citrus species, from primitive and wild to cultivated, through traditional cross-breeding techniques and modern genetic modification tools like gene editing technology. Several agricultural research institutions worldwide, including Indian council of Agricultural research (ICAR)-Indian Agricultural Research Institute (IARI), New Delhi, ICAR-Central Citrus Research Institute, Nagpur, Punjab Agriculture University, Ludhiana (Punjab) in India and universities of other countries like University of California, Riverside (USA), University of Florida (USA), United States Department of Agriculture (USDA), University of São Paulo (Brazil), International Center for Agricultural Research in the Dry Areas (ICARDA) etc. are involved in citrus breeding to develop new citrus varieties with desirable traits. The combining of traits for improved nutraceutical and stress resilience in citrus breeding is a complex challenge, as it requires maintaining



acceptable organoleptic traits, reducing bitterness in peel and pulp, and ensuring longer shelf life of fruit and juice. Matching progenitors can be found in citrus germplasm to combine the desired traits, particularly between Poncirus, Eremocitrus, Microcitrus and Citrus genera. However, varietal improvement through sexual hybridization faces some constraints and nucellar embryony has been a major obstacle in the systematic production of hybrids in citrus. Furthermore, the identification of sexual hybrid embryos usually requires additional analyses such as molecular analysis (Tusa et al., 2002). The situation could be even more complex in interploid crosses owing to post-zygotic incompatibilities that induce endosperm failure and subsequent embryo abortion. Within the perspective of high polyembryony, immature embryo culture (Hu and Ferrira, 1998) along with the application of molecular markers which can expedite the recovery and recognition of hybrid seedlings (Bastianel et al., 1998; Ruiz et al., 2000) are needed in citrus improvement. China, India, Nigeria, Brazil, Mexico, the USA, Spain, Egypt, Italy and Argentina are the world's top ten citrus-producing countries (Zhang et al., 2018). For sustainable growth of the citrus industry, attention is required to develop highly prized scion varieties as well as multi-stress tolerant rootstock hybrids. Various environmental stresses such as soil or water salinity, drought and major biotic constraints include diseases such as CVC, citrus canker, leprosis, tristeza, black spot, sudden death, and more recently, Huanglongbing, in addition to many pests that challenge the citriculture growth of the citrus industry in many commercially citrus growing areas. These environmental factors deserve attention, which are associated to the characteristics of individual citrus groups, expressing the necessity of better understanding to support future work on plant breeding, for instance, fruit quality (juice colour, seeedlessness, nutritional composition). In this crop, rootstocks play an outstanding role in improving yield, quality and protection against environmental stresses. Moreover, most of the environmental factors that limit the growth of citriculture may be combated through use of suitable resistant rootstocks. Citrus is one of the most significant and extensively grown fruit crops. It possesses several special reproductive characteristics, such as nucellar embryony and self-incompatibility. During the past decade, the emergence of novel technologies and the construction of multiple citrus reference genomes have facilitated rapid advances in our understanding of nucellar embryony. To accelerate the breeding efficiency for cultivating more citrus varieties with golden gualities and stable characters, it is essential to develop technologies for better exploitation of nucellar embryony and apomixes for the development of desired traitspecific scion and rootstock variety(ies). In addition, this could only be achieved by collaborative research that updates knowledge on gene function, identifies genes of interest and recognises undesired gene sequences associated with genes of interest across citrus species.



Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Trifoliate orange- A buffer gene source for citrus improvement

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Poncirus, also called trifoliate orange or hardy orange, was earlier thought to be monotypic, until Ding et al. (1984) discovered and described P. polyandra as a new species from Fuming County in Yunnan, China, which differs from P. trifoliata by its larger leaves, some floral differences, and most notably, being evergreen. The flowers of P. trifoliatahave the scent much lesser pronounced than that of other Citrusspecies. The fruits have a very bitter taste due to the presence of ponciridin (7-O-neohesperidoside of isosakuranetin), which is not palatable. Being polyembryonic, Poncirusis preferred as a male parent in citrus breeding to produce an excellent rootstock, besides being used as to produce an excellent protective hedge due to the extreme thorniness. Its hybrids, including citrange, citrumelo, and some other varieties, are also used as citrus rootstocks. This gives to trifoliate oranges the highest degree of cold hardiness among the true citrus fruit trees, surpassing that of kumquats. In addition to cold tolerance, Poncirus exhibits many other characteristics that have been and continues to be used in citrus rootstock breeding, notably stress tolerance (including nematode, some Phytophthora species, CTV, flooding, and cold, but sensitive to high pH, salinity, drought, and exocortis) and dwarfing (P. trifoliata cv. Flying Dragon). Many different selections of this species exist, and have been often categorized either as large-flowered types, which grow upright and develop fewer branches (e.g., Pomeroy), or small-flowered types which are less vigorous and with more branches (e.g., Rubidoux and Australian). Trifoliate orange rootstocks have strong ability to improve internal fruit quality (high Brix and acid contents and a smooth and thin rind) of the grafted scion. Trifoliate orange is currently heavily favored as a rootstock in Japan, China, Australia, New Zealand, and Argentina, and is significantly used in many other citrus-producing regions. Citrus production worldwide is threatened by a serious disease, Huanglongbing (HLB) or citrus greening disease, associated with the phloem-limited bacterium, Candidatus Liberibacter asiaticus (CLas), which is transmittedthrough the psyllid insect vector, Diaphorina citri. There is currently no cure for HLB, and it is generally accepted that the only way to keep Citrus as a viable crop, is by planting tolerant cultivars, obtained by either traditional breeding or by modern gene manipulation. Although some Citrus cultivars seem to be less prone than others to succumb to HLB, no true resistance to the pathogen is known in the genus. Recently, it was observed that some *P. trifoliata* hybrids were not showing typical symptoms of HLB despite being infected, thus creating renewed interest in further evaluating the traits of the many hybrids combining Citrus and P. trifoliata in citrus scion breeding programme. Initial hybrids of Citrus × P. trifoliata typically



produce fruits with unacceptable flavor, but more advanced hybrids (2<sup>nd</sup> to 3<sup>rd</sup> generation) with greater proportions of Citrus in their pedigrees have various degrees of off-flavor and taste. Four Citrus × P. trifoliata hybrids developed by the USDA Citrus Scion Breeding Programme namely1-76-100, 1-77-105, 5-18-24, and 5-18-31had acceptable eating guality and sweet and sour taste, with mandarin, orange, fruity-noncitrus, and floral flavours, however, US 119 and 6-23-20, produced a juice characterized by green, cooked, bitter, and Poncirus-like flavor and after taste. Non-volatile compounds in citrus, mainly sugars, acids and flavonoids and limonoids, contribute to perception of sweetness, sourness and bitterness, respectively. Recently, flavoromics approaches, in which instrumental analyses, sensory evaluation, and statistical modeling are combined, have uncovered key flavour compounds in citrus fruits, which has highlighted the contribution of monoterpene hydrocarbons with citrus/pine odor, sesquiterpene hydrocarbons (including valencene), and esters with floral and fruity odors, combined with the lack of linalool and citrus aldehydes, to the distinctive Poncirus-like flavor. Poncirus-like offflavor is likely due to a combination of higher than typical amounts of sesquiterpene hydrocarbons (woody/green odor), monoterpenes (citrus/pine), and terpene esters (floral), and a lack of aldehydes with typical citrus odor (octanal, nonanal, and decanal). It has been suggested that the bitterness and off-flavor of Poncirus hybrids can be related to high levels of naringinand poncirin as well as limonene in the juice and pulp. More recently, flavonoid analyses has confirmed the higher naringin levels in *P. trifoliata* than in *C. reticulata* (mandarin), along with linarin and rhoifolin. So there is immense possibility to generate advanced Citrus hybrids including P. trifoliata in their pedigrees that approach the guality of common *Citrus* types, having the resistance to HLB disease. The four hybrids of Citrus and P. trifoliata as mentioned abovehave a potential for being released as cultivars, having mandarin, orange, fruity-noncitrus, and floral flavors and sour and sweet tastes.



Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

## Management strategy for citrus genetic resources in India: Accomplishments and challenges

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Citrus in one of the ancient fruit crop of India which finds place in the Sanskrit devotional texts older than 800 BC. Citrus has also been mentioned by different names in the ancient Ayurvedic texts, namely, Dhanwantri (10<sup>th</sup> B.C.), Charak samhita (1<sup>st</sup> century B.C.) and Sushratasahmita (2<sup>nd</sup> century B.C.). Southeast Asia is the home of Citrus as most of the true and ancestral species like C. medica, C. reticulata, C. maxima and C. indica have been originated in this part of the World. The vast genetic diversity of citrus fruits like lime and lemons, oranges, mandarins, pummelos, grapefruit and lesser known types of Citron, Rough lemons, Karna khatta etc. is present in different parts of India. Rare and endangered wild Citrus species, namely, C. indica, C. macroptera, C. latipes, C. assamensis, C. ichangensis (C. cavalerie) and C. megaloxycarpa are reported from northeastern part of India. Among these C. indica (Citrus wild orange) the probable progenitor of present-day mandarin is endemic to this area. This enormous genetic diversity with several local cultivars and indigenous and exotic genotypes present in India and northeast India has been designated as one of the centres of origin and diversity of citrus. Surveys and explorations undertaken by several Indian, British and Japanese explorers during last century indicated the presence of 17 Citrus species, their 52 cultivars and 7 probable natural hybrids from northeastern region of India. Besides this several important species and cultivars are reported from western Himalayas, South of India and eastern India. These include Galgal, Chakotra and Citrus rugulosa (Attani, Chawanni) in the foothills of western Himalayas, Doon valley, Nainital, Chamoli and Garhwal region of Uttarakhand; Gajanima, Kichli and wild mandarin types like Kodaithuli, Nakoor lemon, Mole Puli, Billikichiliin from the Southern India and eastern Ghats. Overall, in India, a vast diversity of Citrus consisting of 24 species, 1 subspecies and 78 varieties of Citrus (including cultivated, wild, endangered and endemic species) has been reported. To safeguard these valuable and unique genetic resources of citrus, ICAR-NBPGR has taken initiative way back in 1981 by establishing the Citrus Gene Sanctuary in the Garo hills of Meghalaya which is the part of Nokrek Biosphere Reserve. In 2009, Nokrek Biosphere Reserve was recognized as a UNESCO World Heritage Site. The reserve has an exceptional variety of endemic Citrus species, especially Citrus indica (Indian wild orange) which has been conferred the Geographical Indications (GI) tag in 2015.

ICAR-National Bureau of Plant Genetic Resources (NBPGR) and ICAR-CCRI have conducted several surveys, explorations and collections in the different states of India during the last 2-3 decades. Collected germplasm has been characterized and successfully conserved using complimentary conservation strategy in the Field genebanks and cryogenebank. During these explorations, collection of wide range of *Citrus* species germplasm including important rootstocks species, cultivars of sweet oranges, mandarins, grapefruit, pummelo and acid lime along



with the indigenous wild and semi-wild species was undertaken. Notably, major focus of collection was wild, semiwild and endangered Citrus species such as C. assamensis, C. indica, C. ichangensis, C. macroptera, C. megaloxycarpa, C. latipes and other natural variants and farmers cultivars of cultivated species from northeastern region. All the collected germplasm is being assigned the national identity or IC numbers and passport information is being maintained in database. Presently, nearly 1670 accessions of diverse Citrus species have been collected from all over India and out of these, 910 accessions have been collected from north-eastern states. During these explorations loss of some species/cultivar from the earlier reported sites was reported and documented and at the same time, many new local types and new locations for already existing species/cultivars and unidentified Citrus types were reported from northeast India. Interestingly, this region has also been identified as one of the hotspots of biodiversity and is under the continuous threat of loss of the existing biodiversity. The surveys and explorations carried out in these areas have observed many Citrus types with uncertain taxonomic identity in northeast region, especially from Garo and Khasi hills of Meghalaya, Manipur and Assam. This is interesting to mention that many of these variants and probable natural hybrids lack taxonomic identity and need further taxonomic and phylogenetic studies. Some of these are being prominently identified by local names like Memangathur, Themachhi, Chimbal, Chamba, Seranga, Salonga, Atol, Bamsim, Kagzi-wak-ma, Soronga, Kampil, Kim, etc. and have significant socio-economic value in narrowed area. Therefore, it becomes a necessity to continuously revisit present status of Citrus genetic wealth of north-east India using modern tools of survey, collection, characterization, evaluation, conservation and utilization.

In situ on-farm conservation indigenous fruit species like Citrus is important and vital to achieve holistic conservation. Several indigenous Citrus cultivars are being maintained on farm in orchards, backyards, farmer's fields etc all over Southeast Asia. Several species of Northeastern India such as *C. reticulata* (Khasi mandarin and Darjeeling mandarin), *C. medica, C. jambhiri, C. grandis, C. limon, C. sinensis* (Tasi and Soh nairange), *C. latipes, C. macroptera* and *C. assamensis* are being conserved and maintained on-farm. In Uttar Pradesh, Madhya Pradesh, Punjab and foothills of Northwestern Himalayas, mainly in the parts of Uttarakhand and Himachal Pradesh, *C. jambhiri, C. grandis, C. rugulosa, C. karna* and a natural hybrid of *C. grandis* locally known as Kimb are being conserved. However, due to the less or no market demand of fruits of these cultivars and pressure of land plants of these traditional cultivars have became unwanted. The local government bodies can play a major role in this area. They can facilitate on farm conservation by introducing appropriate incentive schemes and by becoming a source of encouragement for farmers to maintain traditional Citrus species as well.

For ex situ conservation collection efforts were made to cryopreserve the germplasm. However, during the recent time increased human interventions, climate change, deforestation, shifting cultivation and preference of farmers for growing economically viable crops, this unique genetic diversity of citrus is facing serious threat. Recently, genetic resources of citrus are becoming more vulnerable due to new threats of abiotic and biotic factors in this region. Therefore, it was urgently required to revisit the Citrus genetic resources of Northeast India for their complimentary conservation and effective utilization. Recently, the Department of Biotechnology, Government of India initiated a mega project on citrus germplasm management by involving stakeholders from Indian Council of Agricultural Research and State and Central Agricultural Universities of all the Northeastern States to collect, characterize and conserve the germplasm in the field genebanks and also in the Cryogenebank at ICAR-NBPGR, New Delhi for long-term conservation. Timely implementation of this novel initiative would ensure the conservation and utilization of vast genetic diversity of citrus occurring in Northeast India.



Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

# Three decades of need based Citrus improvement works at Tissue Culture laboratory of CCRI (ICAR), Nagpur

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The Central Citrus Research Institute (CCRI) i.e erstwhile National Research Centre for Citrus (NRCC) was inaugurated at Nagpur in the year 1985 with a mandate to conduct basic, strategic and applied research on Citrus crops and to increase the production and productivity with the intervention of innovative and high-tech citriculture in the country. After the development of required research infrastructure, the attention was focused on providing technological solutions to the problems of Citrus industry of Central India. In order to improve the productivity and the production life of orchards, the Tissue Culture laboratory / wing of CCRI has provided reliable solutions by carrying out the research activities viz.,(i) Selection of horticulturally superior candidate mother plants(ii) Cleaning of elite scions through Shoot tip Grafting (STG) (iii) development of protocols for large scale production of quality planting stock (iv) enhancing the rate of multiplication through the development of Microbudding (v) retrofitting of nursery phase to economize the cost of production (vi) Development of seedless triploid scion cultivars of Nagpur mandarin and Sweet Orange (vii) Development of tetraploids in commercial citrus rootstocks (viii) Increasing the seed production of promising rootstock cultivar Alemow and (ix) marker based screening of root stocks etc. Further quality planting stock production technologies were transferred to eight nurserymen/entrepreneurs. These were discussed in this paper.



Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization in Citrus

# Breeding a brighter citrus future: Advances in citrus breeding for enhanced quality and pest and disease resistance

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Citrus is one of the most widely cultivated fruit crops in the world with high nutritional value, providing a rich source of vitamin C and other essential nutrients. Its economic significance is evident from its global production and consumption, contributing to the livelihoods of millions of people in the citrus industry. As the global citrus industry faces numerous challenges from pests, pathogens, and environmental conditions, researchers are engaging cutting-edge techniques to develop resilient and high-quality citrus cultivars. This lecture explores the pioneering inventions in citrus breeding, including genetic approaches, traditional breeding methods, and novel technologies that are transforming the field. By using these tools, researchers are able to enhance the overall quality of fruits, and fortifying the plants against biotic stresses to ensure a sustainable and prosperous citrus future.

Traditional breeding approaches such as cross-pollination, selection, and hybridization have been significant in citrus breeding. These techniques have enabled breeders to develop citrus cultivars with better-quality traits, including greater fruit quality, high yield, resistance to pests and diseases, and adaptability to diverse growing conditions serving as a foundation for citrus

breeding improvements. Genetic approaches play revolutionary role in the field of citrus breeding, being effective in combating major pests and diseases. Genetic markers, gene pyramiding, and introgression of resistance genes are commonly used genomic techniques in citrus research. Genetic markers are powerful tools used to identify specific genes associated with pest and disease resistance in citrus. Gene pyramiding involves the incorporation of multiple resistance genes into a single citrus cultivar. Introgression of resistance genes involves transferring genes from a wild or closely related citrus species that holds natural resistance to a particular pest or disease into cultivated citrus varieties. Continuous research and application of modern genetic approaches are vital for developing resistant or tolerant cultivars for pests and diseases and enhancing the nutritional quality of citrus fruits.

Further, biotechnology and genomic tools provide breeders with the means to address pest and disease challenges more efficiently. By using these techniques in an appropriate manner, citrus breeding programs are advanced to develop cultivars with higher biotic resistance, promoting sustainable citrus production in addition reducing the reliance on chemical pesticides. However, it is important to consider regulatory frameworks and permissible acceptance of using these biotechnological approaches. Tolerant varieties can withstand the



presence of the disease, resistant cultivars can prevent infection and minimize symptoms, and immune cultivars offer the highest level of protection. These advanced breeding approaches are useful in controlling diseases, minimizing crop losses, and ensuring the sustainability of the production. Further, biosecurity and quarantine practices are also important in breeding programs focused on preventing the occurrence and spread of pests and diseases. Researchers incorporate strategies to ensure that new varieties are tolerant to pathogens and pests before their official release. This includes rigorous testing, screening, and quarantine procedures to detect and eliminate any potential threats. By employing strict biosecurity procedures, they can safeguard the genetic integrity and vigor of citrus cultivars, avoiding the introduction and spreading of harmful pests and diseases. This practical approach is important for sustainability of citrus crops and safeguard the industry from devastating occurrences. Quality improvement in citrus breeding focuses on targeting traits related to taste, aroma, texture, appearance, and nutritional composition. It ensures that consumers obtain high-quality fruits that satisfy their sensory preferences while meeting demands in the market. Most importantly, collaborative efforts and global initiatives are significant in the development of high quality, pest and disease-resistant citrus cultivars. International corporations and projects with expertise, resources, and diverse germplasm from different countries/regions,

would help to tackle common challenges met by the citrus industry. These collaborative activities allow knowledge sharing, exchange of genetic material, and cooperative research to detect and introgression resistance traits from various citrus cultivars across the globe. International associations and organizations enable collaboration among researchers and growers, promoting the development and dissemination of improved cultivars.

These initiatives should focus on sharing information, conducting research trials, evaluating and identifying promising germplasm, and applying advanced breeding methods to develop cultivars with improved quality. With the use of collaborative and global initiatives, the citrus industry benefits from the collective knowledge, expertise, and resources of various stakeholders. These partnerships drive innovation, accelerate breeding progress, and contribute to the development of citrus cultivars with enhanced quality and pest and disease-resistance that can resist biotic and abiotic challenges, safeguarding a sustainable and resilient citrus industry worldwide.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

# Efficient use of potassium through balanced fertilization for sustaining productivity and quality of Nagpur Mandarin on swell-shrink soils of Central India

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The study was conducted on farmer's field to assess the effect of various levels of potassium on yield and quality of Nagpur mandarin on medium deep black soils in Nagpur district of Central India during 2020-21 to 2022-23. The experiment was laid out in RBD with ten treatments replicated thrice. The ten treatments consisted of control (RDNP), 400, 200+200, 600, 300+300, 800,400+400, 200+200+KNO<sub>3</sub>, 300+300+KNO<sub>3</sub>, 400+400+KNO<sub>3</sub>per plant. The RDF was common for all treatments (900:300 g N and P<sub>2</sub>O<sub>5</sub> per plant).

The pooled results revealed that the significantly higher fruit yield, fruit weight, juice content and lowest acidity were recorded with the application of RDF +400 g K at BT (Dec) +400 g K after 60 Days + KNO3 @ 1.5 % spray after 90 Days (T10) followed by treatment T9 ( RDF +300 g K at BT (Dec) +300 g K after 60 Days + KNO3 @ 1.5 % spray after 90 days) which was at par with each other. However, the significantly higher TSS content (%) GMR, NMR and B:C was obtained due to the application of RDF +300 g K at BT (Dec) +300 g K after 60 Days + KNO3 @ 1.5 % spray after 90 days which was at par with treatment receiving RDF +400 g K at BT (Dec) +400 g K after 60 Days + KNO3 @ 1.5 % spray after 90 days which was at par with treatment receiving RDF +400 g K at BT (Dec) +400 g K after 60 Days + KNO3 @ 1.5 % spray after 90 Days (T10).

Hence, it can be concluded that application of RDF +300 g K at BT (Dec) +300 g K after 60 Days + KNO3 @ 1.5 % spray after 90 days was found beneficial for enhancing fruit yield and fruit quality parameter (fruit weight, juice percent, TSS and acidity) along with higher monetary returns in ambia bahar of Nagpur mandarin.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Optimizing citrus farming: Tailored nutrient and water management strategies in India

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Citrus farming plays a pivotal role in India's agriculture sector, yet it grapples with formidable challenges such as soil degradation, erratic weather patterns, and mounting water scarcity. To combat these issues, a holistic multidisciplinary approach, encompassing the realms of soil science, agronomy, hydrology, and precision agriculture, emerges as a beacon of hope. Soil analysis stands as the foundational pillar, identifying nuanced nutrient variations and paving the way for tailor-made region-specific nutrient management plans. This not only curtails the over reliance on inorganic fertilizers but also champions the cause of organic alternatives. The inclusion of biofertilizers such as Vesicular Arbuscular Mycorrhiza, Phosphate solubilizing bacteria, *Azospirillum*, and *Trichoderma harzianum* proves to be a boon, increasing yields while simultaneously reducing the environmental footprint of conventional fertilizers.

Comparing traditional and drip irrigation for various crops highlights the significant advantages of drip systems in terms of water conservation and crop yield. In citrus cultivation traditional methods use 1660 mm of water to yield 100 tonnes per hectare, while drip irrigation only requires 640 mm for an impressive 150 tonnes per hectare. This represents a 61% reduction in water usage and a 50% increase in crop yield, showcasing the sustainability and efficiency benefits of drip irrigation, particularly in water-scarce regions.

The efficient management of water resources emerges as an imperative component in this endeavour. The result is a significant reduction in water wastage and a remarkable enhancement in water use efficiency. Specific recommendations targeting distinct citrus varieties in various regions further solidify this comprehensive strategy. From Sathgudi Sweet Orange in Andhra Pradesh to Kinnow Mandarin in Punjab, the emphasis on standardized stage-wise water requirements, drip irrigation, and smart irrigation strategies consistently improved fruit quality, yield, and water use efficiency while simultaneously preserving this precious resource. Additionally, the integration of nutrient management practices, including a judicious combination of recommended fertilizer doses with beneficial microorganisms, unlocks new avenues for sweet orange cultivation. This not only boosts yield but also extends the shelf life of the produce, ensuring economic viability.

In the pursuit of organic citrus production, the inclusion of vermicompost and beneficial microorganisms emerges as a game-changer, enhancing growth, yield, fruit quality, and pest and disease management. These meticulous irrigation and nutrient management strategies represent a beacon of hope for citrus growers in India. They not only promise increased yields and improved fruit quality but also herald a responsible approach to water conservation, ensuring the sustainability and profitability of citrus cultivation in the country. Furthermore, emphasis is placed on the utilization of advanced irrigation methods such as micro-irrigation, complemented by real-time weather information and remote sensing. This revolutionizes irrigation scheduling and nutrient management to enhance resource efficiency.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## An appraisal: Inter-cropping of vegetable in citrus orchards

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Citrus occupied a prominent place among the extensively grown subtropical fruits as it stands at third place among the major commercially cultivated fruit crop in India after mango and banana. Popularly grown citrus are lemon, lime, sweet orange, mandarin and kinnow; citrus shows a tendency of slow growth at initial stage and having crop geometry of 3 to 8 meter between rows, these condition make possible to grow shallow rooted and short duration crops in between vacant space to utilize the recourses and maximize return per unit area per unit time. Intercropping is one of the potential multiple cropping systems for best use of production factors and maximizing return without much additional inputs. Intercropping signifies growing of two or more crops simultaneously on the same piece of land along with the main crop. Integration of crops with varied growth habits, fruiting seasons and input requirements not only helps in ensuring efficient management of available resources but also offer round the year harvest of a wide range of produce. Integration of seasonal crops with citrus is a viable approach for enhancing system productivity. Among the vegetable crops, onion, okra, cluster bean, cow pea, tomato, brinjal, cole crops and cucurbits can be grown successfully at least during first few years of orchard establishment. There are several rewards in growing of intercrops in orchard as besides providing additional income to the growers, it suppresses weed population, check soil erosion, conserve soil moisture and enrich soil with organic matter. This practice is not always considered to be ideal in all the fruit crops in bearing stage, as there may be competition for moisture and nutrients with the main crop, although it has also been reported that quantity and quality of citrus was found better with intercropping of vegetables as compare to sole crop. Faulty selection of crop and mistimed sowing may impart negative effect on main crop, therefore, selection of suitable intercrops and growing of crops at suitable time is essential to get production from the main crop as well as from the intercrops.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Citrus based agroforestry offers ecosystem services including arsenicphytoremediation in contaminated rice field in Bengal basin

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The use of arsenic (As) contaminated groundwater for irrigation in the Bengal basin resulted in high deposition of As in the paddy soil and rice, threatening the health of billions worldwide. The introduction of agroforestryin contaminated rice fieldsmay help in phytoremediation of As and enhance ecosystem services. A field experiment was conducted from 2018-2022 on an As-contaminated clay loam to evaluate the tree species African mahogany (Khaya anthotheca), fruit tree sweet orange (Citrus sinensis) cv. Malta, and rice – rice cropping system, grown solely or in various combinations in West Bengal, India. In comparison to sole rice-rice cropping system, As load in rice grown in the alleys of red mahogany + sweat orange-based agroforestry system reduced to 226 from 798 µg kg<sup>-1</sup>. Total As removal was 2.6 times higher in this agroforestry system over sole rice-rice. Total As in the 0-20 cm soil layer was also 1.4 times lower under agroforestry system over sole rice-rice system after four years of study. Although sweat orange roots absorbed 28.5 mg kg<sup>-1</sup> As and its shootaccumulated 6.5 mg kg<sup>-1</sup> As, orange juice accumulated the lowest levels of As (11.5 ppb), satisfying the standard for human consumption. This agroforestry system has provided multiple ecosystem goods like edible seeds, fruits, fuelwood and timber sequentially, and was 2.5-3.0 times more profitable compared to the conventional rice-rice system. This system offered greater climate resiliency with 1-1.3 times higher annual biomass, thus offsetting greenhouse gases emission and improving soil nutrient status and biodiversity. Sweat orange + red mahogany-based agroforestry system provided 5.05, 3.20 and 1.99 times higher multifunctional ecosystem services over agriculture (rice-rice), horticulture (sweet orange) and forestry (African mahogany) as reflected by the Composite Evaluation Index and may be recommended in arsenic-contaminated rice field in the Bengal basin.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Deciphering the wild citrus seed microbiome of India

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Citrus crops are widely cultivated across the globe; owing to their significant culinary and nutritional attributes. The Northeastern region of India is widely recognized for citrus genetic resources. However, there is a growing concern regarding the erosion of citrus diversity, which has resulted in a notable decline in the seed microbial diversity. We made first attempt to study wild citrus seed microbiome by metagenomic approach. The findings of the study revealed that the microbiome of citrus is mostly influenced by the host species, and many indigenous varieties exhibit a distinct core microbiome. Wild citrus species have greater alpha diversity compared to the control, specifically Citrus paradisi, which has the highest alpha diversity (Chao1), followed by C. limetta. Taxa abundance indicated that Alphaproteobacteria exhibit a prominent presence with the microbiome of wild citrus seeds. Beta diversity analysis was also conducted, and the results were obtained by the PERMANOVA. These findings indicate that the diversity of the microbiome within a species is highly influenced by the host genotype. The core microbiome consists of *Glucanobacter* and *Bacilli*, which were also examined by culture-dependent approach. Further, we carried out isolation and characterization of culturable seed endophytes from eight citrus species collected from NE India. Bacterial isolates were screened for various plant growth-promoting (PGP) features. Based on 16S rRNA analysis, it was determined that the genus *Bacillus* exhibited the highest level of dominance among the isolates across various citrus species. The following isolatesCG2-1, CME6-1, CME6-4, CME6-5, CME6-9, CJ7-1, CMA10-1, CI11-3, and CI11-4 were identified as potential bioinoculants for the development of a microbial consortium with diverse PGP traits. These traits include the enhancement of nitrogen, phosphorus, and zinc nutrition, as well as the production of indole-3-acetic acid (IAA) hormones. The aim of this consortium is to improve the fitness of citrus crops in acidic soils, thereby providing nutritional benefits.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

# Deployment of internet of things for citrus plant grafting and real-time internal microclimate regulation of the storage house

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Hilly region of Nepal has a good geoclimatic condition for citrus fruits cultivation and production. Many smallholder farmers and commercial farmers are attracted towards citrus fruits production especially mandarin orange and lemon as they fetch good market opportunities as well as good return on investment. The domestic demand for the citrus plant as well as consumption of fruits are increasing every year. Recently there are initiatives to export to foreign countries as well. However, farmers are facing challenges in maintaining the healthy farm and storage of the fruits after harvesting. Some of the major challenges these days farmers are facing include climate change in the form of excess rainfall, drought, Pest and disease control, irrigation and fertilization, labor shortage etc. To overcome many of these challenges an Internet of Things (IoT) based platform has to be deployed. IoT provides real time data to the farmers anytime anywhere so that prompt decision making and action can be taken. This also facilitates analysis and provides a data driven decision making platform. This paper highlights the deployment of the IoT platform for regulating temperature, humidity, soil moisture and proper irrigation for citrus plants. This paper also extends the potential use of IoT for storage houses of citrus fruits maintaining its freshness and avoids the chance of being rotten.



Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies

## Status of citrus rootstocks in Asia

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Rootstocks influence the physiological and biochemical traits of scion cultivars, including plant vigour, guality, fruit production and tolerance against various environmental stresses. It is believed that scions and rootstock are vital components in the fruit production industry. Selecting a suitable rootstock is one of the major decisions in establishing an orchard and achieving maximum returns on a sustainable basis. Trials conducted in various parts of the Asian countries such as India, Bangladesh, China, Japan, Indonesia, Iran and other countries provided detailed information on usefulness of different rootstocks for citrus fruits which varies greatly with scion variety and agroclimatic conditions. Commercial citrus rootstock cultivars are highly polyembryonic but there can be 1-40 zygotic seedlings in the seed bed. Inspite of susceptibility to various phytophthora diseases and restricted longevity, rough lemon has been the most time tested and widely used rootstock in India. However, attempts to develop better substitute rootstock have shown Cleopatra mandarin (Citrus reshnl), Rangpur lime (Citrus limonia), Pectinifera (Citros pectinifera). Some of the citranges and Alemow (Citros macrophy Ua) are found to be promising rootstocks in certain parts of the country. Considering yield and various guality factors, 'Volkamer' lemon, rough lemon (C. limon Burm. f.), 'Palestine' sweet lime, 'Oklawaha' sour orange (C. aurantium L.) and, particularly, 'Carrizo' citrange, are suitable for 'Redblush' grapefruit in the arid regions of the south-western region. Trees on macrophylla (Alemow) (C. macrophylla) produced higher yield 5 years after planting and had significantly higher mean yield efficiency than trees on other rootstocks. In Bangladesh, sweet orange cv. BARI Malta-1 grafted on 7th months old rough lemon rootstock in cleft grafting method for achieving better planting materials. In china the dwarfing rootstocks 'Flying Dragon', 'FA 517', 'HTR-051', 'US-897', and 'Red tangerine' cultivated in various regions allow the design of dense orchards with dwarfing potential. 'Flying Dragon' rootstock with a strong dwarfing effect has been proposed to improve high-density cultivation methods with maximum fruit yield. Grafting citrus cultivars on Flying Dragon trifoliate orange rootstock, which is seldom used in the Mediterranean zone. In Iran the rootstock (Bakraei, Mexican lime, Volkamer lemon and Sour orange) and scion (IAC, Tahiti lime, Deperse lime and Persian lime) interaction regarding the total vegetative traits and mineral elements, Volkamer lemon rootstock is introduced as one of the best scion compounds with scions of IAC, Tahiti lime, Persian lime and Deperse lime, respectively. Different rootstocks are effective on fruit quality and quantity traits of scion variety in Iran. The highest yield efficiency obtained on Troyer citrange rootstock then on sour orange on Satsuma Mandarin (Citrus Unshiu). A significant effort at UF/CREC has been the production of allotetraploid rootstock selections by somatic hybridization in Egypt. In Indonesia, according to measurement of plant height, and diameter of rootstocks and scion stem, the plant growth of Volkamer lemon rootstock grafted with 'Siam Purworejo' scion or 'Keprok Tejakula' scion was better than grafted with Japansche citroen and 'Salam' rootstocks. Japansche citroen rootstock was



more susceptible to HLB than Volkamer lemon and 'Salam' rootstocks. Combination of rootstock/scion varieties affected plant tolerance against huanglongbing (HLB) disease. In Japan rootstocks for satsuma mandarin: Satsuma mandarin trees on trifoliate rootstocks grew rapidly and yielded comparatively large crops. In Pakistan, generally Rough lemon (citronella), Sweet orange rootstocks were used for high fruitful and productive with good fruit quality. Volakamariana was found to be promising rootstocks followed by Rough lemon and Mithi performed well in relation to nutritional status and yield of Kinnow mandarin. In Vietnam, regarding to fruit yield and quality, Duong mandarin trees grafted on Tau lemon rootstock produced highest fruit yield per tree per year in third year after planting. In Taiwan, 'Ponkan' and 'Tankan' make up 83 per cent of the commercial citrus production. *C. sunki*, is the principal rootstock for these varieties. Satsuma mandrills are grown, with trifoliate orange being the favored stock.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Lytic phage in preventing citrus canker on orange caused by *Xanthomonas campestris* pv. citri under green house conditions

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The aim of study to isolate and select bacteriophages capable of degrading X. campestris pv. citri causing citrus canker disease on citrus under laboratory conditions; ability to prevent citrus canker on orange under net house conditions. Firstly, a total of 9 strains (i.e. Xan1, Xan2, Xan3, Xan4, Xan5, Xan6, Xan7, Xan8, Xan9) of citrus canker caused by X. campestris pv. citri in five provinces of Mekong delta. In which, Xan 9 was the best virulent strain on citrus in greenhouse conditions. Secondly, there were twenty one bacteriophages isolated from five provinces of Mekong delta (fourteen bacteriophages were isolated from citrus canker leaves samples, seven bacteriophages were isolated from soil samples under diseased foliage). There were sixteen phages that have a wide parasitic spectrum on many strains of citrus canker bacteria (accounting for about 78% of the total number of citrus canker bacteria). Thirdly, evaluation of the ability to multiply the density of the sixteen phages on Xan9 identified four bacteriophages ( $\Phi$ 9,  $\Phi$ 13,  $\Phi$ 16,  $\Phi$ 20) with the highest multiplication capacity on Xan9. Comparison of degrading X. campestris pv.citri ability of four phages (Ф9, Ф13, Ф16, Ф20) identified three phages (Ф9, Ф13, Ф20) with higher plague diameter than the remaining phages. Lastly, evaluation of the ability to prevent and control citrus canker on oranges caused by X.campestris pv. citri Xan9 of three promising phages ( $\Phi$ 9,  $\Phi$ 13,  $\Phi$ 20) with the treatment of individual phage or a mixture of three phages at 10<sup>8</sup> pfu/ml under greenhouse conditions. The results of all three treatments of single phage and phage mixture treatments showed effectiveness in reducing citrus canker disease compared with the control treatment.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Multi-year comparison of new tools to support establishment of young groves

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Huanglongbing (HLB) disease is one of the most economically important diseases affecting citrus production in Florida. HLB management consists primarily of insecticidal applications to reduce the population of the psyllid vector, *Diaphorina citri*. However, there is still a need to incorporate new tools to existing *D. citri* pest management programs. Over a 3-year period, mesh exclusion covers over trees, reflective ground cover, and red kaolin clay particle film sprays have been evaluated as alternate tools. The efficacy of these treatments was tested on young citrus trees against a grower standard control (monthly insecticides) as well as their effects on other citrus pest incidence. Results show that the exclusion mesh was the most effective at reducing the population of psyllids. Additionally, the incidence of citrus leaf miner (CLM) was also reduced by this treatment. However, spider mites, mealybugs, and scales can also become problematic on trees under the exclusion mesh. While these covers appear to be a promising tool for protecting young citrus trees from the vector of HLB, citrus growers need to monitor for other pests and pathogens that can thrive within the closed mesh environment created by the covers.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Investigation of the presence of citrus tristeza virus and *in-vitro* sanitation trials of local citrus cultivars in chlef valley (Algeria)

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An investigation of *citrus tristeza virus* (CTV), the most destructive disease affecting citrus fruit was carried out in citrus orchards in Chlef Governorate (Northern West of Algeria). Biological indexing and serological analysis (DAS-ELISA and DTBIA) revealed many positive cases of Tristeza. The most affected varieties by CTV in Chlef included sweet oranges and tangerine, the distribution map of the virus was established, and molecular characterization has revealed the presence of exotic strains belonged to different virulent genotypes. Infected trees of different ages and origins were chosen in order to be sanitized and regenerated by a new *in vitro* technique such as somatic embryogenesis. The obtained plants were tested for assessing the elimination of the virus. In order to evaluate genetic stability of regenerants, DNA analyses were performed.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## The citrus-phytoplasma pathosystem: The state-of-the-art

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Citrus, one of the most economically important horticultural cropsgrown in over 150 countries with an annual production of more than 130 million tons, is susceptible to a wide range of pathogens, including fungi, nematodes, viruses, viroids, bacteria, and phytoplasmas. Among these, Phytoplasmas (previously known as Mycoplasma-like organisms) pose a significant threat to the citrus industry worldwide. The never-ending story of citrusphytoplasma association, simply but very destructively, started in the 1970s by observing the witches'- broom disease of lime in Oman, and the following years, became more complex and mysterious through the detection of diverse phytoplasma ribosomal groups with citrus trees showing different symptoms such as leaf interval, vein chlorosis, mosaic or mottling, a reduction in size and curling of leaves, huanglongbing (hlb)-like symptoms, premature fruit fall, twig drying, die-back, witches'-broom and decline, on many citrus varieties and species in a large number of citrus-growing countries in the world. Recent findings on mixed infections of phytoplasma and Liberibacter in citrus trees showing hlb-like symptoms added to the complexity of the story. The interaction between citrus and phytoplasma is a complex phenomenon affected by citrus variety, phytoplasma ribosomal group or subgroup, insect vector, climate conditions, geographical region, and other factors. This review explores the historical background of citrus phytoplasma diseases, their geographic distribution, symptomology, etiology, epidemiology, transmission, natural host range, the reaction of different citrus species and varieties to artificial inoculation, and the pathogenicity mechanisms of phytoplasmas against citrus plants.

#### Introduction

Citrus is one of the most economically important horticultural crops grown in over 150 countries in the tropical and subtropical regions of the world. Citrus fruits are the second most produced fruit, with more than ten million hectares and over 160 million tons worldwide (FAOSTAT, 2022).Citrus is susceptible to a wide range of pathogens, including fungi, nematodes, viruses, viroids, and bacteria. Phytoplasmas (previously known as Mycoplasma-like organisms, MLOs) are wall less phloem limited,single-celled pleomorphic bacteria, poorly culturable on cell-free artificial media (Contaldo et al., 2016), transmitted by insect vectors from the Cicadelloidea (leafhoppers), Fulgoroidea (planthoppers), and Psylloidea (psyllids) families in nature or viathe vegetative plant materials, during agricultural operations such as propagation, grafting, and cuttings.Phytoplasmas are classified in the genus '*Candidatus* Phytoplasma' and the class Mollicutes. More than 150 ribosomal groups and subgroups and 49 '*Ca*. Phytoplasma' species have been identified based on restriction fragment length polymorphism (RFLP) and nucleotide sequencing of the 16Sr gene (Marcone et al., 2002; Bertaccini et al., 2022). Phytoplasmas are associated with more than a thousand diseases in economically important crops, including citrus.



### **Historical background**

The history of phytoplasma-citrus association can be traced back to half a century ago, when witches'-broom, and blastomania and rubbery wood symptoms were observed as possible phytoplasma diseases in Oman (Bove, 1986) and India (Mali *et al.*, 1975;Ahlawat, 1987;Sharma and Singh, 1988) respectively. Although the etiology ofrubbery wood and blastomania is still not molecularly confirmed, witches'-broom disease of lime (wbdl) was further studied, and 16SrII-B phytoplasma '*Ca*. Phytoplasma aurantifolia' was introduced as its causal agent (Zreik et al., 1995). Within two decades, wbdl moved into the neighboring countries, including the United Arab Emirates (Garnier et al., 1991) and Iran (Bové et al., 2000).Wbdl is the most ancient and still, the most destructive phytoplasma disease of citrus in the world causingsignificant economic losses in infected countries.For years, '*Ca*. phytoplasma aurantifolia' was detected in citrus plants in the world. However, in the beginning yearsof the third millennium, a phytoplasma belonging to 16SrIX was detected in citrus trees, showing hlb-like symptoms in Brazil (Teixeira et al., 2008). Afterwards, in a short period of time,more phytoplasmas were reported in association with citrus plants showing different symptomsin almost all of the citrus-producing countries (table 1).It shows that the number of phytoplasmas infecting citrus trees has increased more than five timesduring the last 15 years (Azadvar et al., 2016; Noorizadeh et al., 2022).

#### Symptoms and hosts

Phytoplasma induces the appearance of diverse symptoms on citrus plants such aswitches'-broom, leaf mottling, small chlorotic leaves, multiple sprouting, and shortened internode, prematurely leaf dropping, stunting, abnormal fruits, die-back, decline, and yield reduction (Azadvar et al., 2023). Although the mechanism of symptom development remains poorly understood, phytoplasma strain, citrus species, and geographic region are believed to be the most influential factors in symptom expression. Evidence shows that most, if not all, of the citrus species and varieties are infected by at least one phytoplasma strain in the world. Small-fruited acid lime (Citrus aurantifolia) is the main host for wbdl-phytoplasma in the infected area. Subsequently, this phytoplasma was detected in bakraee, a local citrus variety in southern Iran (Golein et al., 2012), grapefruit, citron, limeguat, sweet orange, and Orlando tangelo (Table 1). However, some citrus species reportedly tolerate to wbdl-phytoplasma (Hassanzadeh Khankehdani et al., 2019). Interestingly, later on, 16SrXIV-A and 16SrI phytoplasmas were detected in acid lime trees showing witches'-broom and proliferation symptoms in India and Pakistan, respectively (Ghosh et al., 2017; Fahmeed et al., 2009). Some cultivated crops, and weedshave been reported as host and reservoir crops for wbdl-phytoplasma (Azadvar et al., 2023). During the last 15 years, diverse phytoplasma groups have been detected in different citrus species showing hlb-like symptoms (Table 1), and this will be a new and very important topic in the symptomology of citrus-associated phytoplasmas. In addition, several reports have demonstrated the presence of phytoplasmastrains in non-symptomatic citrus trees (Table 1). It is worthy to mention that Mexican lime trees infected with 'Ca. P. aurantifolia' (16SrII-B), in the desert area of Oman, show only a growth reduction and dieback symptoms but no typical symptoms of witches'-broom (Al-Ghaithi et al., 2017).

#### **Mixed infections**

In recent years, several reports have been published on natural mixed infection of wbdl phytoplasma or other citrus-associated phytoplasmas with the causal agent of hlb disease (*Ca.* Liberibacter spp.) of citrus trees of different species (Table 1). This has added complexity of the citrus-phytoplasma pathosystem. Although the mixed infection of citrus trees by wbdl-phytoplasma and liberibacter has probably led to a decline and more damage (Alizedeh et al., 2017; Passera et al., 2018), in the mixed infection of other phytoplasmas with hlb agent, the symptoms were identical with wbdl or hlb single infection (Table 1). Critical factors that determine the outcome of mixed infections remain unknown.



Та	ble 1. Phyol	Table 1. Phyoplasma diseases of citrus treesin the world.	of citrus treesin	the world.			
	Disease name	Host plant	Symptoms	Associated phytoplasma	Insect vector	Distribution	Reference
	Witches'- broom	acid lime, bakraee, lemon, limequat, citron, grapefruit, Orlando tangelo, sweet orange, macrophylla, citrange	small chlorotic leaves, witches'- broom, die back	16Srll, 16Srll-B, 16Srll-C, 16SrXIV, Ca.P. aurantifolia	Hishimonus phycitis (for 16Srll only)	UAE, Oman, Iran, India	Bove, 1986; Garnier et al. 1991; Zreick et al. 1995; Ghosh et al. 1999, 2013, 2017; Bové et al. 2000; Djavaheri and Rahimian, 2004; Bagheri et al. 2009;Al-Yahyai et al. 2010; Azadvar et al. 2015, 2020; Faghihi et al. 2017; Al-Subhi et al. 2019
<u>ــــــــــــــــــــــــــــــــــــ</u>	HLB like	mandarin,sweet orange, lemon, pomelo, Nagpur mandarin, citron, grapefruit, tangelo	blotchy mottle, yellowing, vein clearing, fruit malformation	16Srl,16Srl-A, 16Srl-B, 16Srl-S, 16Srll, 16Srlll, 16SrVl,16SrlX, 16SrXlV, Ca. P. palmae	Q	China, India, Brazil, Puerto Rico, Mexico, Ethiopia, Cuba	Arocha et al. 2007; Chen et al. 2009; Arratia-Castroet al. 2014; Lou et al. 2014; Caicedo et al. 2015;Poghosyan et al. 2015; Wulff et al. 2015;Das et al. 2016; Ghosh et al. 2019; Luis-Pantoja et al. 2021; Paredes- Tomas et al. 2023
	Decline or Sudden decline	acid lime, sweet orange	growth reduction, leaf yellowing and necrosis, rapid or slow decline	16Srll-B, 16Srll-C, 16SrlX, Ca. P. aurantifolia	QN	Oman, Saudi Arabia, Iran	Alhudaib et al. 2009; Nascimento Da Silva et et al. 2015; Aliz adeh et al. 2017; Alves et al. 2018; Abbasi et al. 2019; Al Subhi et al. 2020
	Stunting	Citrus sp.	stunting and proliferation	16Srl	DN	Pakistan	Fahmeed et al. 2009
	Phytoplasma and liberibacter mixed infection	grapefruit, Orangelo, pumelo, Persian lime, tangor, sweet orange, eureka lemon, C. grandis	Huanglongbing- like symptoms	16Srl, 16Srl–B and 16Srl S,16SrXII, 16Srll, 16Srll -A, 16Srll–C, 16Srlll, 16SrlV, 16SrlV–A,16SrVI, 16SrVII, 16SrIX, 16SrX, 16SrXII, 16SrXIV, Ca. P. palmae	ı Q	Jamaica, China, Guadeloupe, Cuba, India, Brazil, Mexico, Iran, Puerto Rico	Teixeira et al. 2008; Chen et al. 2009; Lou et al. 2014; Arratia-Castro et al. 2014; Caicedo et al. 2015; Saberi et al. 2017; Bertaccini et al. 2019; Ghosh et al. 2019; Wulff et al. 2019;Lopes-da-Silva et al. 2020; Luis-Pantoja et al. 2021; Paredes-Tomas, et al. 2023
	Ruberry wood	acid lime, lemon	multiple sprouting	Not molecularly detected and identified	QN	India	Ahlawat, 1987
	Blastomania	acid lime, C. <i>limonia</i>	little leaf, defoliation, witches'-broom	Not molecularly detected and identified	Q	India	Mali et al. 1975; Sharma and Singh, 1988
	No phytoplas ma typical symptoms	Mexican lime, Persian lime, fisher novel, rode red Valencia, tangelo	symptomless, reduced growth and dieback	16Srl,16Srll, 16Srll-B, 16Srll-C, 16SrVl, 16SrX, 16SrXl, 16SrXll, Са. P. palmae		Brazil, Oman, Guadeloupe	Silva et al. 2014; Al-Ghaithi et al. 2017; Donkersley et al. 2019; Luis-Pantoja et al. 2021



#### Transmission

Extensivelyconducted investigations demonstrate that *Hishimonus phycitis* leafhopper transmits the phytoplasma in the orchards, although *Recilia schmidtgeni*, *Idioscopus clypealis*, and *Diaphorina citri*were positive for '*Ca*.Phytoplasma aurantifolia' and could be potential vectors for wbdl-phytoplasma (Siampour et al., 2006; Salehi et al., 2007; Bagheri et al., 2009; Hemmati et al., 2020). In Brazil, the leafhopper *Scaphytopius marginelineatus* was identified as a potential vector of 16SrIX-related phytoplasma strain associated with citrus plants expressing hlb-like phytoplasma symptoms (Marques et al., 2012). Natural insect vector of other citrus associated phytoplasmas isstill not identified. while there is currently no evidence supporting transmission of the citrus-associated phytoplasmas through seeds, transmission may occur through the graft inoculation of infected budwoods (Faghihi et al., 2011).

#### **Conclusion and future prospects**

More than five decades after discovering, Phytoplasmas, theyexhibit a most complexfascinating pathosystem in plant pathology and remain the most poorly characterized phytopathogens. Althoughwbdl is considered the most devastating and the main phytoplasma disease affecting citrus, its geographical distribution has thus far been confined to a few countries. Fortunately, there is no evidence to indicate that it is spreading globally. The association of phytoplasmas with citrus trees will be more critical when, besides'*Ca*. Phytoplasma aurantifolia', several phytoplasma strains related to other phytoplasma species aredetected in citrus trees worldwide. It seems a new era of citrus-phytoplasma challenge is going to be started in the world. The data indicate that symptoms, host range, transmission, and distribution of the phytoplasmas in citrus around the globe are distinct and must be studied in depth as a global research megaproject.

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Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## A review of citrus greening disease in the coastal region of kenya

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Citrus greening disease which is also known as Huanglongbin (HLB) disease, is the most destructive disease to citrus fruits worldwide. It is known to affect citrus fruits of all kinds including oranges, lemons, limes, and grapefruits and is believed to have originated from China in the 19<sup>th</sup> century. It has devastated millions of acres of citrus trees in worldwide and continues to establish in regions that have not been reported in the past. Candidatus bacteria which is a vector-transmitted pathogen, is known to cause Huanglongbin (HLB) disease with the primary vector being Asian citrus psyllid (Diaphorina citri). There are three types of HLB, which are currently known i.e. Candidatus Liberibacter asiaticus (CLas), Candidatus Liberibacter africanus (CLaf), and Candidatus Liberibacter americanus (CLam). Candidatus Liberibacter africanus (CLaf) is known to cause devastating losses to citrus fruits in Africa. This bacterium is vectored by the vector Triozaerytreae. which has also been found to have the potential to also transmit Candidatus Liberibacter asiaticus (CLas) together with the closely related vector Diaphorina citri. This paper investigates the extent of spread and establishment of citrus greening disease and its vectors in the coastal region of Kenya. In regards to the occurrence and distribution, the study focuses on; ecological zones, varieties of citrus plants, age of infected trees, and management practices. Farmers' knowledge on the disease was also determined since this might have an impact in spread and establishment. The results thus far indicate increase in spread of the vectors and disease establishment in new regions. Kwale county recorded the highest number of vectors followed by Kilifi county. However, in Lamu county, trees expressed typical symmptoms of HLB but psyllids were not encountered. Psyllids were observed in both young and old trees with majority observed in the young trees. Majority of the farmers' had no knowledge of the disease and thus no control measures were in place. The findings of this report thus far emphasizes the need for capacity building HLB research and control to prevent further losses caused by the disease. Possible areas of funding include; Early detection methodologies for Clas and Claf, vector management. An in-depth understanding of the disease and its associated vectors will consequently provide a breakthrough in development of strategies that could help curb the damage caused by HLB within the region.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Huanglongbing (HLB): The citrus disease of greatest devastation – Obstacles and approaches forcontrol.

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Huanglongbing (HLB), known as citrus dieback, is the most damaging citrus disease globally, with significant economic implications for the citrus industry. The disease manifests through a range of symptoms, including blotchy mottled leaves, shoot yellowing, corky veins, small upright leaves, lopsided fruits, branch dieback, reduced fruit size, abnormal fruit drops, and, eventually, dieback of the entire tree. The primary causative agent behind HLB is the phloem- limited Gram-negative bacterium *Candidatus* Liberibacter asiaticus (CLas), transmitted by Asian citrus psyllids (ACP; *Diaphorina citri* Kuwayama). The understanding of microbiology and virulence mechanisms of CLas poses significant challenges, as it cannot be cultured under laboratory conditions. CLas infection stimulates plant immune responses, leading to the production of reactive oxygen species (ROS). This CLas-induced ROS triggers cell death and necrosis in phloem tissues. Callose deposition within sieve elements, phloem cell mortality, and degeneration collectively disrupt the transport of photoassimilates. The application of exogenous antioxidants and gibberellic acid has exhibited promise in mitigating HLB symptoms through abatement of CLas-induced ROS upsurge.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Biodiversity and eco-friendly management of insect, mite and snail pests of citrus in India

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Citrus is one of the most popular and widely grown fruit crops in the world. Citrus cultivation has been exported throughout the world's tropics and subtropics from its origins in Southeast Asia, unfortunately along with many of its pests but not their natural enemies. The genus is attacked by more than 400 major and minor pests worldwide including insects, mites, mollusks, plant pathogenic bacteria, fungi, viruses, viroids, nematodes and phytoplasma. Major Citrus pests vary by country with over 250 pest species recorded from India. During the past few decades, a lot of information on various pests of citrus has been generated by Entomologists working in the project ICAR-AICRP on Fruits through roving and fixed plot surveys. The date generated from the surveys revealed that citrus psylla, citrus leaf miner, whitefly, blackfly, fruit flies, thrips, aphids, mealy bugs, citrus butterfly, and mites are the major pests of citrus in India. Minor pests include, citrus leaf folder, bark eating caterpillar, citrus trunk borer, citrus scales, termites and snails while fruit piercing moths, American bollworm, grey weevil, hairy caterpillars, chafer beetles and weaver ants are sporadic pests of citrus in India. Some of the emerging pests include root borer, bark borer and citrus looper. To enhance the productivity and quality of citrus fruits for national and international trade, it is of utmost importance to manage these pests in an eco-friendly way and with holistic approach. Besides the management of prevalent pests, there is also need for regular vigilance about the entry of new pests of citrus along with consignments of imported fruits, through implementation of domestic and quarantine regulations. A number of predators, parasitoids, parasites and pathogens have been reported from different pests of citrus in India. The potential biocontrol agents needs to be multiplied in large number for their field evaluation and should be incorporated in IPM programmes of pests of citrus in India in a real sense.



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## Citrus diseases caused by oomycete, fungal and bacterial pathogens in India: Diversity, diagnostics and combat strategies

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Abstract. Incidence of various diseases caused by oomycete, fungal and bacterial pathogens right from bloom to harvesting/ post-harvest stage are the major bottlenecks resulting in incalculable losses and accounting for overall low citrus productivity in India. Some of the most economically important such diseases include Phytophthora, dry root rot, twig blight, scab, canker and greening. The climatic conditions and citrus varieties cultivated in various agroclimatic zones of India (North-west zone, Central zone, Southern zone and North-east zones) are different and thus the diseases occur in these areas are also different. In most arid and semi-arid climate. the diseases attacking aerial portion of citrus plants are relatively less important and cause sporadic damage. In high rainfall areas of North-Eastern part and in hot and humid weather of peninsular India, foliar diseases like powdery mildew, scab, citrus canker, pink and felt diseases cause considerable damage besides the soil-borne diseases which are widely prevalent in all citrus growing areas. Phytophthora spp. induce an array of diseases viz.root rot, crown rot, foot rot, gummosis and brown rot of fruits in citrus causing severe decline and yield losses in India. The major endemic bacterial citrus canker disease is the most devastating in all acid lime growing areas of central and Southern India. Citrus black spot caused by Guignardia citricarpa is an emerging fungal disease of citrus very recently reported from central India. Other fungal diseases like greasy spot, melanose, pink, felt etc. though sporadic in nature can be threatening under favourable climatic conditions. Some of these diseases would become a major threat in view of the recent climate change scenario. For an effective and rational disease management, the correct diagnosis of a disease based on the identification of its causal agent is very essential. Combined morphological characters and molecular data have been lately used for fungal/ fungal-like species identification and taxonomy. In addition to Phytophthora nicotianae, P. palmivora, and P. citrophthora, recent findings revealed presence of P. insolita, P. boehmeriae, P. tropicalis, P. macrochlamydospora and P. lacustris in the citrus rhizosphere in the 15 major citrus growing states of India adding to the *Phytophthora* biodiversity in this country. Morphological characterization coupled with PCR-RFLP based system of the ITS region of the genomic rDNA was used as a tool to identify all the different Phytophthora spp. Sequencing and phylogenetic analyses of theITS region, beta tubulin gene, translation Elongation factor 1 alpha and the region containing the mitochondrial cytochrome c oxidase subunit 1 and 2 gene fragments also confirmed the identity of these species. Integrated disease management (IDM) package for Phytophthora diseases of citrus has been developed. Some tolerant rootstocks like Alemow and some potential effective bio-agent Trichoderma harzianum (strain NRCfBA-44) have been identified against *Phytophthora* root rot. Huanglongbing, HLB (aka Citrus greening disease) is the most important and baffling disease of citrus not only in India but throughout the world. Heavy infestation of HLB in all commercial citrus growing areas is a real concern. PCR detection, sequencing of the signature loci coupled with phylogenetic analyses and Whole-genome sequencing confirmed the bacterial etiology (Candidatus Liberibacter asiaticus) of the disease. Other molecular techniques (nested, multiplex and real time PCR, LAMP) were also worked out for developing more robust and highly sensitive diagnostic tools. Genetic variation of a large population of Indian CLas strains has been evaluated using the PCR markers from the prophage regions. Current diagnostic tools/techniques and integrated management strategies for prevention and control of important fungal, oomycete and bacterial diseases of citrus trees would be discussed.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

## Emerging fungicides to promulgate horticultural crops: Spotlight citrus

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Citrus is one of the most important fruit crops of the world and hold an important economic position in the global fruit industry. These crops are susceptible to a large number of continuously emerging destructive diseases which severely hinder or completely decline the entire production. Control of such diseases are mostly relied on the use of chemicals. Fungicides, despite certain limitations, continue to play a crucial role in the management of these diseases. Systemic fungicides provided novel opportunities for disease prevention. In several instances, the targeted fungi's emergence of resistance has blunted the impact of these breakthroughs. The pathogenic fungi are closely regulated by phenyl amides and azoles. As new areas of chemistry were introduced, each one seeking to outperform the previous one, chemical families were established, with research-based firms frequently employing patent-busting techniques to gain on the burgeoning fungicides market. Farmers are now anticipating the release of new generation, highly specialized fungicides that will attack the disease triangle from a variety of perspectives. Fungicides can affect the carbohydrate levels of the host or imitate the host defense mechanism, in addition to having a therapeutic effect. Fungicides with the aforementioned properties that are now available on the global market include Boscalid, Metconazole Fluxapyroxad, Mefenoxam, and Penflufen. Fungicides like Triflumizole, Amisulbrom, Cyflufenamid, Valifenalate, etc. are expected to take center stage in the fight against oomycetes due to their low doses and ability to limit the risk of resistance. Another class of fungicides consists of substances that, do not produce fungitoxic effects, but which nonetheless manage plant diseases by impeding the mechanisms by which fungi enter plants or by boosting the resilience of their hosts. Examples include probenazole, which makes plants activate their defensive mechanisms, and tricyclazole, which causes fungal appressoria to malfunction. In fungicides history, several fungicide classes have been introduced starting from multi-site inorganic salts to organic compounds with protectant action and then to single-site systemic fungicides with curative activity. New compounds with novel modes of action are introduced to manage resistance to the existing fungicides and to provide more effective options for control of devastating diseases. Technological advances such as combinatorial chemistry, high throughput screening and bio-rational screen designs have revolutionized the synthesis and development of new fungicide active ingredients. In future, natural compounds hold promise to serve as new fungicide leads in place of more toxic synthetic compounds.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Advancing India's citrus industry: A multifaceted approach to address phytophthora challenges

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India, a prominent contributor to the global citrus sector, ranks sixth among leading citrus producing nations, contributing 4.80% to global citrus production. Despite its extensive citrus orchards, India grapples with a paradox of lower production per hectare compared to peers like the United States, Brazil, and Spain. In the 2019-20 seasons, India cultivated 11.2 lakh hectares, yielding 130.5 lakh metric tons of citrus. However, the industry faces a persistent menace Citrus decline, driven by unpredictable environmental factors such as irregular rainfall, prolonged heatwaves, and agro-meteorological uncertainties. The challenge is exacerbated by a limited understanding of the intricate interplay among plant physiology, soil dynamics, water resources, weather patterns, and disease propagation. These factors significantly affect citrus crop production and necessitate prudent water resource management. Effective pest and disease management necessitates a multifaceted approach for disseminating vital information on disease control strategies. Citrus decline, particularly prevalent in central India, is primarily associated with Phytophthora disease, causing a substantial 45% yield reduction and annual losses of approximately \$12.9 million in the citrus industry. Gummosis, another significant challenge, adds to these woes, causing yearly losses of about \$7 million. Phytophthora spp., formidable citrus pathogens, pose a substantial threat to Indian citrus cultivation, leading to diseases such as gummosis and root rot, causing yield losses ranging from 10% to 45%. Ongoing research explores innovative biological control strategies, including Trichoderma spp. and Pseudomonas spp. in conjunction with fungicides, to mitigate the impact on citrus crops. Suboptimal cultural practices, like flood irrigation for flowering induction, disrupt the delicate balance of water uptake and feeder root decay, resulting in sudden declines in plant health. Recent surveys indicate root rot incidence between 10.42% to 62.50%, with intensity from 1.62% to 23.48%, and gummosis incidence ranged from 14.58% to 64.58%, with intensity between 2.08% and 19.91%. Effective chemical management practices exist for controlling these epidemic diseases, but in alignment with organic farming and sustainable agriculture principles, potential bioagents and botanicals are under investigation for Phytophthora disease management. Epidemiological studies are valuable for understanding *Phytophthora* spp. dispersal patterns from disease foci and inoculum sources. To reduce chemical usage, compatibility studies were conducted between commonly used fungicides and fungal and bacterial biocontrol agents, yielding excellent results. Additionally, macroscopic modeling using the Richards equation with root water uptake has aided in understanding soil-water-plant interactions within the rhizosphere. A Citrus Gummosis prediction model was developed using multi-source data, incorporating weather and soilbased disease prediction models. The study considered different plant age groups and introduced Biophysical variables (LAI and Cab) into a statistical prediction model. The Support Vector Regression (SVR) model outperformed the multilinear regression (MLR) model. Furthermore, an Integrated Gummosis Disease Forecast Model (IGDFM) was designed to understand parametric conditions' interconnectivity, achieving promising results. In conclusion, addressing the convergence of environmental uncertainties, Phytophthora infestations, and gummosis calls for innovative, multidisciplinary approaches in Indian citrus farming and disease management. These solutions must incorporate cutting-edge scientific insights, advanced agricultural practices, and holistic resource management, steering India's citrus industry towards enhanced productivity and sustainability on the global stage.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Characterization of *Candidatus* Liberibacter asiaticus and development of simplified method for its rapid detection using recombinase polymerase amplification assay

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North East (NE) region of India is considered as center of origin and biodiversity for many citrus species. Huanglongbing (HLB) is a devastating disease recorded in the citrus orchards of NE region. HLB-associated *Candidatus* Liberibacter asiaticus (*C*Las) strains and genotypes were characterized from the citrus groves of region. 59.59% of the tested samples were positive for CLas infection. Multi-locus sequence typing based on 16S rDNA, omp. rp and CLIBASIA\_01645 genomic loci indicated existence of substantial genetic diversity and existence of 14 genogroups of CLas infecting citrus in the region. Around 13.22-38% of the tested samples in different surveys were having mixed infection of CTV and *Candidatus* Liberibacter asiaticus. A simplified template preparation and isothermal recombinase polymerase amplification assay (RPA) was developed for detection of CLas. The developed RPA assay could detect the *C*Las infection up to 10<sup>o</sup> to 10<sup>-11</sup> dilution of plant extract and up to 1-10 ag  $\mu$ I<sup>-1</sup> of DNA of CLas positive citrus tissues. Developed RPA assay validated using field collected citrus samples wherein 79.19% were CLas positive and was more sensitive as compared to PCR wherein. The developed RPA assay rapidly detected CLas infection and can be performed in resource-poor laboratories for prevalence studies and production of CLas-free planting materials.



Current Approaches in Citrus Health Management, Insect-Pest & Disease Survelliance and Diagnostic Approaches

# Ten years of research on citrus decline disease in Iran: Etiology and sustainable management

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Iran with a total production of 4.5 million tons of citrus fruits ranked 7<sup>th</sup> among 140 citrus producing countries. Climate change resulted in a change in the behavior of plant pathogens as well citrus associated pathogens. In the last decade, citrus decline, the most serious threat to the citrus industry in Iran, destroyed a large number of citrus trees in Kerman, Hormozgan and Fars provinces, in southern Iran. The disease was first reported at 2011 from Jiroft, Kerman, Iran as a newly emerged disease. To find out the causal agent, several research projects in the fields of plant pathology, soil analysis, irrigation, fertilization, and horticulture practices have been conducted. It was observed that, depends on the conditions, within four to 10 years, the affected trees showed sudden death symptoms. The results showed that a complex of citrus greening disease (HLB), *Phytophthora* root rot, *Fusarium* dry root rot, Phytoplasma, citrus root nematode, along with unfavourable climate conditions resulted to citrus decline. Integrated management practices include resistant rootstocks, plant resistance inducers, optimum application of soil fertilizers, improving irrigation system specially during warm season, application of plant-based mulch materials to protect roots against sun radiation during warm season resulted in decrease of citrus decline damage.



Current Approaches in Citrus Health Management, Insect-Pest & |Disease Survelliance and Diagnostic Approaches

## Semiochemical control of tephritid fruit fly pests in citrus cultivation

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The citrus industry is an important contributor to the global and local economy. This is evident from the top citrusproducing countries such as China, United States of America, Brazil, India, Mexico, Spain, Egypt, Turkey, Argentina, Iran, and South Africa. These countries represent the different continents and regions in which citrus is cultivated widely. Thus, with the rise in world population, there has been great demand for consumption of fruits including citrus, making it amongst the highest-value fruit crop in terms of international trade. Currently, key citrus fruits grown for local consumption and export are oranges, tangerines, lemons and limes, grapefruits, and pomelos. Cultivation of those citrus fruits is challenging due to threats of new pests and diseases appearing. With global warming due to climate change and rise in international trade accelerating alien pest incursions, there must be concerted steps to rapidly identify and mitigate the introduction of new pests whilst managing endemic pests to sustainable levels. Tephritid fruit fly pests remain as one of the most destructive insects of citrus cultivation. This is due to their highly invasive and dispersive ability as they are frugivorous and having multigenerational reproductive cycles. Eggs that were laid by females under the skin of the fruit will develop into larvae that will tunnel into the fruit as they consume the tissue thus rendering the fruit unfit for consumption and being rejected for export due to presence of those pest larvae.

Hitherto, fruit fly species from 3 major genus, Bactrocera, Ceratitis, and Anastrepha form some of the most destructive citrus pests. Many species are in the EPPO A1 and A2 lists. This includes those from the genus Bactrocera such as the Oriental fruit fly, B. dorsalis and peach fruit fly, B. zonata, genus Ceratitis such as the Mediterranean fruit fly, C. capitata and the genus Anastrepha such as the Mexican fruit fly, A. ludens and the South American fruit fly, A. fraterculus. Frequent fruit fly incursions require expensive eradication treatments with extensive monitoring networks. In the case of the Mediterranean fruit fly, its establishment in the US could cost over \$1.5 billion dollars a year in losses due to export restrictions, markets lost, treatment costs and crop losses. When alien fruit fly incursions occur, rapid eradication steps must be taken. In the case of the African fruit fly, B. invadens (now synonymized as B. dorsalis), it was first detected in 2003 in Kenya and in less than 5 years, it had spread to over 30 countries in Africa. The use of semiochemicals as behaviour-modifying chemicals when coupled with insecticides of low mammalian toxicity such as spinosad offers effective tools for the surveillance, detection, delimiting, and management of tephritid pest fruit fly populations. The availability of potent male lures combined with insecticides have contributed significantly to tephritid fruit fly control resulting in reduction of male population thus reducing chances of successful matings in female flies. This is evident in the case of B. dorsalis and B. zonata where methyl eugenol as a highly potent male lure has been successfully used in many male annihilation technique programmes in the world. For C. capitata, male lures such the trimedlure is widely used although a



BioLure 3-component lure, which is a combination of three compounds: ammonium acetate, putrescine and trimethylamine is also used. In the case of Anastrepha species, currently there is no identified male lures are available but monitoring for adults utilizes traps with protein-based or other ammonia-emitting lures such as ammonium acetate and putrescine. The search for novel semiochemical compounds is ongoing as more aspects of insect-host plant relationship is elucidated leading to identification of new compounds. In addition, as an insect olfactory-based tactic, much progress in understanding of the mechanisms of olfaction has led to new findings particularly those employing new molecular based approaches. In this talk, I will also highlight the prospects of using novel compounds and other control technologies against those pests that can be integrated whilst addressing key challenges that are faced. Potential emergence of other tephritid fruit fly species as pests is also discussed in light of semiochemical control against those species.



Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus

# Non-thermal and thermal processing to extend the shelf life of citrus fruits from north-east India

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Citrus fruits are well known for their dietary, nutritional, medicinal, and cosmetic properties and are also good sources of citric acid, flavonoids, phenolics, pectin, limonoids, ascorbic acids, etc. In India, the north-eastern Himalayan region and foothills of the central and western Himalayan tracts are rich sources of Citrus genetic diversity. It is well known that Citrus fruits occupy an important position in the context of the fruit trade, considering that both fresh fruits and processed products are produced on a large scale. These citrus fruits are processed at large scale and converted into citrus-based food products such as beverages, canned fruits, and jams. These fruits' high-water activity nature makes them suitable for the growth of various spoilage microorganisms (Salmonella, Listeria spp., Bacillus spp., etc.). Besides microbial spoilage, fruits can also be degraded by respiration, senescence, oxidative reactions, physical damage, starch conversion, etc. Therefore, preserving fresh fruits and their products has become the topmost priority for manufacturers and retailers. Conventional technologies, like heat treatment and drying, aim to prevent microorganism's growth but compromise the nutritional composition and sensory properties. Hence, nonthermal treatments are the most promising techniques for inactivating microorganisms in fruit products and extending their shelf life while retaining the maximum nutrients in the product.

The electric field-based novel thermal and non-thermal technologies include ohmic heating (OH), cold plasma (CP), pulsed electric field (PEF), and pulsed light (PL), in which OH is a combined electric field and thermal. The principle behind OH is that it utilizes the internal resistance of the food samples for heating when an electric current is passed through it. Thus, uniform and rapid heating are achieved. PEF, CP, and PL are non-thermal surface disinfection and sterilization technologies. CP uses energetic, reactive gases to inactivate pathogenic and spoilage microorganisms pertinent to food. It is generated by passing an inert gas through an electric field under applied voltage at room temperature. The electro-permeabilization mechanism of PEF results in the inactivation of microorganisms without thermal effects. A series of short and high- voltage (10-80 kV) pulses for a few micro- to milliseconds break the cell membranes of vegetative microorganisms in liquid media by electroporation. The US FDA (2015) approved Pulsed light for food treatment at a maximum dose of 12 J cm -2. It uses a high-intensity short burst of broad-spectrum light (pulses) ranging in wavelength from 200 to 1100 nm and inactivates the microorganisms by photochemical, photothermal, and photophysical mechanisms. PL is also effective in reducing allergens, maintaining the nutritional value and texture of food, and extending the shelf-life of certain foods alone and in combination with other technologies.



Latest Developments in Technology Outreach, Citriprineurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector

# Uplifting the citrus sector in southern region of India – Opportunities, constraints and roadmap

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India is a major center of diversity of citrus fruits with main regions of diversity being North-Eastern region, North-Western region, the foot hills of Himalayas and Western Ghats. In South India, commercially grown citrus cultivars are Coorg mandarin (Citrus reticulata Blanco) in Karnataka, Tamil Nadu and Kerala, Sweet Orange in Andhra Pradesh and Acid lime in Tamil Nadu. Coorg mandarin (Citrus reticulata Blanco), conferred with the status of 'Geographical Indicator' during 2005-06, is acclaimed for its guality fruits with good shelf life and sugar-acid blend imparting characteristic taste and flavour. It is commercially grown in the hilly regions where, the elevation ranges from 1000-2000m above msl. Commercial cultivation of Coorg mandarin is confined to the states of Karnataka (Kodagu, Chikmagalur, and Hassan), Kerala (Waynad) and Tamil Nadu (Shevroys hills, Lower Palani hills). The area under Coorg mandarin was around 50,000 acres during 1960's and it was contributing more than 50% of the total orange production of the country. But the area decreased rapidly during 1980 to 2000. The area of its cultivation is presently confined to about 3471.93 acres in Kodagu. In Tamil Nadu it is grown in lower Palani & Shevroy hills and adjusting area and total area may be around 3000 acres. In Kerala, Waynad district is known for Coorg mandarin but the area under this is limited to 200 acres. Coorg mandarin plants in traditional producing regions under the multi-storied cropping systems have been progressively declining, in the last few decades. Presently, citrus species are facing the threat of extinction due to widespread prevalence of biotic and abiotic stresses. Phytophthora, Citrus Greening Disease (CGD) and Citrus Tristeza virus (CTV) diseases are the major threats in these regions apart from the apathy of the growers towards the crop with regard to the crop management.

Concerted efforts made at ICAR-Central Horticultural Experiment Station (IIHR), Chettalli, Kodagu, Karnataka indicated the possibilities of revival of Coorg mandarin cultivation in the region through system approach by production and supply of disease-free planting materials, rejuvenation technology and integrating different aspects of improved crop production strategies into a module of critical technology components optimized from 2007 onwards over a calendar time scale were field tested from 2005-2010. This approach indicated hopes of upliftment of Coorg mandarin cultivation which may help in the rehabilitation of the crop. An integrated module was developed and validated that included both management of abiotic as well as biotic factors capable of adversely impacting the Coorg mandarin productivity in multi-storied cropping system. Abiotic variables such as total rainfall, number of rainy days, temperature, relative humidity and biotic variables such as incidence of



Phytophthora, powdery mildew, Citrus Tristeza Virus (CTV), Citrus Greening Disease (CGD) and the vectors dynamics *viz.*, black citrus aphid and citrus psylla on a calendar time scale were considered.

Revival of Coorg mandarin included regular liming for neutralizing soil acidity, application of major and micro nutrients, with focus on soil application of zinc and magnesium. Foliar nutrition of both major and micro nutrients once a month coupled with integrated management of Phytophthora, powdery mildew and vectors resulted in improved tree vigour and yield of declining Coorg mandarin trees. Recommendations were also generated for sustaining the revival package of Coorg mandarin that included reduction of inoculums load through removal of CTV and CGD infected plants; use of disease-free budded plants through STG (Shoot Tip Grafting), bud wood certification thereby propagation on Rangpur lime rootstock; effective management of Phytophthora, powdery mildew vectors and adoption of integrated water and nutrient management practices that improved the general health and production in declining trees. The results of this study may have analogous applications to the situation prevailing in the citrus growing southern regions of the country as well where; similar agro-climate and problems prevail. An overview of the scheduling program of optimized production practices tried under multi-storied Coorg mandarin cropping system of Kodagu and adjoining regions and the *way forward* based on the vision growers presently facing the challenges will be presented and discussed.



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## Agri-preneurship development in India: 'The ICAR's perspectives'

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The approach of developing a culture of agribusiness incubation and agri-entrepreneurs is a prerequisite to accelerate the process to new technologies by the farmers' for realizing the goal of their prosperity. India is the youngest country in the world with her 50% population below the age of 25 and more than 65% below the age of 35. Youngsters are the nation's treasure, which should beutilized in a productive way by ensuring their employment. The Start-up India concept created an environment for Indian youth to start their innovation-based business, which was launched on 16 January 2016 with an objective to build a strong eco-system for nurturing innovation and start-ups in the country that would drive sustainable economic growth and generate large-scale employment opportunities. Since the launch of Start-up India initiative in 2016, DPIIT has recognised 92,683 entities as start-ups out of that 47% from tier two and 3 cities. More than 4,000 recognised start-ups are engaged in sectors relating to emerging technologies such as Internet of Things (IoT), robotics, artificial intelligence, analytics, etc.

India's diverse agro-climatic conditions, which favours the production of agricultural and horticultural produce like citrus in bulk, and availability of production and process technologies and promising infrastructure - are the primary factors that aided the Indian citrus production to attain significant position in the world.India is the third highest citrus producer in the world after China and Brazil. Worldwide, citrus is grown in more than 150 countries, of which 53 cultivate it commercially. India contributes about 7–8% of the global citrus production.Citrus got third position in the country after mango and banana and is cultivated in 10.86 lakh hectares with an annual production of 142.62 lakh tonnes<sup>1</sup>.Citrus production encompasses the production of citrus fruit, which are the highest-value fruit crop in terms of international trade. The fresh fruit market and the processed citrus fruits market are the two main markets for citrus fruits. The orange contributes significantly among citrus production industry.

Indian Council of Agricultural Research (ICAR) had taken the initiative to promote the IP and technology commercialization process, by institutionalization of the policy through development of guidelines and establishment of a suitable mechanism in three-tier mode in the ICAR institutes. Further, support is being given to the activities related to agri-business incubation, which nurtures the technology entrepreneurs and stirrups in 50 ICAR Agri-business Incubation (ABI) centres network all over the country. These centres provide the effective platform for fostering the growth of sustainable business endeavour and provide the service support like research support, business planning, advice on management, and marketing, technical and financial matters.

ICAR institutes in different subject matter divisions are working on their respective mandates related agri-based technologies e.g. Horticultural Science institutes engaged in related research activities including value addition and value chain in all technologies relevant to vegetable and fruits (including citrus), medicinal& aromatic plants and spices research/seed spices etc. and in crop sciences lot of research on value addition & strengthening of value chains has been done on different crops like wheat, maize, millets, pulses, oilseeds and etc. Notable among



them, there is citrus value chain, which has shown significant presence in food, post-harvest and processing industry. Similarly, then animal science division, besides lot of products developed from different institutes, focus has also been given on meat production. Similarly dairy industry, products starting from 'culture for curd making to goat milk based paneer have development and brought laurels to ICAR, whereas fisheries product development has helped in small entrepreneurial development.

Development of various products from fruits (including citrus) and vegetables by ICAR include, shelf life extension strategies to backward linking farmers to value added products in various crops. Engineering division has also developed many technologies comprising of process and design of machines to improve the quality and efficiency in post-harvest operations.

The glimpse of vast diversity in citrus based technology can be seen in different publications across the organizations and ICAR institutes. The research and technology development was done with a vision to integrate them in a larger value chain to create a bigger picture of increasing GDP, employment generation, and consumption of diversified production on one side and giving choice to consumer at affordable price on other through generation of different types of Intellectual Property(IP). The dissemination of these IPs have taken place in discrete ways where agri-preneurs and start-ups ecosystem can play an important role for sustainable, inclusive growth and development of agriculture.

#### Agri-tech management mechanism

IP and Technology Management system implemented in ICAR with effective technology transfer activities through its research institutes. Initially the foundation of these efforts were established with the help of free kit demonstrations to the farming community. However, due to the individual efforts of scientists (from 1996 to 2005) and global dynamism in awareness on IP management and market competition, the agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) played an important role in transferring intellectual assets to the industry. After implementation of the three tier IP management system 38 ICAR institutes were involved in technology licensing activities and signed 341 agreements in 2007-2011. After implementation of XI Plan IPR Scheme and introduction of Business Planning and Development (BPD) Units under National Agricultural Innovation Project (NAIP) boost up this growth with the involvement of 60 ICAR institutes with 1127 licensing agreements. After attaining significant results out of these activities, existing three tier IP system was revitalized by implementation of National Agricultural Innovation Fund (NAIF) in 2012-17. These new dimensions enhanced the technology licensing numbers significantly to 1561 technology licensing agreements with 60 ICAR institutes/ over the period. Out of total 3029 licensing, ICAR-IIHR, Bangalore exhibited as a leading institute in generating the licensing (784), followed by IARI, New Delhi (558); IIWBR, Karnal (170), CPCRI, Kasargod (112), and DOGR, Pune (92).

In order to discussion, 3029 licensing partnerships were classified in twelve different subject specific domains of agriculture and allied sciences viz. Seed and Planting Material (34.66%); Crop Protection Technologies (27.90%); Food Processing and Post-Harvest Technologies (12.81%); Farm Machinery and Tools (07.82%); Crop Husbandry and Biotechnology Processes (06.21%); Fish Farming and Processes (03.30%); Animal-Health and Nutrition (2.77%); Fibre and Textile Technologies (2.58%); Poultry Breeds and Production Technologies (1.19%); and other three groups viz. Agri-Based Value-Added Products, Sea Weed and Marine Based Products, and Pig Farming Processes and Products having less than one percent share.

#### **Agri-Business Incubation Centres (ABIC)**

Agri-business Incubation (ABI) Centres were established in year 2016 in 25 institutes which is further expanded to 50 ABIs, considering the spectrum of technologies, available infrastructure and competency of the institutes.



These incubators provide an effective platform for fostering the growth of sustainable business endeavour and provide a wide range of services such as research support; business planning; office space; access to information and communication technologies; and advice on management, marketing, technical and financial issues. In line of national campaign of Start-up India and stand-up India, to further strengthen its ecosystem, new dimension of agribusiness incubators has been added to facilitate start up in agri-sector.

There is a crucial need to encourage start-ups for transforming the agriculture sector of the country by linking farmers and producers to the markets, new product development, value addition in agriculture and horticulture produce, efficient processing for reducing wastages and creating effective supply chain models. In order to increase success quotient of the start-ups and enterprises, strengthening and opening up of new incubators in agricultural food including horticultural fruits and vegetables processing sector to provide backup technical support is of utmost importance for accelerating, nurturing and investing in innovative early stage start-ups

#### Conclusion

The effective implementation of agripreneurship and startup ecosystem in field of agricultural including horticultural science and technologies have been made possible with rigorous and continuous efforts of ICAR agribusiness incubators network which functions in line of related policies of Government of India. The citripreneurship significantly contributes a lot in agri-entrepreneurship development programmes of the country in providing entrepreneurs, industry and startups in respect of food and agriculture sector which gives an opportunity and further prospecting new marketing linkages, technology and financial tie-ups and partnership. The citripreneurship culture provides ecosystem for startups of country's finest mentors, angel investors, venture capitalists and startup fund organizers.

Start-ups in the agricultural as whole and horticultural food processing and post-harvest sector in particular through aggregation models on both demand and supply side in B2B and B2C segments can provide efficient solutions across the value chain, thus helping the farmers in getting better prices for their produce and also efficient discovery of prices.

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Latest Developments in Technology Outreach, Citriprineurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector

## Elevating the citrus sector in north-western region of India – Needs and focus

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Citrus is one of the most widely cultivated fruit crop. These are good source of nutrients, dietary fibre, vitamins (vitamin C, folic acid, thiamin, niacin, vitamin B6, riboflavin, pantothenic acid) and minerals (K, Ca, P, Mg and Cu). Citrus fruits also possess health promoting phytochemicals like carotenoids, flavonoids and limonoids, which are useful in preventing onset of various chronic diseases (Liu *et al* 2012). The tropical and subtropical areas lying between the geographical coordinates of 35°N to 35°S latitudes are suitable for cultivation of citrus. The northwestern region of India is mainly represented by the states of Punjab, Haryana and Rajasthan. The citriculture in this part of the country is largely flourishing under sub-tropical climate. The region is characterised by a single time synchronised flowering for most of the citrus fruits (mandarin and sweet oranges) in the spring.

#### STATUS OF N-W INDIA VIS A VIS NATIONAL CITRICULTURE

Citrus is the second most important fruit crop in terms of area and third most important in terms of production in India (Anonymous, 2022a). In India, citrus occupies an area of about 1.09 million hectares with the annual production of 14.8 million metric tonnes (Anonymous 2022a). The north west Indian states, Punjab, Rajasthan and Haryana collectively accounts for about 11.0% and 16.8% of the national citrus area and production, respectively (Table 1 and Table 2). The agro-climate in most parts of this region is a typical subtropical climate.

Citriculture region	Area ('000 ha)	Production ("000MT)	Productivity (MT/ Ha)
All India	1095.0	14810.0	13.5
N-W India	121.0 <b>(11.0 %)</b>	2486.2 <b>(16.8 %)</b>	20.5

Table 1: The comparison of N-W Indian citrus industry with the National citriculture

#### Major citrus growing areas

In Punjab, though citrus can be cultivated in all the districts, but the major cultivable area is in south-western (S-W) districts of Fazilka, Muktsar, Bathinda, and Faridkot. The climate in these districts is of arid to semiarid subtropical type. The second belt is present in sub-montaneous region (mainly Hoshiarpur), which has a humid subtropical climate. The soil condition of two belts is also contrastingly different. The soil in S-W districts is saline to alkaline in nature with pH reaching up to 8.5 while soil of sub-montaneous regions is acidic to neutral.

In Rajasthan, citrus is being cultivated under two different pockets: arid irrigated plains (Ganganagar and



Hanumangarh)and hot humid plains of Jhalawar. In arid region, Kinnow mandarin is cultivated while in Jhalawar, Nagpur mandarin is the main cultivar.

In Haryana, citrus is grown under Sirsa, Bhiwani, Hisar, Narnaul and Fatehabad districts. The agroclimatic condition of these districts has resemblance with the commercial citrus growing areas of Punjab.

State	Mandarins		Lime and lemons		Sweet oranges	
	Area	Production	Area	Production	Area	Production
	(ha)	(MT)	(ha)	(MT)	(ha)	(MT)
Punjab#	47,900	1284000	3100	25000	4000	35000
Rajasthan##	38,718	646077	3028	19942	302	5321
Haryana#	23, 920	470852	-	-	-	-
Total	1,10,538	2400929	6128	44942	4302	40321

Table 2: Area and Production o	f citrus in dffe	rent states of N-W	India in 2022-23
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Source: <sup>#</sup>Anonymous (2023a, b), <sup>##</sup>Anonymous 2022b

#### Varieties and Rootstocks

The Punjab Agricultural University (PAU) after thorough evaluation has recommended different citrus varieties under different groups along with their compatible rootstock for cultivation in Punjab. The varieties along their maturity periods under Punjab conditions are mentioned in the Table 3 below.

Table 3: Various varieties along with the compatible rootstock recommended for cultivation in the N-W Region

Citrus group	Cultivars	Recommended rootstock	Maturity Periods
Mandarin	Daisy	Rough lemon	Early November
	PAU Kinnow-1, Kinnow, W-Murcott	Rough lemon#	January
Sweet	VanigliaSanguigno		End October –Mid November
oranges	Early Gold		End October
	Mosambi		November
	Jaffa		December
	Valencia Late		February-March
	Mosambi	Pectinifera	Early ripening in November
	Blood Red	Cleopatra mandarin	December January
Lime and	PAU Baramasi lemon	Rough lemon	July-August
Lemons	Kagzi lime		August
	Sweet lime		September
Grapefruit##	Star Ruby (Seedless)		Last week of November
·	Red Blush		Last week of November
	Foster		November-December
	Marsh Seedless		December-January

<sup>#</sup>For mandarin growing in soil with pH < 8.0, Carrizo is recommended as rootstock; <sup>#\*</sup>Cultivated on home scale in Punjab.



These recommendations are adopted equally by the adjoining states. In Rajasthan, mandarins are the main citrus fruit with a minor portion of area under sweet oranges, limes and lemons cultivation (Table 2). Among the mandarins, Kinnow and Nagpur mandarin are the two major cultivars. Nagpur mandarin is the cultivated in Jhalawar area. In Punjab, from the tablelisted varieties, Kinnow among the mandarins, Mosambi, Jaffa and Blood Red in the sweet orange group, Baramasi lemon in the lemon and lime group are the preferably adopted varieties by the growers. The adoption of Daisy mandarin on account of its earliness is growing up gradually. In recent, PAU has also developed a low seeded Kinnow mutant, PAU Kinnow-1. In Haryana, Kinnow mandarin is the major citrus cultivar.

#### NEEDS AND FOCUS FOR ELEVATING THE CITRUS SECTOR IN N-W INDIA

In view of the fact that climate favoring citrus cultivation, there is a need to expand citrus cultivation in this part of the country. The depleting natural farm resources under conventional paddy-wheat based cropping pattern in states like Punjab suggest an urgency to diversify towards fruit crops cultivation. Citrus fruits are vital option for diversification. But, for expanding area under citrus and keeping citrus cultivation remunerative for various stakeholders, certain needs to be addressed, which are discussed below:

#### **Quality Planting Material**

Citrus cultivation is a long term venture. To meet the needs of the area expansion under citrus, large number of high quality planting material is required. There are public sector nurseries in the three states to cater the needs of quality planting material. However to meet the large planting material requirements, the respective states have also set up Indo-Israel Centre of Excellence for citrus or Fruits (Table 4). These centres also have a mandate of production of quality planting material of citrus fruits.

#### Table 4: Public sector citrus nurseries under N-W India1.

- 1. Department of Fruit Science, Punjab Agricultural University (PAU),
- 2. Ludhiana
- 3. PAU-JC Bakhshi Regional Research Station, Abohar, Fazilka
- 4. PAU-Fruit Research Station, Jallowal
- 5. PAU-Regional Research Station, Bathinda
- 6. Centre of Excellence for Fruits, Khanaura, Hoshiarpur, Punjab
- 7. Centre of Excellence for Citrus Fruits, Kota, Rajasthan
- 8. Centre of Excellence for Fruits, Mangiana, Sirsa (Haryana)

#### Diversification within citrus

Most of the N-W Indian citriculture revolves around the high yielding Kinnow mandarin. In recent, PAU has come up with a low seeded Kinnow mutant, PAU Kinnow-1. To avoid market glut at a single time, there is a need of mandarin varieties with different maturity periods. PAU has recommended Daisy mandarin to address this. The early maturing sweet orange varieties like Early Gold and VanigliaSanguigno can also prove a good candidate for this diversification. The baramasi lemon matures early, hence should be a good genotype for citrus diversification. There is also a need to start programs on breeding of new set of varieties.



#### **Technological Interventions**

In arid areas, water remains a precious resource. Focus should be on the use of water and fertilizer saving technologies like drip cum fertigation technology and mulching. The adoption of drip cum Fertigation technology in citrus orchards can save water and fertilizers. Additionally, in this region, foot rot/gummosis is the major culprit behind lower citrus productivity and longevity of citrus. The pathogen of these diseases, *Phytophthora* travels through water. The use of drip technology prevents spread of pathogen from infected plant to the other. The soil health should also be given a due care. The organic manure should be consistently applied while the chemical fertilizers should be applied based on the plant needs.

#### Management of pesticide load in citrus

Citrus crop is affected by different insect pests and diseases. To protect the citrus plants from insect pests and diseases, growers use a variety of recommended and un-recommended pesticides without knowing their residue implications. The presence of residues above the prescribed maximum residue limits (MRL's) in fruits render them unfit for export. The consumers demand for high quality and safe food is constantly increasing, which is a challenge for growers to keep up with the standards of quality. So the rationalization of current spray schedules and development of IPM and IDM strategies is regarded as an immediate priority. Good Agricultural practices may be adopted to manage the pesticide load. Further to facilitate, the analysis of pesticide residues, residue analysis laboratories needs to be established in these states.

#### Value addition of the citrus

To meet the primary uniform quality of produce as per international standards, automatic color size and fruit health based sorting and gradingis required. Fungicides based waxing need to be used for extended shelf life of citrus produce during export. One such state of art optical grader is being run in the service of farmers by, Punjab Agro Industries Corporation Limited (PAIC) at Sito, Abohar in Punjab.

To diversify Kinnow marketing, it should be processed into different value added products. PAIC has taken initiatives in this regard. The agency in collaboration with a French firm, has produced an alcoholic beverage from Kinnow juice and has launched it under the brand 'Origin'. The agency is also preparing a bitterness free Kinnow juice enriched with beet root and carrot juice. The high seed content of Kinnow has been a contributory factor towards juice bitterness. The availability of low seeded Kinnow mandarin should also help getting rid of some treatments in developing a bitter free Kinnow juice.

#### **Other Industrial applications**

Kinnow peel and extracted pulp are the waste of Kinnow juice industry. Fresh juice industry generated 30-34% fruit peel waste.Kinnow peel is a rich source of pectin and dietary fibers (Ghosal and Negi, 2020). It can be used for pectin extraction. Pectin is used as a stabilizer in a range of products like jams, jellies and also the other milk, soya and cereal based beverages.

Besides it, the peel can be used for extraction of bio-flavonoids. Citrus flavonoids are useful in prevent coronary heart disease and serve as anti-inflammatoryand anti-carcinogenic agent. Being rich source of essential oils, the peel and seeds of citrus also find use in cosmetic and personal care products (Suri et al., 2022).



#### **New market Avenues**

Beside domestic market, international markets like European Union, Russia, Middle East countries and Bangladesh are few destination for Indian Kinnow export. These markets should be explored well with Government support.

In conclusion, keeping in view, the avenues available for proper utilization of citrus fruits as table purpose, its value addition into different edible product, and using its by products for development of food and non-food products holds a bright future for citrus expansion in North Western India.

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# Thematic Area -1

Recent Trends in Improvement, Genetic Diversity, Conservation and Utilization of Citrus



Advancing Citriculture for Agro-economic Prosperity

### **Oral Presentation**

## TS-1-O-01

# Impact of leaf stomatal conductance and photosynthetic rate features on fruit yield in grapefruit (*Citrus paradisi* Macf.) varieties

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A study was conducted on nine grapefruit (Citrus paradisi Macf.) varieties to see the impact of leaf morpho-anatomical traits and photosynthetic traits on fruit yield. This was achieved through the analysis of leaf morpho-anatomical traits using Optical and scanning electron microscopes as well as measuring chlorophyll content and photosynthetic traits with a SPAD-502 chlorophyll meter and Infra-red Gas Analyzers instrument in field conditions. The results showed that despite having the same pedigree, the grapefruit varieties displayed variation in leaf anatomy and photosynthetic traits. The highest stomatal conductance was found in Ray Ruby (0.23 mole H<sub>2</sub>O m<sup>2</sup>/sec), which also noticed a maximum photosynthetic rate (13.79  $\mu$ mole CO<sub>2</sub> m<sup>2</sup>/sec) and relatively higher transpiration rate (4.76 mole H<sub>2</sub>O m<sup>2</sup>/sec). Significantly higher chlorophyll content was observed in Oroblanco (52.33 SPAD unit) and it was at par with Ray Ruby (48.67 SPAD unit). The dendrogram was able to distinguish the varieties based on their characteristics, regardless of their geographical origin or pedigree. The varieties in cluster 'A' i.e., Rio Red, Ray Ruby and Red Blush were maximum in photosynthetic traits such as stomatal conductance, photosynthetic rate and intercellular CO2 concentration, subsequently contributed to higher yield of 80.65 kg/tree, 69.71 kg/tree, 67.41 kg/tree, respectively. The principal component 1 (PC1) accounted for 35.5 per cent of the total variance and the main dominant features were stomatal conductance, photosynthetic rate, yield, and open to closed stomatal index, while, principal component 2 (PC2) represented 27.5 per cent of the total variation which was primarily dominated by leaf stomatal density, stomatal pore length, total stomata, and transpiration rate. The study concluded a positive correlation of fruit yield with leaf stomatal conductance, photosynthetic rate and transpiration rate.

**Keywords:** Chlorophyll content, Morpho-anatomical traits, Principal Component, Scanning Electron Microscopy and Stomatal conductance



# Growth, physiology and biochemical alterations in citrus rootstock hybrids as influenced by polyethylene glycol (PEG) mediated progressive drought under *in vitro* condition

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Drought is articulated as a physiological state of water deficit and is arguably the most critical abiotic factor limiting citrus growth and productivity. Present investigation was conducted in 2020-21 at Indian Agricultural Research Institute, New Delhi to assess drought tolerance potential of five citrus rootstock hybrids by assessing morphological and physio-biochemical responses under in vitro conditions. Onemonth-old in vitro germinated seedlings were cultured in MS medium with increasing concentration of PEG 6000 (0, 6, 8 and 10%). Result suggested inadequate alternations in most studied morphological and physiochemical traits under mild drought (6% PEG) than control (without PEG). Moderate (8% PEG) and peak (10% PEG) droughts, alternatively, caused significant variation ( $p \le 0.05$ ) in majority of traits. Hybrid CRH 21-13/14 (Pummelo × Troyer citrange) proved the better performer in terms of plantlet growth (12%), lowest leaf wilting index (1.2), leaf injury (29.4%) and analogous leaf count under peak drought than control. It also sustained > 70% relative water content (RWC) and > 60% membrane stability index (MSI)at the peak PEG concentration. Pummelo × Morton hybrids viz., CRH 23-9/17 and CRH 23-14/16 inhibited highest plantlet height (> 37%), RWC (40%) and MSI (> 45%) as well as highest wilting and injury symptoms at higher PEG concentrations. At the peak PEG concentration, CRH 21-13/14 outperformed its counterparts in terms of chlorophyll 'a' (0.74 mg/g FW), total chlorophyll (1.91 mg/g FW) and chlorophyll a/b ratio (1.66). Although most hybrids showed > 100% increase in total soluble sugar levels compared to control under the highest PEG levels, it was more pronounced (> 150%) in CRH 21-13/14 and CRH 21-14/14. While CRH 23-5/15 and CRH 21-13/14 perceived superior up-regulation (> 150%) in leaf proline level during peak drought than control. Inclusively, we propose greater drought resilience of CRH 21-13/14 which could be tested further in field conditions.

Keywords: Drought, Hybrids, In vitro, PEG, Physio-biochemical, Rootstock screening



# Efficiency of RAPD and SSR markers in diversity analysis of acid lime (*Citrus aurantifolia* Swingle) cultivars

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Genetic diversity in twelve acid lime cultivars collected from different parts of the country was studied at Citrus Research Station, Tirupati using markers utilizing 13 RAPD and 15 SSR molecular markers. A total of 54 and 32 polymorphic bands were observed with RAPD and SSR primers, respectively with mean polymorphism observed between RAPD and SSR primers was 72.28% and 50 %. The SSR markers viz., AG14 (150bp), CAC33 (170bp), CAC15 (150bp) and RAPD markers namely, OPA01 (1700bp), OPA05 (1900bp), OPA13 (1600bp), OPB09 (2000bp), OPC01 (2100bp, 1500bp), OPT08 (2000bp) specifically amplified acid lime cultivar TAL-94/14. Grouping of genotypes using combined data of RAPD and SSR primers indicated that twelve cultivars clustered into different groups showing that individual cultivar possess wider variability. The Jaccard's similarity coefficient for the combined data ranged 0.37 to 0.90 and the dendrogram also placed TAL-94/14 in a separate cluster. The combination of SSR and RAPD markers also guarantees few additional benefits and may be a suitable method for cultivar characterization as each cultivar cannot be defined by a single marker but a set of markers and the results arrived can form the basis for further citrus genetic improvement programme.

Keywords: Acid lime, Marker diversity, RAPD, SSR



# *Ex vitro* root induction in micro-propagated shoots of rough lemon (*Citrus jambhiri* Lush.)

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Micro-propagated shoots can be rooted in vitro as well as ex vitro. The ex-vitro method can help achieve rooting and hardening simultaneously. This method has been attempted in many plants. In this study, ex vitro root induction was attempted in rough lemon, a popular citrus rootstock. Approximately 8 months old, 5 cm long shoots with 4-5 leaves were treated with NAA and IBA at different concentrations (0, 200, 400 and 600 mg l<sup>-1</sup>) for 10 minutes. The treated shoots were planted in jars containing potting mixture, irrigated with <sup>1</sup>/<sub>4</sub> MS solution and kept at  $25\pm2^{\circ}$ C. The rooted shoots were transferred to styrofoam pots in green house. The pots were covered with diposable plastic glasses and were gradually opened. The data on rooting was recorded after 40 and 80 days. No rooting occurred in control. From the two auxins, NAA induced higher percent rooting. Maximum rooting (57.1%) occurred with 400 mg l<sup>-1</sup> NAA and followed by 53.8% in 200 mg l<sup>-1</sup>NAA. After 80 days, rooting increased to 71.6% and 75% in400 and 200 mg l<sup>-1</sup>NAA treatments, respectively. Number of roots per shoot ranged from 1-2 under different treatment. The maximum root length (3.7 cm) was observed in NAA (400 mg l<sup>-1</sup>). An average 1-2 lateral roots were also observed with NAA (400 mg l<sup>-1</sup>) and IBA (400 mg l<sup>-1</sup>) treatments. The optimized method should help reduce the propagation time of micro-propagated plants in rough lemon.

Keywords: Micro-propagation, Ex vitro rooting, Growth hormone



# Evaluation of promising clones of acid lime (*Citrus aurantifolia* Swingle) at Rahuri region

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The field experiment was carried out at All India Coordinated Research Project on Fruits, Department of Horticulture, MPKV, Rahuri in RBD design with three replications and nine treatments (promising clones) during the year 2022-23 with an objective to evaluate the performance of promising clones of acid limes and to identify suitable clone of acid lime for Rahuri region. The results revealed that, the treatment ( $T_9$ ), Phule Sharbati (check) recorded significantly maximum thorn intensity (63.00 thorns/m length) as compare to other clones of acid lime, while, treatment ( $T_2$ ) i.e. PDKV Chakradhar recorded significantly minimum thorn intensity (13.33 thorns/m length). Whereas, there were non-significant differences between the treatments for plant height, E-W spread, N-S spread, canopy volume and stem girth. In case of pest and disease incidence results revealed that, Phule Sharbati (check) showed least infestation of leaf miner by recording 1.00 live mines/leaf and was at par with the NRCC AL-8( $T_4$ ) and Patlur Sel-1( $T_8$ ) which recorded 1.13 and 1.40 live mines/leaf, respectively as compared to other clones under investigation.

Keywords: Acid lime, Canopy volume, Clones, Plant height, Thorn intensity



## Mapping of phytophthora resistance in intergeneric citrus rootstock population

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Citrus gummosis, caused by Phytophthora, is one of the major diseases of citrus. Commercially used rootstock Rough lemon is highly susceptible to Phytophthora, whereas Poncirus trifoliata and its hybrids are resistant to it. However, the genetics of Phytophthora resistance in P. trifoliata is not known. To gain insight of Phytophthora tolerance in tolerant rootstock, we developed an intergeneric population using in vitro embryo rescue from the cross of 'Rough lemon' and 'X639'. The F<sub>1</sub> population of thirty-nine hybrids, was evaluated using both in vitro and in vivo methods against Phytophthora. Ten phenotypic traits, correlated with Phytophthora infection, were screened using in vivo method, while two traits were screened using in vitro method. A genetic linkage map of 2645.66 cM length with an average of 21.12 cM distance between adjacent markers was made having genome coverage of 83% using SSR markers. The phenotypic data obtained from screening along with the genotypic data was used to find the QTLs related to Phytophthora tolerance. Eighteen QTLs were found, nine of them were considered minor QTLs and nine QTLs were considered major QTLs. Knowledge about these QTLs can help in future mapping studies as well as the development of novel, regionally adaptive citrus rootstocks that are Phytophthora tolerant.

Keywords: Citrus, Rough lemon, X639, Phytophthora, SSR, QTL



## Assessing stability and performance of mandarin varieties in AICRP trials

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The AICRP trials are conducted across diverse locations, employing standardized test treatments, with the goal of identifying consistent performers aligned with specific objectives. Employing the Eberhart-Russell approach, stability models were developed in this study. Data from seven mandarin varieties across five AICRP(F) centres were analysed individually for plant height, stem girth, canopy volume, fruit weight, number of fruits per tree, and fruit yield. Utilizing a four-year dataset from 2018 to 2021, R codes calculated stability measures including regression coefficients, bi, and squared deviations. Results revealed that Mudkhed seedless, Nagpur mandarin, Kinnow mandarin, and Coorg mandarin demonstrated stability in fruit weight across all locations. Similarly, Mudkhed seedless, Nagpur seedless, Nagpur mandarin, Kinnow mandarin, Coorg mandarin, and Darjeeling mandarin displayed stability in fruit yield across all locations. For the number of fruits per tree, Nagpur seedless, Kinnow mandarin, and Coorg mandarin were identified as stable choices for cultivation across locations. Furthermore, by assessing environmental index values, optimal locations were determined. Akola ranked first for fruit weight, followed by Ludhiana and Nagpur. Ludhiana was the top-performing location for fruit yield, succeeded by Akola, Sriganganagar, and Nagpur. Sriganganagar emerged as the favourable location for the number of fruits per tree, with Ludhiana and Akola close behind. These initial results would be helpful to suggest stable lines for further exploitation in mandarin crop improvement studies.

Keywords: AICRP, Centres, Mandarin, Stability



# Collection, characterization and conservation of native indigenous citrus germplasm of upper Brahmaputra valley zone of Assam, India

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Numerous tribes from various communities live in the North Eastern region of India, which is home to a wealth of Citrus species. These tribes use various Citrus germplasm to treat a variety of human ailments. Number of species, landraces, and probable hybrids of Citrus can be grown without any special attention due to the region's edaphic and climatic conditions as well as its physiographic state. However, as a result of shifting farmer's attitude toward some recently introduced cash crops, which is a result of population growth, important resources are now being genetically eroded, and most of them are on the edge of extinction. Therefore, it is imperative that the scientific community utilize all the unique and endangered Citrus resources of the North-Eastern region while also conserving them. AAU-CPCRS, Tinsukia, Assam conducted a roving survey in this area to determine distribution, examine variability, and collect various native and wild germplasms. Two Citrus germplasm were collected viz. Bor Tenga and Bira Jora from Malu gaon and Pengeri respectively. Additional Citrus germplasm including Luipop sui, Khasi mandarin, Mousambi, Suka tenga, and rough lemon was also gathered from the Maj Gaon area and from various locations of Upper Brahmaputra Valley Zone. The morphological analysis of the leaves, fruits, and seeds revealed that all seven Citrus germplasms collected accessions showed a significant amount of variation. However, due to the lack of information on how to cultivate these species and the worrisome rate of forest cover loss, it is vital to implement complementary conservation policies to protect these species and guarantee their viability for use in the future. Furthermore, propagation, evaluation need to be done to safeguard the existing population of Citrus and for future Citrus improvement programmes.

Keywords: Conservation, Characterization, Germplasm, Indigenous, Wild species



# Evaluation of sweet orange cultivars (*Citrus sinensis*) and rootstocks (Stionic combinations) under high density orchards at Jalgaon, Maharashtra conditions

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Modern horticulture technologies, High Density Planting (HDP) as well as suitable stionic combinations for processing type sweet orange cultivars play an important role in future citrus industry. Evaluation of five processing type sweet orange cultivars, viz. Hamlin, Valencia, Natal, Pera and Westin on four rootstocks, viz. Volkameriana, Limao cravo (Rangpur lime type), Swingle citrumelo and Cleopatra tangerine was conducted during 2017-18 to 2022-23 on a four year old plantation with planting distances (4m x 3m) in central India (Jalgaon) under sandy loam soil. Maximum plant height was recorded in Hamlin on Rangpur lime while Pera was dwarfest on Cleopatra tangerine rootstock. Hamlin on Swingle Citrumelo showed higher canopy volume and Pera on Cleopatra tangerine produced the smallest canopy. Maximum scion girth was observed in Valencia and minimum in Westin. Natal on Swingle Citrumelo and Valencia on Cleopatra tangerine recorded maximum fruit weight. Juice content was highest in Natal while maximum TSS level was recorded in Hamlin. Natal followed by Hamlin and Valencia budded on Rangpur lime rootstock registered higher yields during 5 th year of high density plantation. Lowest yield was recorded in Valencia on Volkameriana. The objective was to find out response of sweet orange varieties when planted in high density as various stionic combinations coupled with proper training and pruning, hi-tech irrigation system with suitable orchard management to achieve significant production. This paper describes performance of exotic stionic combinations of sweet oranges under agro-climatic conditions of Jalgaon, Maharashtra.

Keywords: Sweet orange, High density planting, Scion rootstock combination, Yield



## Performance of different mandarin clones for yield and quality

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An experiment was conducted to evaluate the yield and quality performance of 29 different mandarin clones at Research Farm, ICAR-Central Citrus Research Institute, Nagpur, India. The experiment was laid out in Randomized Block Design with three replications. These clones were planted at 6 x 6 m spacing. The package of practices recommended by ICAR-CCRI was uniformly followed for all the clones. The observations on yield and quality parameters were recorded. Analysis of data revealed that significant variations were observed among the twenty nine mandarin clones for all the traits except acidity. The maximum fruit weight was recorded by JK-5 (186.77 g) and the minimum fruit weight was recorded by DM-16 (121.66 g). The maximum fruit length (66.71 mm) and fruit diameter (73.08 mm) was recorded by N-107 and the minimum fruit length (42.36 mm) and fruit diameter (48.25 mm) was recorded by KO-7. The minimum number of seeds were recorded by DM-8 (2 seeds/fruit), whereas highest juice content (51.48 %) and TSS (10.33 <sup>o</sup>Brix) were recorded by N-105 and SM-3, respectively. KO-7 recorded the highest TSS:acid ratio (18.54). It was concluded that DM-16 was performed better in yield and DM-8 was preferred for seedlessness. KO-7 was better in terms of fruit size and TSS:acid ratio.

Keywords: Mandarin clones, Yield, Quality, Seedlessness



# Citrus improvement in ICAR-Central Citrus Research Institute, India: Achievements and future prospects

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Biotic and abiotic stresses, climate change and emergence of new pathogenic strains cause huge economic losses to citrus growers in India. Further, drought, salinity and unseasonal rains are severely hampering the citrus industry in India which leads to low productivity and poor quality produce. Indiscriminate use of these chemicals not only increase the input cost and also affect the environment and human health. Since, there is no resistant or tolerant scion and rootstocks available, genetic improvement of citrus is very much important. In this context, citrus improvement programme was initiated at ICAR-CCRI, Nagpur during early 1990. Identification of elite clones, introduction of new varieties, improving the existing varieties and breeding of new scion and rootstocks has been undertaken for quality improvement. Several elite clones of mandarin cv. Nagpur mandarin, sweet orange cv. Mosambi, pummelo, and acid lime were selected for yield and guality. ICAR-CCRI has identified N-4 seedless as commercially seedless (0-2 seeds/fruit) and N-28, N-34, N-38, N-51 as high yielding Nagpur mandarin clones. Mosambi clone TM-33 is high yielder with high juice content. NRCC-7 and NRCC-8 acid lime are high yielding with high juice content and suitable for processing industry. Besides, few pummelo and grapefruit clones have been identified for quality. Scion breeding programme resulted in two hybrids *viz*. Citron x acid lime (fruit weight – 164.5 g, TSS – 6.9 <sup>o</sup>Brix, acidity –3.84 %. and juice content – 27.64 %)and Citron x Adajamir (fruit weight – 119.1g, TSS - 6.7 <sup>o</sup>Brix, acidity -7.36 %. and juice content – 15.96 %). The rootstock breeding to develop Phytophthora tolerant rootstocks using Rough lemon and Rangpur lime as female parent and Trifoliate orange and its hybrids as male parent resulted in five hybrid rootstocks (NRCC roostock 1 to 5). Alemow (Citrus macrophylla) has been identified as drought and Phytophthora tolerant rootstock for Nagpur mandarin. Recent past, genetic engineering, marker assisted breeding, CRISPR, and genomics research on citrus resulted in cutedged results. We have taken initiation to develop citrus varieties and rootstocks that are more resilient to stresses, have improved fruit quality, and offer sustainable solutions for the citrus industry in India and beyond using these biotechnological methods.

Keywords: Citrus breeding, Stresses, Biotechnology



## Citrus species diversity – Resource of varied nutrients and essential oils

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India's diverse landscapes and climates harbour a rich array of Citrus genetic resources, making it one of centers of origin. The wide variability for important nutrients in fruit juice and aroma constituents of fruit peels can enable species delineation and promote utilization of resources. In this study, a total of 38 samples belonging to 19 species, 6 hybrids representing diverse agro-ecologies were evaluated following standard methods for ascorbic acid content, antioxidant activity, total titrable acidity (TTA), total soluble solids (TSS), TSS/TTA ratio and peel essential oil yield (% v/w), the mean values were 97.77 µg/ml, 117.67 µg gallic acid equivalent (GAE)/ml, 3.71%, 7.81°Brix, 7.81 and 0.80% respectively. The significant positive correlation was observed between ascorbic acid, antioxidant activity, and total soluble solids while total titrable acidity and total soluble solids were negatively corelated. Essential oils extracted from fruit peels by hydro-distillation were profiled by gas chromatography (GC-FID). A total of 137 essential oil components were identified and validated using reference standards and Kovat Retention Index, across the studied accessions. Principal Component Analysis (PCA) of essential oil components revealed first 17 components accounted for nearly 85% of the variance. Hierarchical Cluster Analysis (HCA) using ward linkage generated three clusters and three major chemotypes - limonene, 1,8cineole and Z-β-ocimene rich were identified. The artemisia ketone was found associated with Citrus *jambhiri* collected from north-eastern India. while  $\delta$ -cadinene and  $\alpha$ -thujonone could effectively distinguish intra-specific variation in Citrus limon. The variation in the essential oil profile of two hybrids was observed in comparison to their respective parents. The combination of volatile profiling and chemometrics can be a valuable tool for identification and characterization of the cultivars at interspecific and intra-specific levels.

**Keywords:** Antioxidant activity, Chemotypes, Essential oil Profile, Gas Chromatography, Kovat Retention Index, Titrable acidity, Total Soluble Solids, Vitamin C



## Genomic insights into mandarin varieties: A comprehensive study of re-sequenced Indian accessions and global population analysis

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Mandarin oranges, known for their vibrant color, sweet flavor, and easy-to-peel nature, hold a significant place in the world of citrus fruits. India boasts a rich history of citrus cultivation, with mandarins occupying a prominent position among citrus fruit varieties. In this study embarks on a genomic exploration of four distinct mandarin varieties, namely Nagpur Mandarin, Sikkim Mandarin, Khasi Mandarin, and Coorg Mandarin, employing Next-Generation Sequencing (NGS) technology. With sequencing depths exceeding 28x, we conducted a comprehensive analysis of the entire genome, capitalizing on the chromosome level assembly of Citrus sinensis L. v3.0 (sweet orange) as a reference. A total of 13,63,8900 single nucleotide polymorphisms (SNPs) were identified. Augmenting our dataset, we integrated genome resequencing data from 109 publicly available mandarin accessions, merging them with the four Indian mandarin genomes. This fusion enabled us to undertake a multifaceted investigation into population dynamics, demographic shifts, inbreeding patterns, introgression events, and genetic burdens within the mandarin population. Furthermore, through phylogenetic analysis involving 113 mandarin accessions of diverse origins (includes wild, cultivated and mandarin hybrids) we unveiled the intricate evolutionary journey of mandarins. The genomic sequences of the resequenced mandarin accessions now stand as invaluable resources, poised to drive forthcoming studies in functional genomics and molecular breeding, with the ultimate aim of enhancing mandarin cultivars and fortifying the future of this beloved citrus fruit.

Keywords: Mandarin oranges, Citrus cultivation, NGS technology, Mandarin plant genome



# Assessment of phenology, productivity and insect pests and diseases in citrus grown under climatic conditions of Rayalaseema region of Andhra Pradesh

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To study the trend of recent variation in the climate under Rayalaseema conditions of Andhra Pradesh, the decennial average maximum and minimum temperature (°C), rain fall (mm) and relative humidity were computed for different months for the period 2007-16 and were compared against the respective average monthly maximum and minimum temperatures over three years. A noticeable change has occurred in the average maximum temperature during April, and may months as compared to the decennial average maximum temperatures. When compared to average maximum decennial temperature the current year maximum temperature was reduced to 0.78°C and minimum temperature dropped to 0.69°C. The upper temperature limit during April is critical for fruit set and during may and June for fruit development of ambe bahar crop. The decennial average annual rainfall is 1049.98 mm distributed in 55 rainy days. Total rainfall of 1039.06 mm (61 rainy days) was recorded during 2022. Highest rain fall of 160.26 mm was recorded during August 2022 (160.26 mm) with 9 rainy days. However, the observation of past years showed that maximum rain fall was recorded during November months (1343.4 mm) followed by September months (1261.6 mm). Due to receipt of higher rainfall during November prolonged to December affected the generative growth phase and also due to continuous and heavy rains during harvesting period (Aug-October) the fruit quality was affected and premature fruit drop also observed. The average minimum and maximum relative humidity levels were ranged in between 33.84 to 87.44 %. However during October to January average relative humidity levels ranged in between 60 to 87.4 % and where citrus greening disease incidence levels were high and ranged from 65 to 84.44 %. Month wise pest and disease incidence calender prepared based on the occurrence and degree of infection.

Keywords: Citrus, Climate, Phenology, Relative humidity, Temperature



#### Host genotype plays crucial role in citrus seed microbiome

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North eastern region of India known to be the "Origin of Citrus" and it is important to study wild seed microbiome. We collected wild seeds belongs to seven different species of citrus, during May-June in 2022 from Nokrek Biosphere Reserve, Garo Hills, Meghalaya, India. Seeds of current cultivars of citrus i.e., C. sinensis, C.maxima, C. limon and C. paradisi were also collected from farmer fields in South India. Metagenome was isolated from seed and sequenced using Miseg Illumina platform. Sequencing analysis observed that wild and cultivated exhibits diverse microbiome assemblages and clear differences were identified. Alpha diversity of wild is higher than cultivated (Shannon 1) and T test and pair wise comparison results are p-value: 3.6068e-18; [ANOVA] F-value: 121.18. Beta diversity at genus level calculated and PCO has been calculated and the PCO1=25% and PCO 2= 65% which shows highest variation among genotypes. Pairwise comparisons using PERMANOVA shows highest difference obtained between wild vs. cultivated (F value = 14.26, R squares = 0.262 at p<0.001). Diverse assemblages have been observed with wild and cultivated but core microbiome has been identified. Machine learning algorithm has been employed for Biomarker discovery for both wild and cultivated has been calculated using Linear Discriminant Analysis Effect Size (LEfSe) by adjusting False Discovery rate (FDR). Pearson correlation has been calculated at taxonomy levels with sparCC permutation (100), at Pvalue threshold = 0.05 and correlation threshold = 0.3. The present study provides the deeper insights in citrus seed microbiome composition and differences in wild vs. cultivated.

Keywords: Origin of citrus, Seed Microbiome



#### **Citrus breeding in Corsica**

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CIRAD and INRAE institute develop a Citrus breeding program in Corsica (France). The objective is to create and select excellent varieties of mandarin, blood mandarin, Lemon/lime, grapefruit like, and new kind of fruits. Fruits should have a nice color, good flavor and perfume, good TSS/TA, long shelf life and should be easy peeler, juicy. To produce seedless varieties, we focused on triploid hybrid selection and self-incompatible varieties. Marker-assisted selection increases significantly the efficiency of our breeding program for colored varieties or/and disease tolerant character. Several thousand hybrids are on the field in France, but also in foreign country. Selections of new varieties are in process and some are under evaluation in participatory selection.

Keywords: Citrus breeding, mandarin, shelf life, hybrids



#### Seedlessness imparted in Nagpur mandarin through mutation breeding

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Mutation breeding is an essential approach in the advancement of seedless mandarin oranges. The characteristic of seedlessness is often regarded as advantageous in mandarins due to its ability to increase consumers' acceptance as fresh fruits. The fruits of Nagpur mandarin (C. reticulata Blanco) contain 8-10 numbers of seeds. Through mutation breeding techniques, genetic mutations can be induced in Nagpur mandarin that lead to the production of seedless varieties without altering other desirable characteristics like flavor and texture. Given this background, dormant buds of Nagpur mandarin were treated with different radiation doses of gamma - irradiation (20, 40, 60, 80 and 120 Gy) using Cobalt – 60 source at Bhabha Atomic Research Centre (BARC), Mumbai to evolve seedless variety. The irradiated bud scions were grafted onto Rough lemon (C. jambhiri) rootstock using the side-graft technique and planted at ICAR-Central Citrus Research Institute, Nagpur during July-August 2015. Evaluation of different mutant lines resulted in one commercial seedless mutant (N-74/10Gy) and the MV2 generations were raised from seedless mutants during 2019. The physico-chemical properties of commercially seedless mutant and the control revealed that maximum weight, length, diameter, axis diameter and rind thickness of fruits (145.90 g, 60.87 mm, 67.36 mm, 13.68 mm, 2.41 mm) were recorded with control as compared to N-74/10Gy where the values were 79.00 g, 49.12 mm, 56.55 mm, 9.38 mm and 1.93 mm, respectively. However, the N-74 fruits received 10Gy irradiations were found to be seedless (1 seed/fruit). No variation was observed in terms of no. of segments per fruit in 10Gy irradiated (10.00) and control (10.50) fruits. Qualitatively, fruits harvested from control block exhibited little higher juice content (46.06%) and TSS (7.95°B) than N-74/10Gy having 43.03% juice content and 7.20°B TSS. However, N-74/10Gy fruits had less acidity (0.51%) as compared to 0.97% in control. Therefore, N-74/10Gy has been identified as promising mutant line which needs to be further carried forward to towards development of a seedless variety of Nagpur mandarin for a satisfying consumer demands and enjoyable fruit experience.

Keywords: Nagpur mandarin, Mutation, Seedlessness, Gamma - irradiation



Advancing Citriculture for Agro-economic Prosperity

#### **Poster Presentation**

#### TS-1-P-01

#### Hybridization studies in mandarin

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The study on floral biology and crossing efficiency of various mandarin varieties was conducted at the Center of Excellence for Citrus, Nagpur, and Regional Fruit Research Station, Katol from December 2021 to November 2022. Promising varieties for flowering were Minneola (60-65 days) and Beauty of Glen Retrate (55-60 days). Maximum anthesis observed from 8 am to 12 pm in all the varieties during which maximum anther dehiscence occurred between 10 am to 12 pm. Stigma receptivity was observed for 3-5 days with maximum receptivity on the day of anthesis. Both Nagpur mandarin and Beauty of Glen Retrate showed maximum stigma receptivity (4-5 days). In Nagpur mandarin, maximum pedicel length (0.47 cm) was observed whereas minimum pedicel length (0.42 cm) was observed in Minneola. In case of number of stamens per flower variation was observed among the different varieties from 11-16 stamens per flower, Nagpur seedless had maximum stamens (14.97) whereas Minneola had minimum stamens (13.27) per flower. Minneola variety showed maximum staminate flower (1.56%) and minimum perfect flower (98.44%) whereas Nagpur seedless showed only (0.31%) staminate flower and maximum perfect flower (99.69%). Pollen germination was found superior in Nagpur mandarin (64.83%). Beauty of Glen Retrate showed maximum pollen fertility (89.04%) and also had maximum pollen size (31.68 µm). In crossing efficiency study, ten crosses found with successful fruit set. Maximum fruit set (22.22%) was observed in case of Nagpur seedless as female parent and Beauty of Glen Retrate as male parent. In all, 20 crosses were attempted, out of which 10 crosses set the seeds. The Nagpur mandarin and Nagpur seedless were good female parents, while Nagpur seedless and Beauty of Glen Retrate were good male parents in direct crosses. In reciprocal crosses, Beauty of Glen Retrate and Nagpur seedless were good female parents, and Nagpur mandarin and Minneola were good male parents. These varieties are suitable for further mandarin hybrid development.

Keywords: Floral biology, Mandarin varieties, Crossing effciency



#### Survey, collection and evaluation of elite types of Nagpur mandarin

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From September to December 2020, an experiment focused on Ambia bahar fruits. Five talukas in Nagpur district—Kalameshwar, Saoner, Parseoni, Bhiwapur, and Ramtek—were chosen for surveying superior Nagpur Mandarin types. In each taluka, farmers were selected based on specific criteria such as tree size, fruit weight, high yield potential, uniform deep orange color, tight skin, and good fruit storage. Among the 75 genotypes, NM-19 excelled in various fruit characteristics. NM-59 displayed favorable traits in fruit weight, seed count, cavity diameter, shelf life, and juice content. NM-8 stood out for fruit weight, length, breadth, segments per fruit, and TSS. NM-4 exhibited positive attributes in fruit weight, breadth, segments per fruit, and shelf life. NM-11 excelled in length, acidity, juice percentage, and TSS:acidity ratio. NM-14 performed well in weight, breadth, and shelf life. NM-20 showed good traits in length, segments per fruit, TSS:acidity ratio, and color. Lastly, NM-60 displayed superiority in weight, length, breadth, and segments per fruit. All these genotypes were found significantly superior over Nagpur mandarin (check). Nagpur mandarin genotypes can be categorized based on fruit color: NM-7, NM-17, NM-20, NM-46, and NM-49 have orange fruit; NM-1, NM-3, NM-4, NM-6, NM-8, NM-10, NM-12, NM-13, NM-15, NM-32, NM-33, NM-34, NM-59, NM-60, NM-65, and NM-71 have yellowish-green fruit; NM-2, NM-9, NM-11, NM-25, NM-42, and NM-62 display yellowish-orange fruit; NM-5, NM-14, NM-16, NM-18, NM-19, NM-24, NM-26, NM-27, NM-30, NM-31, NM-37, NM-40, NM-48, NM-50, NM-51, NM-53, NM-54, NM-63, NM-64, NM-66, NM-67, NM-68, NM-69 have greenish-yellow fruit; NM-28, NM-36, NM-41, NM-44, NM-47, NM-52, NM-56, NM-57, NM-58, NM-70, NM-72, NM-73, and NM-74 bear pale green fruit; and NM-21, NM-22, NM-23, NM-29, NM-35, NM-38, NM-39, NM-43, NM-45, NM-55, and NM-61 have green fruit. All observed fruits exhibited a compact nature. In terms of essential traits like fruit weight and number of segments per fruit, genotypes NM-19, NM-59, NM-8, NM-4, NM-14, and NM-60 stood out as superior. Notably, NM-20 displayed a deep orange rind color

Keywords: Nagpur mandarin, Mandarin genotypes



#### Cryopreservation for conservation and preservation of citrus germplasms

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To safeguard the genetic diversity and ensure the availability of citrus germplasm for future generations, cryopreservation offers an effective method to store and conserve citrus germplasm. In the context of citrus conservation, cryopreservation can be applied to preserve important genetic resources, such as seeds, embryos, shoot tips, and pollen, for future use. Cryopreservation allows for the long-term storage of citrus germplasm, including seeds, which contain the genetic information of the plant. This ensures the preservation of diverse citrus varieties, including those that are rare, endangered, or important for breeding programs. Cryopreservation techniques can be used to freeze and store shoot tips of citrus plants, preserving their genetic material and allowing for the production of new plants in the future. Cryopreservation can also be applied to preserve citrus embryos at early developmental stages. This technique is useful for rescuing embryos from hybridization experiments, rare or slow-growing citrus varieties, or plants that are difficult to propagate. By cryopreserving the embryos, they can be stored until they are ready for germination and subsequent plant production. Frozen pollen can be stored for extended periods, ensuring its availability for controlled pollination, breeding programs, and the production of hybrid plants. Cryo-banking their genetic material helps safeguard their diversity, enabling future research, breeding programs, and potential reintroduction efforts.

Keywords: Cryopreservation, Ctriculture, Conservation, Embryo rescue, Pollen preservation



### Evaluation of acid lime (*Citrus aurantifolia* Swingle) cultivars under Bundelkhand conditions

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Citrus is one of the most important fruit crops of the world and grown in more than 100 countries. Global citrus production was 139.189 million metric tons and top citrus producing countries are China (35.47 million tons), Brazil (19.07 million tons), India (11.15 million tons) and USA (8.54 million tons) (FAO, 2017). Globally India ranks first in production of acid lime (Citrus aurantifolia Swingle) with 2.54 million metric ton from 0.282 million ha area and productivity of 10.07 metric tons per ha (NHB, 2017). Evaluation studies were carried out in acid lime for the selection of cultivars with a wide range of morphological characteristics under agro-ecological conditions in Banda, Uttar Pradesh. Nine acid lime cultivars, viz., NRCC-7, NRCC-8, Kagzi Lime, Ganganagar Lime-1, Kagzi Kalan, Sai Sarbati, PhuleSarbati, PusaUdit, and Bala Ji were planted for the study in the year 2019. During 2022-23 the tree growth parameters were recorded. The maximum plant height (365.00 cm), stem height (77.00 cm), canopy height(301.66 cm), trunk girth(11.66 cm), plant spread N-S(306.66 cm), plant spread E-W(316.66 cm), av. Spread(311.66 cm) and canopy volume(4.93 m<sup>3</sup>) were noted with NRCC 7Minimum plant height (204.33 cm), stem height (50.00 cm), canopy height & trunk girth (153.33 cm & 8.16 cm), Plant Spread (N-S) (215.66 cm), Plant spread (E-W) (203.33cm), av. spread (210.33 cm) and canopy volume (1.69 cm) were recorded with PhuleSharbati. Maximum canopy volume was recorded with NRCC 7, followed by Kagzi Lime and lowest with Bala Ji. Plants are only 4 years old fruiting started with Ganganagar Lime 1 and NRCC 7. Ganganagar Lime started 2 years onwards.

Keywords: Acid lime (*Citrus aurantifolia* Swingle), Cultivars, Varietal evaluation.



### Unleashing the potential of pummelo: Survey, selection and propagation of elite genotypes

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The study was conducted during the year 2021-22. The survey was done in Devanahalli and Bengaluru rural areas of Karnataka, where 40 elite pummelo genotypes were identified and analysed for their physico-chemical composition. The canopy spread was maximum in DBS-1 in North-South (10.60m) and DNR-1 in East-West (11.40 m). The genotype DBS-1 recorded maximum number of fruits (480) and yield (451 kg) per tree. Most of the genotypes were having spheroid fruit shape (20), truncate type of fruit base (20), truncate type of fruit apex (16) and smooth skin surface (24). The maximum fruit length (18.87 cm) and width (17.38 cm) were observed in HSR-1. The maximum fruit weight and volume were observed in DNM-1 (1746.24g and 3528.34mL respectively). The pulp weight recorded maximum in DSR-2 (1069.67 g), peel weight in CHES-1 (1109.21 g) and number of segments in DSR-2 (17.00). The seeds were having varied shapes, the majority of them (29) have a cream colour and smooth surface (26). The maximum ascorbic acid content was recorded in DSP-8 (60.00 mg/100g), Titratable acidity in genotype RHREC-7 (2.07%) and maximum TSS in DSP-17 (11.07°B) while DAK-1 (7.64%) recorded the maximum total sugar, the maximum amount of reducing sugar was recorded in RHREC-1 (3.93%) and non-reducing sugar in HSR-2 (5.60%). Based on physico-chemical attributes, top ten elite genotypes were identified and grafted (softwood) on six-month-old rootstock. Graft success and survivability percentage were found maximum (96.67% and 100.00%, respectively) in DSP-8 genotype and significant variation was observed among genotypes for growth parameters like number of leaves, number of sprouts, graft girth and height. Overall, this study contributes to the identification of elite pummelo providing valuable insights for the improvement of the crop in this region.

Keywords: Citrus grandis L, Genotype, Physico-chemical characters, Propagation, Pummelo, Survey



### Correlation and hierarchical cluster analysis of pummelo genotypes based on biochemical traits

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The present study was carried out during 2019 to 2021 at Punjab Agricultural University, Ludhiana. The results showed that TSS exhibited a significant positive association with maturity index (r = 0.868) and total sugars (r=0.827) whereas, a significant negative correlation was observed with titratable acidity (r=-0.789). Furthermore, titratable acidity exhibited a significant negative correlation with maturity index (r = -0.965), total sugars (r = -0.829), pH (r = -0.809), sucrose (r = -0.805), total fructose (r = -0.744), reducing sugars (r = -0.725) and glucose (r = -0.621). However, pH showed a significant positive correlation with maturity index (r = 0.765) and total sugars (r=0.762). The maturity index revealed a significant positive correlation between total sugars (r = 0.859) and sucrose (r = 0.838). Total sugars showed a significant positive correlation with total fructose (r = 0.884), sucrose (r = 0.783), reducing sugars (r = 0.775) and glucose (r =0.706). The correlation analysis of the present study showed that sucrose significantly contributed to the accumulation of total sugars in pummelo fruits, followed by total fructose and glucose. Hierarchical Cluster Analysis of pummelo genotypes based on titratable acidity stood distinct from all the other analyzed biochemical constituents related to its sugar profile and maturity. With regard to genotypic classification, two main clusters M and N were observed. Cluster 'M' represented four pummelo genotypes namely NRCC Pummelo-3, PTF-2, PTF-4 and Local. Cluster 'N' comprised six pummelo genotypes: NRCC Pummelo-1, NRCC Pummelo-2, NRCC Pummelo-4, NRCC Pummelo-5, PTF-1, and PTF-3. Within cluster 'M', NRCC Pummelo-3 stood out distinctly and grouped with PTF-2 and PTF-4 based on morphological clustering. However, NRCC Pummelo-1 and NRCC Pummelo-4 clustered with the other genotypes. The smaller fruit size of NRCC Pummelo-3 likely concentrated sugars in a smaller area. PTF-4, with optimal fruit size, showed high sugar content, making it ideal for fresh consumption.

Keywords: Correlation, Biochemical constituents, Pummelo, morphological parameters



#### Characterization of promising citrus rootstocks.

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Investigation was carried out at BUA&T, Banda, U.P.with available sixteen citrus rootstocks viz.Rough lemon (C.jambhiri Lush.), Rangpur Lime (C.limonia Osbeck.), Carrizo citrange [C.sinensis (L.) Osbeck. × Ptrifoliata (L.) Raf.], Sour orange (C.aurantium L.), Cleopatra mandarin (C. reshine T.), Karna khatta (C. karna), Troyer citrange (C. sinensis × P. trifoliata), Trifoliate orange (P. trifoliata L.), Calamondin (Citrus mudurensis Lour.), Pomrey (P. trifoliata var. pomrey), Hill lemon (C. pseudolemon Tan.), Billikichali (C. mederaseantana Tan.), Meethikhatti (C. jambhiri var. Mitthi), Pumelo (C. maxima), Tawainica (Citrus × taiwanica Tanaka) and Yama mega were selected for the study. These exhibited variations for different physico-chemical fruit and seed attributes. Qualitative variations were recorded for fruit shape, fruit base, fruit apex, skin (epicarp) colour, surface texture, adherence of albedo to the pulp, albedo colour, cross-section shape of axis, pulp colour, juice colour, seed shape, seed surface, seed colour and cotyledons colour. Variations were also noted for quantitative traits like fruit weight (9.53 - 510.93 g), width (29.75 - 112.52 mm), length (25.16 - 113.92 mm), circumference (92.45 - 338.40 mm), the width of epicarp (0.93 - 15.62 mm), number of segments per fruit (8 - 14), the diameter of fruit axis(3.30 - 16.27) mm), fruit volume (10 - 570.64 cc), the specific gravity of fruit (0.69 - 0.98 g/cc), TSS (6 - 10 <sup>o</sup>Brix), juice percentage (28.30 - 58.00 %), no. of seeds per segment (1 - 4), no. of seeds per fruit (5 - 38), seed length (6.84 - 21.05 mm), seed width (3.26 - 10.52 mm) and seed weight of hundred seeds (5 - 70 g). The study revealed that a significant amount of genetic variability exists among the available rootstocks. Interrelationship of characters can be utilized for commercial multiplication and improvement of citrus plants.

Keywords: Citrus spp., Rootstock, Seed, Morphological characterization, Genetic variability



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#### TS-1-P-08

# *In vitro* micro-shoot tip grafting of Khasi mandarin (*Citrus reticulata* Blanco) for production of disease-free quality planting materials using three indigenous rootstocks of North East India

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Khasi mandarin (Citrus reticulata Blanco) is the one of the most commercially important Citrus crops in north eastern region of India. The production of the crop is plagued by various biotic and abiotic factors of which Citrus Tristeza Virus (CTV) is considered one such biotic factors contributing to overall Citrus decline in the region. The present study is conducted to explore the applicability perspective of three indigenous citrus species viz. Rough Lemon (Citrus jambhiri Lush), Melanesian papeda (Citrus macroptera Montr.), and Pummelo (Citrus maxima Merr.) as rootstocks for Khasi Mandarin using in vitro micro-shoot tip grafting for production of CTV free quality planting materials. The STG procedure for optimization of various culture parameters such as effect of sucrose, age of rootstock, pretreatment of scion, scion size on virus elimination and grafting method were analyzed. The pretreatment of scions with 5 µM Kinetin gave overall highest graft success in all the rootstocks.14 days old Rough lemon seedlings and 12 days old seedlings in both Melanesian papeda and Pummelo were recorded to be ideal age of seedlings for in vitro micro-shoot tip grafting. Liquid MS medium fortified with 6% sucrose was found to be suitable medium for culture and growth of micrografts with a success rate of 62.10 % for Rough lemon, 44.29 % for Melanesian papeda and 40.21 % for Pummelo. Cleft grafting method was found to be more suitable over Inverted-T grafting. RT-PCR results using coat protein gene specific primers for CTV confirms the complete elimination of virus with 0.2-0.5 mm size scions. With regard to all the above factors pertaining to graft success, Rough lemon was observed to best compatible rootstock while Melanesian papeda and pummelo can also be considered as potential rootstocks for further studies.

**Keywords:** Citrus Tristeza Virus, Indigenous rootstocks, Khasi Mandarin, Murashige and Skoog medium, Plant Growth Regulators, RT-PCR, Shoot Tip grafting



### Morphological and biochemical traits for distinguishing ecotypes of Khasi mandarin

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Khasi Mandarin (Citrus reticulata), is a prized citrus species of the North Eastern Himalayan region including Assam. It is propagated commonly by seeds and exhibits tremendous variation in morphobiochemical traits which are used as indicators to identify different ecotypes of Khasi mandarin. A sample size of thirty five plants was studied from seven major Khasi mandarin growing districts of Assam with five plants selected from each district. Significant variability was observed among the selected accessions for gualitative and guantitative characters of tree, leaf, flower, fruit and seed. Maximum fruit weight (145.29g) was recorded in AKM03T2 and minimum (110.80g) was in AKM01T4. The highest TSS was recorded in AKM07T2 (11.9 °Brix) and lowest in AKM06T2 (6.22 °Brix). Dendrogram based cluster analysis of the 35 accessions considering 33 polymorphic qualitative traits and 4 traits of disease (powdery mildew, citrus canker) and pest (trunk borer and leaf minor) occurrence resulted in 6 groups and 6 subgroups. The highest coefficient of dissimilarity (11.08) was observed between accessions AKM01T5 and AKM02T3 from Jorhat and Tinsukia district with eleven characteristics features. The lowest coefficient of dissimilarity (6.00) was recorded between accessions AKM03T3 and AKM05T4 which were from the Golaghat and Dima Hasao with twelve characteristics features signifying close relatedness. No groups were formed based on geographical location indicating that gualitative traits were largely influenced by genetic factor. The principal component analysis revealed 33 components, with 4 capturing 91.7% of variation in quantitative traits, and the other 16 accounting for 90.2%. Among 66 characters, 30 minimum descriptors were identified, simplifying the evaluation and characterization of Assam Khasi Mandarin germplasm and its genetic diversity.

Keywords: Khasi Mandarin, Variability, Ecotypes, Germplasm, Genetic diversity



#### **Ovule degeneration studies in mandarin varieties**

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The complex reproductive biology involving self-incompatibility and characteristic partial or discrepant parthenocarpy leads to production of seedless fruits in mandarin. Parthenocarpy in mandarin is governed by two dominant complementary genes with the concentration of phytohormone (GA<sub>3</sub>) in unfertilized ovary (Parthenocarpy ability) as one of the prospective traits among the Mandarin genotypes. The present study aimed to investigate the extent of ovule degeneration at different developmental stages viz. 4, 30 and 90 days after pollination among six mandarin varieties (Kinnow, PAU Kinnow-1, Nagpur, Nagpur seedless, Kishu and Owari) during the year 2022. The transverse sections revealed the variation in the carpel polymorphism which was recorded number of segments and ovule numbers per ovary. Post 4 days of pollination, all the six genotypes including the seedy and seedless cultivars exhibited nearly similar number of segments and ovule numbers per segment. However, after 30 days of pollination Ovule degeneration was observed in three seedless cultivars (PAU Kinnow-1, Kishu and Nagpur seedless) manifested as reduced shrunken size and number of ovules/ seeds per segment. While viable seeds occurred in Kinnow and Nagpur orange cultivars. The other variation was recorded in the dimensions of the central core region which diminished in PAU Kinnow-1 while Owari exhibited biggest central core though the size of the ovules was reduced in Owari. Post 90 days of pollination no viable seeds could be obtained from Kishu cultivar. While 1-2 seeds, 2 to 3 and 3 to 4 seeds were observed in Owari, Nagpur seedless and PAU Kinnow-1 cultivars. The seeded cultivars exhibited the presence of fully mature and viable 15-18 and 20 to 23 seeds in Nagpur and Kinnow genotypes. This study though identifies the variation among genotypes for the extent of degeneration of ovule/ seed post pollination, the underlying mechanism(s) are required to be elucidated which will be useful for understanding the type and extent of parthenocarpy in Mandarin.

Keywords: Optical microscopy, Ovule degeneracy, Parthenocarpy, Seedlessness, Transverse sections



#### Evaluation of elite genotypes of Nagpur mandarin in Vidarbha region.

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Nagpur mandarin is one of the best mandarins in the world. Production of this fruit crop in central and western part of India is increasing every year. High yielding variety act as catalyst for more yield therefore scope of increasing production and productivity by adopting better varieties. A study on varietal selection and evaluation has been underway at Nagpur and Amravati district of Vidarbha region during the year 2022-2023. Ten Nagpur mandarin genotypes (i.e., NM-20, NM-19 NM-14, NM-04, NM-02, NM-45,NM-22, NM-03,NM-01and Nagpur mandarin)of local origin have been evaluated in the study. This investigation describes the growth and fruit bio-chemical characteristics of Nagpur mandarin genotypes observed during 2022-23. The genotypes showed the highest plant growth for height, canopy spread and plant volume were observed in NM-02 (6.25 m), NM-14(34.21 m<sup>2</sup>) and NM-14 (104.92 m<sup>3</sup>) respectively. The average fruit number per plant (1150 fruits plant<sup>-1</sup>) and fruit yield (193.02 kg plant<sup>-1</sup>) were observed inNM-01. while the highest fruit weight (219.02 g), equatorial length of fruit (80.20 mm), polar length of fruit (70.40 mm), fruit firmness 4.38 (kg cm<sup>-1</sup>), fruit volume (221.60 cm<sup>3</sup>), acidity (0.77 %) and ascorbic acid (31.20 mg 100g<sup>-1</sup>) were observed in NM-20. The highest number of segments (12), juice content (55.86%), TSS (9.92) and TSS:acidity (16.97)was observed in NM-02 genotype. The highest epicarp width (4.64 mm) and number of seeds (11.40) were found in NM-02 genotype. Therefore, NM-20, NM-19, NM-02 and NM-01 genotypes seem to have good characteristics in all respects for further improvement and selection in the future.

Keywords: Evaluation, Genotypes, Nagpur mandarin, Fruit bio-chemical characteristics



### Citrus germplasm exploration and morpho-phenological reveal presence of unique and underexplored citrus sps in the state of Manipur

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Germplasm exploration of citrus resources in Manipur come to light occurrence of diverse citrus resources in different locations. A total of more than 150 citrus accessions belonging to nine different citrus species has been collected and morphologically characterized following IPGRI protocol. The survey come to light the existence of wide diverse citrus resources in the state of Manipur. Despite wide occurrence of citrus forms, only certain citrus species having good economic return and palatable ones observed cultivated. While many a citrus forms having low economic values and poor consumer preference is observed in a neglected state and has higher chance of lost from nature. Exploratory survey and morphological studies revealed presence of unique citrus types possibly of hybrid origin from sweet orange, citron, or pumelo, and existence of C. indica, Papeda sps in the hilly and interiors of the state in an underutilized state. Analysis of 50 morphological characters in terms of seed, leaf, fruit revealed wide variation among the citrus accessions. Diverse characters such as seed shape (7 types), leaf lamina (3 types), fruit apex (5 types), fruit base (4 types), fruit shape (4 types). has been recorded from the morphological studies. The survey also come to light existence of primitive sps Citrus indica in 10 locations of six district and presence of Papeda sps in 13 locations of six districts. Fragmentation of habitats, poor preference and low marketability due to bitter and acidic taste further lead to erosion of once bounty citrus resources. Surveys for collection and proper documentation of their unique characters will lead to know the extent of citrus spp present in this natural home garden and status of the existing diversity. Large number of variant forms originates due to complex behavior of cross-pollination and high compatibility among the citrus forms.

Keywords: Citrus, Species, Manipur, Survey, Bitter and acidic taste



### Identification and diversity analysis of interspecific citrus rootstock hybrids with combination of morphological traits and microsatellite markers

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Rough lemon is indigenous and has wide adaptability in Indian citriculture. Rough lemon and Volkamer lemon are comparable in horticultural performance and their resistance against biotic and abiotic stresses are almost similar but Volkamer lemon has better adaptability in different agroclimatic conditions. To improve Volkamer lemon hybridization has been attempted with Kinkoji (for citrus greening tolerance), Swingle citrumelo (Phytophthora resistance), and Cleopatra (salinity tolerance). Polyembryony in Volkamer lemon and absence of the heritable morphological markers (Cleopatra) are limiting factors for hybrid identification and morphological characters are not being segregated in Kinkoji and Swingle citrumelo at early stage, then molecular markers are employed for detection of putative zygotic seedlings. We performed hybridization between Volkamer lemon ( $\mathcal{Q}$ ), Kinkoji (58 F<sub>1</sub>) ( $\mathcal{E}$ ), Swingle citrumelo (71 F<sub>1</sub>) ( $\mathcal{J}$ ), Cleopatra (83 F<sub>1</sub>) ( $\mathcal{J}$ ) and discriminate the hybrids by using 25 SSR markers. The fifteen markers were found efficient for hybrid identification (> 71% hybrids detected). CCSME23 (88.21 %) marker has detected 187 hybrids in all crosses thereafter, 184 hybrids detected each by HvSSRCS-2, HvSSRCS-135, HvSSRCS-237, HvSSRCM-92 and HvSSRCM-466 with 86.79 %. A total of 44 qualitative and 18 quantitative traits were analyzed, revealing significant variability in 15 qualitative and six quantitative characters related to leaf, spine, branch, and stem. Phylogenetic and population structure analyses detected ample genetic variability, showcasing two subpopulations in the B (Volkamer lemon × Kinkoji) and N (Volkamer lemon × Cleopatra) crosses, and five subpopulations in the D (Volkamer lemon × Swingle citrumelo) cross. The practical application of microsatellite markers allows early discrimination of zygotic F1 seedlings in citrus, saving resources, time, and space

Keywords: Microsatellite, Polyembryony, Hybridization, Zygotic seedling, Variability, Swingle citrumelo



Advancing Citriculture for Agro-economic Prosperity

#### TS-1-P-14

### Molecular and biochemical analysis of Indian mandarin varieties: Exploring diversity and quality traits

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A study conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, in 2020-21, focused on molecular and biochemical characterization of diverse Indian mandarin types, including Coorg, Darjeeling, Kinnow, Nagpur, and Khasi mandarins. The study aimed to understand variability within mandarins and specifically within Khasi mandarins across Indian states like Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Sikkim. Genetic diversity was assessed using ten simple sequence repeat markers, revealing high polymorphism and allelic diversity. Analysis, including dendrogram and population structure examination, revealed clusters based on mandarin types and their geographic origins. Biochemical parameters, encompassing total soluble solids, acidity, vitamin C, total carotenoids, sugars, organic acids, and vitamins, were evaluated. Profiling of sugars, organic acids, water-soluble vitamins, and fat-soluble vitamins exhibited notable distinctions among mandarin types, highlighting substantial genetic diversity. Khasi mandarins grown in Meghalaya showcased superior attributes, including higher total soluble solids, sugars, and organic acids, signifying their favourable fruit quality. Additionally, molecular characterization confirmed distinct genetic diversity. These findings emphasize the genetic distinctiveness and biochemical variations among mandarin types, encompassing differences within Khasi mandarins. Such insights hold potential for selecting parent plants in upcoming breeding programmes.

Keywords: Genetic diversity, Mandarins, Molecular characterization, Biochemical characters



### Morpho-physiochemical studies of Khasi mandarin (*Citrus reticulata* blanco) genotypes in Tinsukia district of Assam, India

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A variability study conducted in 2021-2022 in Tinsukia district, Assam, aimed to identify superior genotypes based on morphological and physiochemical parameters. Seven Khasi Mandarin accessions were analyzed for fifteen morphological characters. The study revealed significant variability in the studied Khasi Mandarin genotypes in Tinsukia district, Assam. The maximum fruit diameter (64.20 mm) was observed in CRS/KS/22/52 (IC. No.: 0647197) which was at par with MD-11/78 (IC No.: 591437), CRS/KS/22/50 (IC. No.: 0647196), CRS/KS/22/55 (IC No.: 0647513), CRS/KS/22/53 (IC No.: 0647198) and CRS/KS/22/54 (IC No.: 0647512) while minimum fruit diameter (60.19 mm) was recorded in CRS/KS/22/56 (60.19 mm). The maximum fruit length (mm) was observed in CRS/KS/22/52 (IC No.0647514) while the minimum was in genotype CRS/KS/22/54 (49.60). The maximum fruit rind thickness (mm) was recorded in CRS/KS/22/52 (3.90) whereas, minimum was in genotype CRS/KS/22/56 (2.66). The highest total soluble sugar content (%) was found in genotype CRS/KS/22/52 (10.69) and the lowest content was recorded in genotype CRS/KS/22/53 (10.33). Maximum acidity content (%) was observed in CRS/KS/22/54 (0.48) and it was statistically at par with CRS/KS/22/56 (0.47), CRS/KS/22/55 (0.43), MD-11/78 (0.42), CRS/KS/22/53 (0.42) and CRS/KS/22/50 (0.41) while, minimum acidity per cent was observed in CRS/KS/22/52 (0.39%). Maximum juice content (ml) was recorded in CRS/KS/22/52 (108.60 ml) whereas, minimum juice content was observed in genotype CRS/KS/22/54 (96.80 ml). Genotype CRS/KS/22/52 (136.20 g) had the maximum fruit weight and CRS/KS/22/54 (119.60 g) had recorded the minimum weight of the fruit. The study indicates that genotype CRS/KS/22/52 surpasses other Khasi Mandarin types, making it a promising candidate for further research in crop improvement and horticulture technology development

Keywords: Accessions, Genotypes, Khasi mandarin, Morphological, Physiochemical

#### Study on genetic diversity of citron (*Citrus medica*) group of citrus in North Eastern



#### region of India

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*Citrus medica* also known as Citron belongs to the Rutaceae family, one of the important medicinal Citrus fruits of the world. It is native to India and is found to grow in a wild and semi-wild along with the foothills of the Eastern Himalayas and North-East India. In the present investigation using 12 accessions of Citron germplasm (IC No.; 591406, 591407, 591410, 591421, 591422, 591424, 591425, 591433, 591442, 591444, and 591406) were collected from different parts of the North Eastern region. The collected germplasm was established at Field Gene Bank block at AAU-Citrus and Plantation Crops Research Station, Tinsukia, Assam based on variability of genetic parameters viz., plant height (cm), leaf lamina length (cm), leaf lamina width (cm), fruit length (cm), fruit diameter (cm), fruit weight (g), fruit rind thickness (mm), number of segment/fruits, average number of seed/fruits, juice content (ml). The present results revealed that some of the accession of genetic parameters viz., plant height, fruit weight (g), average number of seed/fruits, and juice content (ml) positively show a high value of the phenotypic coefficient of variation. This variability parameter, it reveals that variation in Citron genotypes. Based on the study, it is helpful for the selection of high fruit-yielding genotypes and selected genotypes can be used in further breeding programs and also be used for commercial purposes.

Keywords: Citron, Genotype, Germplasm, Morphological, Phenotypic



#### Genetic diversity of pummelo (Citrus grandis) germplasm in Assam, India

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The quality of pummelo [Citrus grandis (L.) Osbeck] in India is not homogeneous across the regions and morphological characteristics are very different and the genetic variability has not been fully reported. There is an urgent need to collect, characterize and conserve the existing vast genetic resources of Citrus for safe conservation and utilization in crop improvement programmes of existing genotypes and rootstocks based on both conventional and biotechnological methods. The genetic diversity in morphological and physico-chemical traits of ten genotypes of pummelo growing under the agroclimatic conditions of Assam, India, was studied during 2021-23. Significant variation was observed for physical parameters, viz. fruit weight ranged from 550 to 1100 g; fruit diameter 125 to 200 mm, rind thickness 12.1 to 22.0 mm, number of segments 10 to 18 and seed number 10 to 14. The shape of the fruits of all the collections is found to be spheroid except CRS/KS/21/43 which is obloid. Similarly, among the guality parameters, juice content varied from 18.45 to 20.87 %; ascorbic acid 38.16 to 42.62 mg/100 g; acidity 0.59 to 0.76 %, TSS 7.02 to 7.82 <sup>o</sup>Brix and sugar: acid ratio from 8.82 to 12.43. Collection CRS/KS/21/44 exhibited the maximum fruit weight (1100 g), CRS PUM 2 recorded maximum juice content (20.87 %) and CRS PUM 6 recorded highest TSS (7.82 <sup>o</sup>Brix). Wide range of variation in physical and quality parameters of pummelo collections indicated the potentiality of superior collections for commercial plantation and large scale consumption besides the scope of individual plant selection based on these characters for future genetic improvement programme.

Keywords: Genotypes, Genetic diversity, Pummelo



### Molecular diversity and marker assisted selection of elite genotypes of Khasi mandarin (*Citrus reticulata* blanco.) using SSR markers

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Khasi Mandarin is one of the most popular and commercially grown mandarin cultivar in Arunachal Pradesh including other states of North-Eastern India and is known for their unique aesthetic, organoleptic and nutritional characteristic in all over the world. The present experiment comprises thirty elite genotypes of 'Khasi Mandarin' which were collected from different districts of Arunachal Pradesh, North East India. Twenty-two SSR markers were employed for screening the genotypes to confirm the variability within the selected genotypes. UPGMA dendrogram clustering revealed 10 distinct groups of populations based on the distribution of genotypes between the different geographical regions. Analysis of molecular variance diversity resulted in moderate molecular variance (62%) within populations and low molecular variation (38%) among the populations. Four SSR loci with low PI have been identified as universal markers for 'Khasi Mandarin' characterization. The Polymorphic Information Content (PIC) values ranges from 0.22 to 0.34 in genotypes and found to be informative primers for estimating variability in citrus as they revealed 66.67 to 100 per cent polymorphism.

Keywords: Elite genotypes, Khasi mandarin, Molecular diversity, SSR markers, Variability



#### In vitro seed germination of different citrus rootstocks species in MS media

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An experiment was carried out at the Tissue culture laboratory, micro-propagation unit under RKVY, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal during the year 2021-2023. Eight different citrus species i.e., NRCC 1, NRCC 2 (Rough lemon x Troyer citrange), NRCC 3 (Rough lemon x Troyer citrange), NRCC 4 (Rough lemon x Trifoliate orange), Rough lemon (Citrus Jambhiri), Rangpur lime (Citrus limonica), Pummelo (Citrus grandis) and mandarin (Citrus reticulata) were used in MS basal media to observe the seed germination behavior and to standardize the rootstock seedling for in vitro shoot-tip grafting. Seeds were collected from healthy ripe fruits, decoated and surface sterilized with 1-2 drop of tween-20 for 15 minutes in a running tap water followed by 50 gram bavistin treatment for 1 hour and 50 milligram plantomycin for 10 minutes and sodium hypochlorite 100 mg for 2 minutes in 100 millilitre distilled water. After each treatments the seeds were thoroughly washed with distilled water for 2-3 times. From each rootstocks species 100 seeds were placed in MS basal media to observe the germination behavior. Highest seed germination percentage was observed in Rangpur lime (83.00 %) followed by NRCC 4 (81.00 %). The lowest seed germination behavior was observed in rough lemon (74.00%) followed by pummelo (75%). However the seed germination percentage was in the range of 75.00 to 83.00 % in all the citrus species used for rootstock purpose. Rapid seed germination was observed in NRCC 2 (9.37 days) followed by NRCC 3 (11.14 days) whereas very slow germination was observed in Pummelo followed by rough lemon.

Keywords: Germination, Media, Micropropagation, Rootstocks, Seed, Treatment



### Critical study of *in vitro* micrografting using Kachai lemon and Rangpur lime and improving its survival through double grafting

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Citrus industries today focus on the plantation of disease free planting material eliminating the unproductive juvenile phase. Plantation of grafted khasi mandarin using rootstocks having tolerance to endemic stress can give an impetus to fruitful production with increase orchard longetivity. To know the compatibility and factors affecting the micrografting, in vitro micrografting has been undertaken using Rangpur lime and Kachai lemon in MT liquid media supplemented with 6% sucrose and 0.5 mg/l BAP. Three experimental trials consisting of twelve graft operation for each rootstocks in each trial recorded a success rate of 50% and 53.78% in 'Rangpur lime' and 'Kachai lemon' respectively following wedge cut. The diameter of the epicotyl portion was observed strongly influencing the micrograft with 1-1.5 mm highly favourable for success of micrograting while less than 1 mm is found not favourable for micrograft success. Smooth and gently insertion of scion to cut portion of rootstock result in better union of the two genotypes while repeated handling of scion gave bruises to scion resulting in browning and unsuccessful graft union. Trimmed scion with slight pointed in the portion of scion joining the rootstock give faster union of the two genotypes. Keeping the interconnecting genotypes in dark for two weeks, successful graft with better union might start giving flush of the attached scion. Poor survival rate of the micrografted plantlets during hardening and slow growth of the micrografted ones, it is found favourable to do double grafting. Improvement in survival of micrograft is achieved through double grafting to pencil size rootstock through humidity maintenance using plastic cover and keeping in a cool place of 18-24° C.

Keywords: Citrus, Micrografting, Double graft, Scion, BAP, MT



### Young tree performance of grapefruit varieties (*Citrus paradisi*) under central India conditions

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An experiment was conducted at Research Farm, ICAR-Central Citrus Research Institute, Nagpur to evaluate performance of grapefruit varieties under Central India conditions. Seven grapefruit varieties viz. NRCC Grapefruit – 6, Flame, Star Ruby, Red Blush, Marsh Seedless, Foster and Imperial budded on Rough lemon rootstock were plated at a spacing of 5 x 5 m during 2017. The experiment was laid out in Randomized Block Design with three replications. The observations on plant growth, yield, and quality parameters were recorded during 2022. The analysis of the data suggested that significant differences were found among the grapefruit varieties for the traits studied. The highest plant height was found in Red Blush (1.71 m) followed by Marsh Seedless (1.60 m). However, the highest canopy volume was found Red Blush (4.84 m<sup>3</sup>) followed by Marsh Seedless (3.51 m<sup>3</sup>), which were at par. Among seven varieties, the highest fruit weight was found in NRCC Grapefruit 6 (470.67 g) followed by Red Blush (370.00 g) and Flame Grapefruit (319.33 g). The highest TSS was found in Foster (8.60 °Brix) and NRCC Grapefruit-6 (8.60 °Brix). The highest juice content was in NRCC Grapefruit-6 (37.79 %). There was no significant a difference was found for no. of fruits per tree and the highest yield was recorded in Red Blush (5.87 kg/tree) which was at par with Flame (5.71 kg/tree). Based on the findings from first year of fruiting, Red Blush and Flame are performing better.

Keywords: Grapefruit, Early stage, Growth, Yield



## Evaluation of galgal (*Citrus pseudolimon*) germplasm for growth, yield and quality under black soils of central India

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Thirteen galgal (*Citrus pseudolimon*) germplasm collected from different parts of India were evaluated for their performance under black soils of Central India at Germplasm block, ICAR-Central Citrus Research Institute, Nagpur, India during 2021. The experiment was laid out under Randomized Block Design with three replications. The germplasm were planted at a distance of 5 x 5 m spacing. Observations on growth, yield and quality parameters were recorded. The analysis of the data revealed that the significant variations were observed among the 13 germplasm for all the traits studied. The maximum plant height (5.02 m) and canopy volume (92.51 m<sup>3</sup>) was recorded by IC-311360. The maximum fruit weight was recorded in IC-322100 (751.6 g) and the minimum fruit weight was recorded by IC-346969 (180.97 g). The maximum fruit length was recorded by IC-322260 (129. 2 mm) whereas the maximum fruit diameter was recorded by IC-285440 (111.3 mm). The maximum no. of segments per fruit was observed in IC-311386 (13.1) and IC-322260 (13.0). The highest juice content was recorded in IC-32260 (44.39 %) which was closely followed by IC-326669 (44.35 %) and IC-285430 (43.8 %) and the lowest juice content was recorded in IC-311386 (31.8 %). The highest TSS was recorded in IC-311360 (8.8 <sup>o</sup>Brix) whereas the highest acidity was recorded in IC-311377 (5.18%). The lowest seed content was recorded in IC-311377 (12.8 seeds/fruit).

Keywords: Galgal germplasm, Randomized Block Design



#### Genetic diversity analysis in Rough lemon using SSR markers

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Rough lemon (Citrus jambhiri Lush.) is an important species widely used as a rootstock for commercial citrus cultivation in India and some parts of the world. The rootstock is a crucial part of today's citrus production. Rough lemon can be used with citrus varieties including orange, grapefruit, and lemon. Because of its superior impact on scion vigour and yield, good suitability for sandy soils, tolerance to drought and salinity, good growth under deep soil conditions, Rough lemon is still the most commonly used rootstock in the majority of the citrus growing belts of North, North East, Central, and South India. The presence of genetic variety, which can be found in wild species, closely related species, breeding stocks, mutant lines, etc., may act as a source of beneficial alleles and help plant breeders create better rootstocks that are more tolerant to climatic change. In this study, thirty-seven C. jambhiri accessions were characterized using SSR markers for diversity analysis. A total of forty seven primers were screened, and eleven SSR primers were identified as polymorphic based on repeatability and banding patterns. By using DNA banding pattern a dendrogram and an unweighted neighbouring joining tree was generated, which was separated into 3 clusters. Each cluster was partitioned into 2 subclusters within these clusters which shows genetical diversion in rough lemon genotypes. There is very little genetic difference across the various sites of the collection, suggesting that it started from a single, huge population and scattered vegetatively with little introgression. Citrus jambhiri, a highly polyembryonic species, has minimal genetic variety unless it is geographically diversified. Citrus jambhiri is a cross pollinated crop with a large amount of genetic variation within accessions that can be used efficiently for gene tagging and genome mapping of desirable traits such as larger fruit, high juicy content, disease and insect resistance.

**Keywords:** Rough lemon, SSR markers, Genetic variety, Breeding stocks, SSR primers, Gene tagging, genome mapping



### Studies on the evaluation of promising acid lime (*Citrus aurantifolia swingle*) cultivars for commercialization under Tamilnadu condition.

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Acid lime cultivation in India is a significant agribusiness, with major states like Andhra Pradesh, Gujarat, Rajasthan, Bihar, Maharashtra, Tamil Nadu, and Arunachal Pradesh contributing to its production. This citrus group is highly sought after for pickles, food products, and beverages. Its richness in ascorbic acid, salts, and minerals provides various health benefits. The experiment on was carried out at between 2017 to 2022 at Horticultural College and Research Institute, TNAU, Perivakulam, Tamil Nadu under the All India Coordinated Research Project on Fruits (ICAR-AICRP-Fruits). Nine acid lime clones were collected from different AICRP (Fruits) centres across the country and evaluated for various growth, yield and quality parameters. The clones evaluated are TAL-94/14, TAL-94/13, Phule-Sharbati, Akola lime (PDKV lime), NRC – Nimboo 2, NRCC – Nimboo 2, NRCC – Nimboo 3, NRCC-Nimboo 4, KL-12 and PKM-1 used as check variety for this experiment. Among the acid lime clones evaluated at Tamil Nadu condition, the clone NRCC – Nimboo 3 recorded the enhanced performance at various growth parameters viz., plant height (4.27m) and canopy volume (31.82m<sup>3</sup>). It also showed increased performance in number of fruits per tree (396.02 /tree), yield per tree (21.53kg/tree), fruit weight (47.11g), ascorbic acid content (38.84mg/100g), yield per treeper cent over check (3.26). Next to NRCC – Nimboo 3, the check variety PKM 1 performed well with number of fruits per tree (298.62), yield per tree (20.85kg/tree) and ascorbic acid content (36.53mg/100g). The clone NRCC – Nimboo 3 performed well under tropical climatic condition of Tamil Nadu. In future it may be recommended for commercial cultivation along with the existing promising cultivar PKM 1 acid lime released at Horticultural College and Research Institute, TNAU, Periyakulam, Tamil Nadu.

Keywords: Acid lime, Varieties, Evaluation, Commercialization, Tamil Nadu



#### "PDKV mandarin " a new variety of Nagpur mandarin

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Nagpur mandarin is famous in central India for its taste, however varied range of yield and quality reported amongst the clones. In the view of find suitable clone having better yield as well as quality potential, survey was conducted during period 1997-2002 .Initially in-situ study was conducted to select the elite clones of Nagpur mandarin having better yield and quality. Total ten elite clones selected were evaluated against the check Nagpur mandarin in RBD replicated thrice. The results indicated clone No. 5 which was renamed as PDKV mandarin remained superior over the years (2007-2017) over the other clones and check Nagpur mandarin, and recorded significantly maximum yield parameter (no of fruits 626.19 and fruit yield 80.48 kg/tree i.e. 20.19 t/ha) as well as stood best for quality parameters (juice 49.74 %, TSS 11.13% and TSS: acidity ratio 14 ). The variety was identified as PDKV mandarin during 4 th GD of AICRP , Fruits , IIHR, Bengaluru as wel as recommended for release during Maharashtra Agril. University Joint Agesco during year 2016-2017 for its high yielding ability , better quality besides having attractive apricot orange color.

Keywords: Clone, Nagpur mandarin, PDKV mandarin



#### "PDKV Bahar" a prolific bearer new variety of acid lime

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Acid lime is one of the prominent crops of Maharashtra. Very few varieties of acid lime prevails and varied clones exists which envisages scope for the selection of elite one for yield as well as quality. After sixteen years of constant nurturing of elite lime clones at AICRP (Fruits), Dr.PDKV, Akola was fruitful in the form of release of PDKV Bahar a prolific bearer variety for Maharashtra. Followed by initial in-situ evaluation, total ten elite clones were selected. Finally five most superior clones were evaluated(2009-2014) along with commercially popular varieties viz. PDKV lime and Sai Sarbati as check in RBD replicated thrice for comparison. Study revealed PDKV Bahar (Clone-2) was superior over other clones as well as check varieties and recorded significantly maximum yield (124.92 kg/plant i.e.34.54 t/ha), no of fruits (1833/plant),TSS(8.39°B) and acidity(7.18%). PDKV Bahar reported 14.71 and 21.32 % increase in yield over the PDKV lime and Sai Sarbati respectively, in all the fruiting seasons over the years.

Keywords: Acid lime, Clone, PDKV Bahar, PDKV lime, Sai Sarbati



#### "PDKV Trupti" a novel variety of acid lime for pickle

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Pickle is one of the commercial product prepared from the lime, pickle is prepared for most of the lime varieties, however some of the local types are famous for the pickles. A view of same different local types were collected considering suitability for the pickle type and were evaluated for pickle purpose during period 2014-16 at AICRP (Fruits') Dr. PDKV, Akola in CRD. Similarly, the field evaluation of 14 genotypes included five cultivated varieties and nine elite clones were done (2009-2017) in RBD replicated thrice. For the evaluation of the lime for the pickle purpose, the parameters were decided taking help from scientist from different corner of country, processing industries, and consumer as well as farmer preferences. The score sheet was prepared. While scoring the genotypes for pickle, due consideration was given to yield and quality of fruit (thick peeled and large fruit) desired for pickle. Akola lime 3 had obtained maximum score for quality attributes for pickle and pickle quality (93) as well as overall consumer acceptance (8.37) which was ranked first and came under the category of liked very much on Hedonic Scale by the referee and consumers. This was largely because Akola lime 3 fruits were thick peeled (0.28cm), having highest peel percentage (22.25%), less juicy (49.75%), maximum rag % (28.73%) . Similarly pickle had highest pickle recovery (67.50 %), maximum acidity (7.30 %) better retention of TSS (27.10 0B) and ascorbic acidity(28.34mg/100 g) in the pickle compare to the ruling varieties PDKV lime, PDKV Bahar, Saisarbati, Pramalini, Vikram and PDKV Chakradhar used as check which were mostly thin peeled. Akola lime 3 further released for Maharashtra as PDKV Trupti having characters round to oblong large size (weighing 59 to 60 g, average 53.50 g) fruits , yellow at maturity, fruit segments : 9-10 no, peel warty very thick, adherence very strong and having yield 25 to 27 tones/ha.

Keywords: Acid lime, Pickle, PDKV Trupti



### Studies on floral biology, yield and phytochemical characterization of different sweet orange genotypes under Bundelkhand region

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Sweet Orange in Bundelkhand region is now a promising fruit crop for utilizing the up and medium fallow lands. This area is suitable for sweet orange cultivation as a commercial basis. There is a need to identify high yielding variety with good guality for commercial cultivation. The present study was carried out at Fruit Research Station, RLBCAU, Jhansi during 2021-22. The experiment was laid out in randomized block design with four treatments (Sathgudi, Mosambi, Pusa Round and Pusa Sharad) and six replications. Among all the genotypes of sweet orange under study, the flowering takes place in February to March under Bundelkhand climatic conditions. Sathgudi was early to flower (23 February) followed by Mosambi (25 February) and flowering in Pusa Sharad and Pusa Round were recorded late i.e. on 2<sup>nd</sup> March and 4<sup>th</sup> March. Full bloom period ranged from first week to Second week of March. Terminal, axillary and mixed type of bearing habit were observed in all the genotypes. Two types of flowers viz., hermaphrodite (perfect) and staminate with five sepals, five petals were recorded. In all genotypes of sweet orange, maximum anthesis and dehiscence took place in between 10 A.M. to 12 P.M. Maximum pollen viability was recorded in Pusa Round (82.0%) while it was minimum in Sathgudi (52.0%). The highest yield attributes characters were recorded in Pusa Round, followed by Mosambi, and Sathgudi. Highest juice percentage was observed in Pusa Round, followed by Pusa Sharad. The data on fruit quality parameters showed that the highest TSS/acid ratio content was found in Mosambi, followed by Sathgudi. From the above findings, it can be concluded that the variety Pusa Round and Mosambi can be recommended for commercial cultivation in Bundelkhand region.

Keywords: Flowering, Quality, Sweet orange, Yield



#### *In vitro* culture of Kinnow for improved clonal propagation

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Kinnow mandarin, a prominent citrus fruit renowned for its exceptional taste and high nutritional value, faces several challenges in conventional propagation methods. This study explores the potential of micropropagation as a viable technique for the rapid clonal multiplication of Kinnow. Micropropagation, offers numerous advantages, such as disease-free plantlets, year-round availability, and the production of genetically uniform offspring. The objective of this research was to establish an efficient in vitro regeneration protocol for Kinnow, optimizing the essential factors influencing in vitro growth and development. In the present investigation different explants (leaf, node and shoot tip) were used for in vitro shoot regeneration in Kinnow. Nodal segment when cultured on Murashige and Skoog's (1962) basal medium supplemented with BAP(3.0 ppm) in combination with Kin (0.5 ppm), GA3 (1.0 ppm), Ads (40 ppm) and activated charcoal (500 ppm) produced the maximum number of shoots, shoot length, and number of leaves. The in vitro regenerated shoots were transferred in to half strength MS medium fortified with with IBA (4.0 ppm) and NAA (1.0 ppm) for root formation.For hardening process, well rooted plants were transferred to Soil+Soilrite+Vermiculite (2:1:1) which showed maximum 70 % survival. The protocol yielded disease-free, genetically stable plantlets of Kinnow.

Keywords: Citrus, Kinnow, Micropropagation, Mandarin, Phyohormone



#### The induction of seedlessness in acid lime using gamma-irradiation

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Acid lime (Citrus aurantifolia) is one of the most important citrus fruits in India. The fruits of acid lime are mainly used for making beverages and pickle. The presence of limonin in the acid lime seeds often makes the juice slightly bitter. Therefore, developing a seedless acid lime variety would definitely enhance the consumer preferences and boosting the fruit's marketability. Mutation is a well-recognized breeding method for inducing of seedlessness in fruit crops. In this context, the present experiment was undertaken to develop seedless acid lime varieties, while ensuring the retention of other favourable traits such as acidity and flavour through gamma irradiation. Given this background, seeds of acid limevar. NRCC Acid Lime-7 were treated with different radiation doses of gamma-irradiation (20, 40, 60, 80 and 120 Gy) using Cobalt-60 source at Bhabha Atomic Research Centre (BARC), Mumbai to evolve seedless variety during 2015. The germinated seeds were transplanted in the polybags. The one year old plants were transplanted in the farm of ICAR-Central Citrus Research Institute, Nagpur during July-August 2016. Evaluation of different mutant lines resulted in one commercial seedless mutant (5Gy). The mutant 5Gyrecorded only 1-2 seeds per fruit as against 10 seeds per fruit in control. The average fruit weight of mutant was 26.8 g whereas control recorded 48 g weight. The mutant has TSS of 5.0°Brix, 8% acidity and 26.3% juice content whereas control registered TSS of 7.1°Brix, 7.1% acidity and 45.2% juice content. Therefore, 5Gyhas been identified as promising mutant line which needs to be further carried forward to towards development of a seedless variety of acid lime with improved quality characters for a satisfying consumer demand.

Keywords: Acid lime, Mutation, Seedlessness, Gamma - irradiation



#### Evaluation of parents using seedlings characteristics crossed with blood oranges

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In order to develop citrus varieties with high anthocyanin content in the flesh and easy peeling, crossbreeding was performed with blood oranges as pollen parents and `Finasodea' (Citrus clementina `Finasodea') and `Ehimekashi No. 28' (C. hybrid `Ehimekashi No. 28') as seed parents. Fruit quality and appearance rate of red flesh fruits after fruiting of them were investigated. When 'Moro' (C. sinensis `Moro') was used as apollen parent, fruit diameter and weight were high, but there was no difference between seed parents. In terms of the number of seeds, significant differences were recognized between seed parents, between pollen parents, and between seed parents and pollen parents. So in order to reduce the number of seeds, `Arnold Blood' (C. sinensis `Arnold Blood') was suitable as a pollen parent and `Ehimekashi No. 28' as a seed parent. The appearance of red flesh was high in `Arnold Blood' than in `Moro´, but there was no difference between the seed parents as in the case of fruit weight. Among UPOV guidelines of mandarins, the presence of neck and number of radial grooves at stalk end showed significant differences between pollen parents. In addition, presence of depression at distal end, rind thickness, and adherence between flesh and rind showed significant differences between seed parents. Since the adhesion between the flesh and rind is related to the ease of peeling, it was found to be good to select clementine as a seed parent. So far, it is not possible to suggest a cross combination to obtain red flesh and easy-to-peel seedlings, but it is expected that some conclusions can be drawn when more seedlings are investigated in the future.

Keywords: Citrus, crossbreeding, Blood orange, Red flesh, Easy peeling, Fruit quality



### Development of a new citrus cultivar 'Woorihyang'(C. spp) harvested within the year.

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Citrus is an evergreen fruit tree mainly cultivated in areas worldwide where the lowest temperature is -7°C or higher. The citrus industry in the Jeju region covers a cultivation area of 19,978 hectares and generates an income of KRW 1,027.1 billion. It is an important crop that holds the top position in domestic fruit production. The satsuma mandarin (C. unshiu) accounts for 79.6% and 84.8% of the total cultivation area and production in open field cultivation. This leads to a concentration of shipments and a decline in prices every year. Accordingly, the Jeju Special Self-Governing Province Agricultural Research and Extension Services was development of 'Woorihyang(C. spp.)'that can be harvested within the year and of good quality to solve this problem. Artificial pollination was conducted in 2011. 'Ehimekashi No. 28 gou(C. spp)' and 'Kanpei (C.spp)' were used as the seed and pollen parent. The first and final selections were made in 2017 and 2021, respectively, and applied for variety protection to the Korea Seed & Variety Services in 2022 (Application No. 2022-259). Trees' vigor is strong, and thorns are generated during the juvenile stage and summer shoots. Therefore, there is convenience in cultivation management when they are removed at the beginning. The harvest season is in late November, and the soluble solid content is 13.6°Bx, while the acidity is 0.80%. This variety does not have any seeds, and it is easy to peel. The fruit drop rate was 92.2% in 2022, and it is believed that maintaining a low temperature in the facility before the fruit enlargement stage will help reduce fruit drop. We promoted demonstration cultivation of 'Woorihyang' variety in 1.5ha and transferred a non-exclusive license to seedling companies for rapid supply to farms. 'Woorihyang' is expected to contribute to the diversification of citrus cultivar because it can be harvested earlier and it has superior quality than the control variety 'EhimeKashi No. 28 gou'.In addition, this cultivar can reduce production costs because it can be harvested within a year. It is also expected to be highly preferred by both farmers and consumers due to its high sugar-to-acid ratio.

Keywords: Citrus, Ehimekashi No. 28 gou, Kanpei, Satsuma mandarin, Woorihyang

### Thematic Area -2 Advances in Citrus Production Technology, Smart Citriculture and Application of Cutting-edge Technologies



Advancing Citriculture for Agro-economic Prosperity

### **Oral Presentation**

## TS-2-0-01

# Effects of plant growth regulators and micro-nutrients on fruit set and yield of mandarin in Dhankuta district of Nepal

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The foliar applications of three plant growth regulators, viz. GA<sub>3</sub> @ 20ppm; 2,4-D @ 15ppm; and NAA @ 50ppm; alone and their combinations with three plant micro-nutrients, viz. B @ 0.4% + Zn @ 0.2% + Ca @ 1% were evaluated to determine the best foliar spray of growth hormones and plant nutrients for the increased fruit set and yield of mandarin. The foliar sprays, which were applied five times, started from full bloom until late September (pre-harvest stage). The experiments were conducted in Dhankuta, Nepal during 2019 and 2020. The highest bloom fruit set of 6.1% was recorded at the foliar application of GA<sub>2</sub> 20ppm + NAA @ 50ppm, compared to the control treatment of 4.6%. Likewise, there were significant effects of plant growth regulations and micro-nutrients applications on the fruit drops across the fruit growing periods. The foliar application of  $GA_3 + 2,4-D + micronutrients$  resulted in the lowest fruit drop during the months of June (63.9%), July (65.9%), and September (20.8%), as opposed to the control treatment's fruit drops of 77.6, 80.4, and 41.5%. In addition, the fruit drops occurred at the foliar sprays of  $GA_3 + 2,4-D$  were statistically at par with the  $GA_3 + 2,4-D + micro-nutrients$ . Also, the  $GA_3 + 2,4-D + micro-nutrients$ . micronutrient spray treatment had the largest number of fruits (995 nos.) and total fruit weight (64.2 kg) per tree, which were considerably higher than the control by 201.5 and 175.5%, respectively. To sum up, a combined foliar application of gibberellic acid, 2,4-D and micro-nutrients is recommended for improving the fruit set, retention, and yield of mandarin.

Keywords: Fruitlets, Fruit set, Mandarin, Plant growth regulators, Plant micro-nutrients



# Comparison for carbon sequestration potential between citrus orchard and traditional orchard system in Sikkim

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Experiment was conducted during the year 2018-2023 to estimate soil organic carbon stocks and fractions in different orchards at ICAR-Sikkim Centre. The oxidizable organic carbon (Mg C ha<sup>-1</sup>) in soils (0-15 cm soil depth) of the selected fruit orchard was highest in peach (20.91) followed by kiwi (20.58), citrus (20.16), guava (20.10), pear (19.71), plum (19.66) and lowest in mandarin (19.61). The similar trend was observed in the entire soil layer (15-30, 30-45, and 45-60 cm). Active carbon pools (Mg ha<sup>-1</sup>) in soils in 0-15 cm layers of different fruit orchard was significantly higher in citrus (15.48) followed by kiwi (15.45), peach (15.08), guava (14.42), mandarin (13.56), pear (13.40) and lowest in plum (12.57). Here also similar trends in active carbon pool were observed in all the below ground different soil profile. The very labile fraction of carbon (C frac,) contributed the largest percentage of total soil organic carbon, leading to the more active carbon pool in the surface soil can reasonably be used as good indicator for assessing soil for its crop productivity. Passive carbon pools in soils (Mg ha<sup>-1</sup>) in 0-15 cm soil layer of different fruit orchard was highest in peach (9.98) followed by guava (9.80), kiwi (9.75), pear (9.42), mandarin (8.56), citrus (8.46) and lowest in plum (8.26). After four years of the experiment the carbon pool index, lability index and carbon management index were calculated for recognition of best fruit orchard production system to sequester more carbon in the mid hill of Sikkim. The lability index was highest (0-15 cm) in kiwi orchard (1.64) followed by peach (1.57), guava (1.56) and lowest in plum (1.32). As mentioned above the similar trend for lability index was also observed in other soil depth. The carbon pool index was also higher in kiwi (1.15) followed by peach (1.14), guava and citrus (1.11) and lowest in mandarin and plum (1.08). Finally, the carbon management index was higher in kiwi orchard (186.96) followed by peach (180.55), citrus (179.82), guava (173.16), pear (161.32), mandarin (154.44) and lowest in plum (142.56). Among the orchards, kiwi orchard had greater amount of total soil organic carbon, carbon pool index, lability index and higher carbon management index and hence, considered the best orchard production system to sequester carbon in the Sikkim Himalaya. The second and third position was achieved by peach (180.55) and citrus (179.82) with respect to carbon management index.

Keywords: Soil carbon fractions, Orchard, Kiwi, Guava, Peach, Pear, Mandarin, Citrus, Plum



# Stress and toxicity of zinc in mandarin orange (*Citrus reticulata* Blanco) seedlings – A morpho-biochemical study

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Mandarin orange (Citrus reticulata Blanco) is one of the most widely and commercially grown fruit crop in different countries including India and known as Darjeeling Mandarin, Khasi Mandarin, Nagpur Mandarin, Coorg Mandarin etc. as per the growing locations of the fruit. This fruit is rich source of vitamin A, C, B and plenty of phosphorus as well as digestive fibres. Healthy plant growth of citrus depends on judicious nutrient management with respect to organic manures, inorganic fertilizers and micronutrients as well. Inadequate or excessive levels of zinc can induce stress and toxicity that reduces crop yield. Several physiological disorders of citrus (e.g. chlorosis, little leaf, mottling of leaf etc.) are attributed to Zn deficiency and moreover toxicity of Zn inhibit metabolic activity and stunted growth of plants. The level of concentration at which the deficiency as well as toxicity of zinc occurs in citrus especially the mandarin orange is not yet reported. Thus, the present study has been conducted with application of different levels of zinc (ZnSO<sub>4</sub>,7H<sub>2</sub>O) @ 5.0, 7.5, 10.0, 12.5 and 15 mM/l as pot application of solution. Zinc application has improved the growth and health of mandarin orange seedlings up to an optimum level (10.0 mM/L). Lower growth rate, biochemical deficiency indicators and visual symptoms (viz. chlorosis, mottling) indicated zinc deficiency under control (no zinc application) treatment. Better growth of mandarin orange seedlings, healthy biochemical indicators (SOD, proline, phenol, free amino acids, electrolyte leakage, RWC etc.), absence of critical visual symptoms (mottling, wrinkling, scorching of leaves etc.) and zinc content of leaves denoted the optimum nutrition of zinc under in T<sub>2</sub> (ZnSO<sub>4</sub> @10.0 mM/l) and T<sub>3</sub> (ZnSO<sub>4</sub> @7.5 mM/l) (particularly in T<sub>3</sub>).Poor biochemical indicators, appearance of critical visual symptoms (viz. chlorosis, mottling, wrinkling, scorching of leaves and wilting of plants) as well as excess zinc content in leaves indicated toxicity of zinc in the plants under the treatments T<sub>4</sub> (ZnSO<sub>4</sub> @12.5 mM/l) and  $T_{s}$  (ZnSO<sub>4</sub> @15.0 mM/l).

Keywords: Mandarin orange, Zinc nutrition, Deficiency, Toxicity



## Influence of fertigation with water soluble fertilizers on growth, yield and quality of Nagpur mandarin (*Citrus reticulata* Blanco)

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Nagpur Mandarin is the most popular fruit crop cultivated in Vidarbha region of Maharashtra and is well known as "Nagpur Santra". Due to its special taste and aroma, it has got geographical indication. It is water and nutrient sensitive crop. Precise and timely application of water and nutrients plays crucial role in its production which leads to water and nutrient savings. A field experiment was conducted during 2015 and 2018 at a farmer's orchard. The experiment aimed to study the impact of different levels of fertigation and soil application of fertilizers on the growth, yield and guality. The experiment was carried out on Ambia bahar. The experiment was set up using a randomized block design with six treatments and four replications. The treatments comprised of one treatment involving the soil application of the Recommended Dose of Fertilizers (RDF) and five treatments of fertigation with different percentages (115%, 100%, 85%, 70%, and 55%) of RDF. Soil application was done in two splits as per the recommendations, while fertigation doses were applied in six different splits based on nutritional requirements as per the stage. The pooled analysis of the recorded data revealed that fertigation with 115% of RDF recorded the highest values for growth parameters, including an increase in plant height (0.582 m), increase in stock girth (6.05 cm), increase in plant spread (0.579 m) and increase in canopy volume (18.21 m<sup>3</sup>) which were statistically comparable to fertigation with 100% and 85% of RDF. Fertigation with 85% of RDF exhibited the highest the number of fruits, average fruit weight and actual fruit yield - 596, 168.22 g and 100.13 kg/plant, respectively. However, these were found statistically at par with fertigation using 100% and 115% of RDF. The highest fruit yield of Nagpur mandarin (27.74 t/ha) was recorded in fertigation with 85% of RDF, followed by fertigation with 100% and 115% of RDF - 27.38 t/ha and 26.99 t/ha, respectively. However, these yields were statistically comparable. Fertigation with 85% of RDF exhibited the highest Total Soluble Solids (TSS) content (11.17 °Brix), the lowest value of acidity (0.798%), and the maximum TSS: Acidity ratio (13.97), indicating superior fruit guality. Fertigation with 100% of RDF followed closely with values of 11.05 °Brix, 0.808%, and 13.71, respectively. Fertigation with 85% of RDF resulted in a significantly higher juice content (50.97%) compared to all other treatments. The maximum benefit-cost ratio (3.20) was obtained in fertigation with 85% of RDF, followed by fertigation with 100% of RDF (2.99) and fertigation with 115% of RDF (2.85). The study suggests that the sustainable production of Nagpur Mandarin can be achieved through fertigation technology using 85% of the RDF (1020–340–510 NPK g/plant) in six splits, which leads to 15% reduction in fertilizer usage and a 23.6% increase in yield compared to traditional soil application of fertilizers. These findings emphasize the importance of proper nutrient management for optimizing the growth, yield and guality of Nagpur Mandarin crop.

Keywords: Citrus, Nutrient, Vidarbha, Fertigation, Ambia, Nagpur mandarin



## Citrus crops in Bundelkhand region of central India: Prospects and challenges

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Citrus is an important commodity worldwide. Annually, the total citrus fruit production is estimated to be 146.86 million tonnes worldwide with China (38.39 million tonnes), Brazil (19.59 million tonnes) and India (12.04 million tonnes) ranking first second and third, respectively. As per first advance estimate 2021-22, India holds 1.09 million hectare area under citrus fruits with production 14.15 million tonnes. Citrus production contributes 13 per cent of total fruit production in India. Citrus area in Uttar Pradesh is 3.931 thousand ha with production of 15.10 thousand MT and productivity 3.89 Mt/ha whereas, in MP it is 135.48 thousand ha with production of 1793.50 thousand MT. Percentage share of citrus area in Uttar Pradesh is mere 0.84 with growth rate of 4.94. The Bundelkhand covers a geographical area of around 70,000 sq km comprising 02 important states i.e. Uttar Pradesh and Madhya Pradesh, 04 divisions and 13 districts. The region is marked by extremes of temperature reaching 48°C during summers and as low as 1°C in winters. Average rainfall per year is 800-900 mm. Cultivated area in the U.P. part of Bundelkhand is about 60% and 42% in the part of M.P with 40 to 42% irrigated area. Black, red laterite, mixed black- red laterite soils are predominant in Bundelkhand with pH ranged from 6.5 to 8.4. Despite its potential, the region has been facing several economic challenges in recent years, including drought, poverty, and unemployment. Soil and climatic conditions are suitable for growing mandarins, sweet oranges and acid limes in Bundelkhand. In recent years, promising results have also been received in districts of Banda, Jhansi, Lalitpur, Hamirpur, Jalaun, Mahoba,, Chitrakoot, Tikamgarh, Chhatarpur. The paper deals with the potential prospects and challenges in citrus cultivation in Bundelkhand region of Central India.

Keywords: Citrus, Bundelkhand, Red laterite, Black soil, Calcium carbonate, Drought resistant



# Precision in water and nutrients management through drip fertigation is need of hour to improve productivity of sweet oranges - The case study

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Citrus group fruits are cultivated in 10.91 lakh ha area and productivity is 12.97 MT/ha. Among all citrus fruits crops, Sweet Oranges are cultivated in 2.20 lakh ha and productivity is 17.70 MT/ha in India. In Maharashtra, productivity of Sweet Orange is just 9.98 MT/ha. Sweet Oranges are mostly cultivated conventionally, water and fertilizers use effciency in conventional farming is just 30 – 35 % and under drip irrigation is 90 - 95%. Drip irrigation technology has proved its merits in increase in yield, improve in quality, saving of water, fertilizers, labour, power and time. Cyclic wetting and drying in conventional irrigation method, imbalanced nutrition and negligence towards pests and diseases are main constraints of low yields. Krishi Bhushan awardee farmer Shri Hemachand Dagaji Patil from village Panchak, Taluka Chopada, Dist Jalgaon, has planted Jain Sweet Oranges 1500 plants on raised beds in 4.5 acres at 4 x 3 M spacing under drip fertigation in medium black soil, Plant population increased from 112 to 337 plants / Acre. He started harvesting of fruits from  $4^{th}$  year. Due to precision in water and nutrients management with drip fertigation, there was no cyclic wetting and drying was observed in soil. Due to regular drip fertigation no nutrient deficiency and water stress at any crop growth stage was observed n which helped to explore more genetic yield potential of varieties used. He harvested bumper production 45 - 50 Kg / plant i.e 15 MT / Acre on 5th year. It's eye opening to farmers who are cultivating Sweet Oranges conventionally who gets production just 4 - 5 tonnes per acre in Maharashtra. Adoption of drip fertigation is needed to improve productivity of sweet Oranges.

Keywords: Drip irrigation technology, Drip fertigation, Citrus fruits, Sweet oranges,



## Nutrient management with bio-stimulant on Nagpur mandarin on farmer's field of Central Vidarbha

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Field investigation was carried out during 2017 on the farmer's field in Katol tehsil. Five locations viz., Fetri, Katol, Katol (RFRS), Wandali-wagh and Hatla village were selected for study. Considering management practices of organic, inorganic and integrated nutrient by farmers; fruit guality, yield of Nagpur mandarin and soil fertility were studied. Fifteen orchards were selected and fruit samples were collected for quality parameters. D-extract prepared in proportion of neem leaves + nirguli + maharukh + custard apple + wild tulasi + castor leaves + karanj leaves (2 kg each) + 1 kg cow dung + 2 lit cow urine in 200 lit water (Dashparni extract). J-extract prepared with 10 kg cow dung+ 10 lit cow urine + 2 kg jaggary+2 kg chickpea flour + half kg of organic rich soil in 200 lit water (Jivamruit extract). Highest fruit yield (17.0 t ha<sup>-1</sup>) of Nagpur mandarin was registered with the combined application of 20 kg FYM+ Dextract 10 lit in 100 lit water + J-extract 60 lit in 100 lit water and Neem extract 10 lit in 100 lit water and applied it @ 500 lit ha<sup>-1</sup>every month through eight foliar spray from June to January. Fruit yield of Nagpur mandarin increased by 8.10 and 22.72 % with the application of N:P:K-502:460:160 g tree<sup>-1</sup> and 15 kg FYM + N:P:K:Mg:S-450:70:150:25:50 g tree<sup>-1</sup>, respectively over organic alone. Quality parameters viz, juice recovery %, TSS, fruit weight and ascorbic acid found maximum with the application of FYM 20 kg tree <sup>1</sup>coupled with inorganic fertilizers. Maximum soil organic carbon (6.7 g kg<sup>-1</sup>) was observed under orchard treated with combined application of 15 kg FYM+N:P:K:Mg:S-450:70:150:25:50 g tree<sup>-1</sup> and it found decreased with depth. Exchangeable cations follow order of Ca<sup>2++</sup>>Mg<sup>2++</sup>>Na<sup>+</sup>>K<sup>+</sup>.CEC of soil ranging from 49.2 to 62.2 Cmol (p<sup>+</sup>) kg<sup>-1</sup> among the locations. Micronutrients played supportive role in augmenting the fruit yield of Nagpur mandarin. Practice of INM used by farmers with 15 kg FYM + N: P: K :Mg: S-450:70:150:25:50 g tree<sup>-1</sup> to age of eight year orchard found useful for sustaining the fruit yield of Nagpur mandarin, guality and fertility status of soil.

Keywords: Biostimulant, Jivamrut, Nagpur mandarin, Quality, Yield



## Response of sweet orange (*Citrus sinensis* Osbeck) to varied nutritional regimes under high density planting in diverse Indian states: Andhra Pradesh and Maharashtra

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An experiment was carried out within the ICAR-All India Co-ordinated Research Project on Fruits, with an aim of investigating the optimal nutrient requirements for enhancing the growth and yield of sweet orange cv. Sathgudi in Andhra Pradesh and Mosambi in Maharashtra. This investigation focused on high density planting (HDP), was conducted at two research centres, namely Tirupati in Andhra Pradesh and Rahuri in Maharashtra. The primary objective of this study was to determine how productivity could be increased by manipulating the number of plants per unit area and adjusting nutrient levels using different nitrogen (N) sources. The specific agricultural practices and preferences of the respective regions were taken into account during the study. The experimental design employed a factorial randomized block design, replicated thrice, with three different planting densities (referred to as S1, S2, and S3) and three nutrition levels (referred to as L1, L2, and L3). The planting densities were defined as follows: S1 with a spacing of 6x6 meters, accommodating 277 plants per hectare; S2 with a spacing of 6x5 meters, accommodating 333 plants per hectare; and S3 with a spacing of 6x4 meters, accommodating 416 plants per hectare. The nutrition levels were categorized as L1 with 75% nitrogen from inorganic sources, 25% nitrogen from organic sources (FYM), and full inorganic P & K fertilization, excluding the contribution from FYM; L2 with 50% nitrogen from inorganic sources, 50% nitrogen from organic sources (vermicompost), and full inorganic P & K fertilization, including the contribution from vermicompost and L3 with 100% recommended dose of fertilizer (RDF) through inorganic sources only, serving as the control. The findings of the experiment revealed that the HDP (S3) led to increased productivity when compared to the conventional planting system at both the research centres. Notably, there was a significant interaction between nutrients and high density planting at Tirupati, particularly with treatment L1. This combination demonstrated reduced guantities of P fertilizer input by 42.85% and K by 93.75%. However, these interaction effects were not significant at Rahuri center. Consequently, it is recommended that, for the agro-climatic conditions of Tirupati, adopting a HDP of 416 plants per hectare along with 75kg of FYM, 1125g of N, 200g of P<sub>2</sub>O<sub>5</sub>, and 25g of K<sub>2</sub>O per plant per year can lead to productivity increase of 33.94% and an improved benefit-cost ratio of 20.27%.

Keywords: HDP, Nutrition, Sweet orange



## Effect of sensor based fertigation scheduling on water productivity and nutritional density of Nagpur mandarin (*Citrus reticulata*)

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Nagpur mandarin (Citrus reticulata) is globally considered as a premium fruit crop and future growth of citrus industry will be marked not only by a simple increase in yield. In light of many newly emerging challenges, changing the production pattern paired with input use efficiency must be seen as a huge challenging undertaking. Nagpur mandarin cultivation is popular among citrus growers due to its constant demand in the domestic market and easy adaptability to varied agro-climatic conditions in Vidharbha region of Maharashtra. In these parts of Maharashtra water is scarce commodity and hence there is need to apply water judiciously as per the water requirement of the crop and the actual water requirement of Nagpur mandarin crop depends on age, season, location and management strategies. In view of this, an experiment was conducted at the ICAR-CCRI, Nagpur (latitude 21° 08<sup>°</sup>, longitude 79° 01<sup>"</sup> and 349 m msl) is to be developed sensor based fertigation scheduling across phenological/critical stages for enhancing water productivity and nutritional density of Nagpur mandarin in Ambia Bahar. Based on the atmospheric demand, the average Nagpur mandarin evapotranspiration are 16,284 litres tree<sup>-1</sup> and water were applied to Nagpur mandarin tree at various stages ranged from 736 to 3438 Liters tree<sup>-1</sup> month<sup>-1</sup>. For which, 4 main irrigation and 3 sub-fertilizer treatments were designed under split plot. In sensor based fertigation scheduling, at 25% \*AMC & N, P, K, B for new leaf initiation, 40% \*AMC & N, P, K, Fe, Mn, Zn for crop development, 55%\*AMC & K, Fe, Mn, Zn for maturity and 40% \*AMC & P, K, Zn for harvesting is the best for improving water productivity and nutritional density of Nagpur Mandarin. In conclusion, In sensor based fertigation scheduling, Single Lateral with ring type micro-irrigation was the best for 11<sup>th</sup> year old Nagpur mandarin tree is recommended, applicable water saving strategy and good alternative with respect to other techniques of water managements when water resources are limited in order to increase water productivity and nutritional density while other physiological growth, roots, soil moisture and water use parameters are maintained at an acceptable level.

**Keywords:** Nagpur mandarin, Sensor based, Fertigation schedule, Micro-irrigation, Water use, Water productivity, Nutritional density.



## Evaluation of sweet orange varieties on raised bed and conventional flat system in plains of Assam

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An experiment on evaluation of sweet orange varieties on raised bed and flat planting system was conducted at RRCC during 2019-22. Nine different sweet orange varieties viz., Cutter Valencia, Mosambi, Hamlin, Pineapple, Natal, Valencia, Pera, Jaffa and Westin which were budded on rough lemon rootstock were evaluated for plant growth, fruit yield and fruit physico-chemical parameters. Results revealed a significant variation in growth and fruit physico-chemical parameters among the varieties. Cutter Valencia recorded significantly the maximum plant height (4.50 m) on both raised bed and flat system, however canopy volume (33.6 m<sup>3</sup>), stock girth (50.7 cm) and scion girth (49.7 cm) was recorded maximum on flat system. Fruit weight was noted maximum in Cutter Valencia (218.16 g) which also registered the maximum fruit length (72.0 mm) and fruit diameter (73.3 mm) with lowest seed content (2.33). However, seeds per fruit (26.6) and juice content (52.0%) was recorded highest on raised bed in Pineapple. Significantly, the maximum TSS was recorded on both systems in Mosambi (10.2 °B) followed by Westin (9.2 °B) while maximum titratable acidity was noted in Valencia. Almost all varieties registered higher yield on raised bed system as compared to flat system and significantly highest fruit yield was obtained in Mosambi (31.26 t/ha) followed by Cutter Valencia (28.34 t/ha). Conclusively, results of the present study indicated the significant effect of raised bed under plains of north-eastern region.

Keywords: Sweet orange, Raised bed, Flat bed, Fruit quality, Yield



## TS-2-0-11

## Design and performance evaluation of a tractor front mounted hydraulically operated pruning and shaping machine for orchard crops

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Hand tools are inefficient and time-consuming for pruning small orchards, prompting commercial orchards to explore power-driven equipment. However, existing solutions have not significantly reduced the need for manual labour. To address this gap, a tractor front-mounted hydraulically operated pruning and shaping machine was developed. This innovation aims to enhance pruning efficiency, speed, and effectiveness while minimizing manpower requirements. The machine comprised a PTO-driven hydraulic power pack, hydraulic cylinders, motors, control valves, and circular saw blades. This system allows for adjustable cutting height and blade angles via hydraulic cylinders, facilitating precise pruning and canopy shaping. The pruner was fixed to the front of the tractor with four bolts and nuts. The developed pruner can prune the plant up to heights of 3.5 m. The hydraulic system is designed to rotate the blades at about 2500 rpm. The system has been preliminary evaluated at ICAR-CIAE farms with guava orchards. The inter- and intra-row spacing of guava plants was 6.0 m. The average height of guava trees was about 4 meters. The pruner is able to cut the branches in the range of 3 to 65 mm at a forward speed of 1.3 km/h. Field capacity and fuel consumption were found to be 0.15 ha/h and 4.2 l/h, respectively. The machine has a pruning capacity of 32 plants per hour. The average turning time loss of the pruner is 32 s/turn. The cost of the developed pruner and its operating cost were found to be Rs. 450,000/- and Rs. 4910/ha, respectively. The payback period of a hydraulic pruner is 400 h, the break-even point is 44 h/year, and the benefit-cost ratio is 1.34. This innovation addresses the shortcomings of manual and existing mechanical pruning methods, offering a more effcient and cost-effective solution for orchard maintenance.

Keywords: Orchard, Hydraulic motor, Pruner, Saw blades, Field capacity



## Effect of foliar application of micronutrients on growth, yield and quality of acid lime in *hasta bahar*

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The present investigation was conducted at the Experimental Farm Department of Fruit Science, Dr. PDKV, Akola during 2022 on a seven-year-old orchard of acid lime cultivar PDKV Bahar spaced at 6 X 6 mt. The orchard is already regulated by hasta bahar treatment by application of GA<sub>3</sub> 100 PPM in month of June and foliar application of Cycoceal 1000 PPM in September, while during October 1 % KNO<sub>3</sub> application in combination with different micronutrient treatments with the objective of enhancing flowering, yield, and fruit quality of hasta bahar-regulated acid lime. There were seven treatments and replicated thrice with RBD Design. Foliar application of treatment T<sub>1</sub>-Borax 0.3%, T<sub>2</sub>,-Zinc Sulphate 0.5%, T<sub>3</sub>-Ferrous Sulphate 0.3%, T<sub>4</sub>-Borax 0.3% + Zinc Sulphate 0.5%, T<sub>5</sub>-Borax 0.3% + Ferrous Sulphate 0.3%,  $T_6$ -Borax 0.3% + Zinc Sulphate 0.5% + Ferrous Sulphate 0.3%, and T-Control The foliar application of Borax 0.3% + Zinc sulphate 0.5% + Ferrous sulphate 0.3% on acid lime variety PDKV Lime during hasta bahar is significantly increase the parameters like fruit set per cent (50.25%), fruit weight (48.90 g), fruit width (4.53 cm), fruit length (4.66 cm), fruit volume (46.48 cc), specific gravity (1.027 gm/sg.cm), number of fruits per tree (179.33), yield per tree (11.84 kg/tree). The fruit quality parameters studied revealed significant results: TSS (10.12 °B), reducing sugar (0.89 %), non-reducing sugar (0.71 %), total sugar (2.03%) and ascorbic acid (62.92 mg/100 g) with minimum fruit drop percent (6.10 %) were obtained under T6 and followed by the application of Zinc sulphate 0.5% i.e. T<sub>4</sub> as compared to the control treatment.

Keywords: Acid lime, Hasta bahar, Micronutriensts, PDKV Bahar, Yield



## Growth regulator and promoters for development of climate resilience in mandarin crop in *ambia bahar*

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Besides biotic factors, causing threat to mandarin crop specially during ambia bahar, fruit drop in ambia bahar due to heavy or longer duration rains or long dry spell coupled with higher temperature has become common feature in recent years in mandarin. In Central India where soils are heavy poor drained soil, losses are more. An experiment conducted during period from year 2019 to 2022 laid out in Randomized Block Design comprised 10 treatments alone and different combinations as well as concentrations of new generation growth regulators with main objective of to find out suitable concentration and combination of growth regulators to improve yield and guality in ambia bahar. The first spraying was done when acute drop was noticed due to continuous rains when fruits were at immature to pre-harvest stage, followed by two sprays at an interval of 15 days. The results indicated treatment consisting of N-ATCA 10 ppm+ Brassinolide 4 ppm+ NAA 10 ppm+ Folic acid 100 ppm was most effective, the pooled results indicated that treatment recorded minimum fruit drop (12.38 %), which is reflected in maximum yield parameters (119.48 kg/tree for 801.94 fruits) and was at par with treatment N-ATCA 10 ppm+ Brassinolide 4 ppm+ GA 15 ppm+ Folic acid 100 ppm and treatment N-ATCA 10 ppm+ Brassinolide 4 ppm+ 2-4,D 15 ppm+ Folic acid 100 ppm. Similarly maximum net return (Rs 3.16 lac/h) obtained with treatment in reflected in N-ATCA 10 ppm+ Brassinolide 4 ppm+ NAA 10 ppm+ Folic acid 100 ppm receiving more cost benefit ratio (3.43). Thus results suggestive of NAA (10 ppm) or 2-4, D (15 ppm) or GA (15 ppm) could brings about effective check of physiological fruit drop in adverse situation, only when applied along with N-ATCA (10 ppm) + Folic acid 100 ppm + Brassinolide (4ppm)

Keywords: Ambia bahar, Brassinolide, Folic acid, Fruit drop, Mandarin, NAA, N-ATCA, 2-4, D



## Effect of severity and time of pruning on fruit yield and quality of *hasta bahar* in acid lime

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An experiment was carried out to evaluate effect of severity and time of pruning on yield and quality of *hasta bahar* in acid lime in Factorial Randomized Block Design with two factors, time with three levels viz., 1<sup>st</sup> September, 15<sup>th</sup> September and 1<sup>st</sup> October and severity with three levels viz., 5 cm, 10 cm and 15 cm with overall 9 treatment combinations and replicated three times. In time of pruning, pruning at 1<sup>st</sup> September found significantly superior in regards to average weight of fruit, volume of fruit and length / breadth ratio (Fruit index).While, pruning at 15<sup>th</sup> September found significantly superior in regards to average weight of fruit and length / breadth ratio (Fruit index).While, pruning at 15<sup>th</sup> September found significantly superior in regards to average weight of fruit, volume of fruit and length ratio (Fruit index).While pruning with 10 cm found significantly superior in regards to fruit, volume of fruit and length / breadth ratio (Fruit index).While pruning with 10 cm found significantly superior in regards to fruits per tree, yield per tree, yield per tree, fruit juice per cent. TSS and ascorbic acid. Pruning with 10 cm severity at 15<sup>th</sup> September time was found significantly superior in regards to fruits per tree.

Keywords: Pruning, Fruit yield, Hasta bahar, Acid lime



## Pneumatic based plucking force measurement setup for development of robotic end effector in citrus

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In India, the majority of fruit harvesting is carried out manually, and the adoption of modern technologies remains a distant prospect. As a result, a partial-automated fruit picking approach has been developed as an effort to address this gap. The solution involves a vacuum-based fruit picking system that is linked to a tractor's three-point linkage and powered by a PTO (power take-off) mechanism. The system was designed to evaluate the force needed to detach oranges from their branches. A shut-off valve is integrated into the connection between the flexible tubing and the centrifugal blower, allowing for the regulation of vacuum pressure. To analyze the physical attributes of the fruits, including parameters like area, perimeter, length, width, and RGB values, an image processing system was developed in conjunction with the MATLAB IDE. A testing setup was established, featuring a cantilever beam load cell. This load cell setup was configured to hold the stem (peduncle) of the orange fruit. Additionally, an Arduino UNO-based system was created to capture the initial weight of the fruit suspended on the load cell, as well as the subsequent pulling force exerted until the fruit became detached from the peduncle. Comparative tests were conducted for both manual pulling and manual twisting methods of fruit detachment. For vacuum-based fruit detachment, the analysis revealed that among the chosen samples, the highest, lowest, and average pulling forces were recorded as 31.03 N, 1.59 N, and 11.21 N, respectively. In the case of manual twisting combined with pulling, the corresponding values were 24.89 N, 0.99 N, and 6.68 N. Notably, during vacuum-based picking, it was observed that the peduncle, along with some internal fruit material, detached in nearly 20% of the cases, regardless of the fruit's level of maturity

Keywords: Orchard, Pneumatic, MATLAB, Twisting, Pulling



# Delayed harvesting and creasing physiological fruit disorder in Nagpur mandarin (*Citrus reticulata* Blanco): Nature and alleviation

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Delayed harvesting to avoid glut in the market is preferred by citrus growers to fetch better prices late in the harvesting window which results in creasing of fruits thereby reducing the transit and shelf life of fruits. A field experiment was conducted at ICAR-CCRI, Nagpur during 2020 and 2021 to improve fruit yield, quality characteristics and to manage the creasing disorder incidence encountered in delayed harvesting of one month in Mrig bahar of Nagpur mandarin (Citrus reticulata Blanco) through foliar application of different concentrations of gibberellic acid (10, 15, 20 and 25 ppm). Maximum number of fruits harvested per plant (716), yield per plant (105.60 kg/plant), total estimated yield per hectare (29.25) t/ha), fruit weight (147.25 g), fruit length (58.53 mm), fruit breadth (67.60 mm), fruit volume (145.37 cm<sup>3</sup>), juice per cent (45.67 %), vitamin C (40.73 mg/100ml) content and minimum per cent of creasing (3.74%) were noted in the treatment GA<sub>3</sub> 25 ppm + urea 1.5% over the control. Maximum fruit firmness (2.55  $kg/cm^2$ ) was recorded in the treatment GA<sub>3</sub>20 ppm + urea 1.5% followed by treatment GA<sub>3</sub>25 ppm + urea 1.5% (2.36 kg/cm<sup>2</sup>) indicates the delayed ripening of fruits, which helps in storage of fruits on tree by controlling the incidence of creasing disorder whereas minimum fruit firmness (1.62 kg/cm<sup>2</sup>) was recorded in control. Maximum TSS (11.63%) and TSS/Acid ratio (15.11) were recorded in control followed by treatment GA<sub>3</sub> 10 ppm + urea 1.5% (10.32 % and 13.05, respectively) indicates fruits were more ripened compared to other treatments whereas minimum TSS (9.86 %) was recorded in GA<sub>3</sub> 20 ppm + urea 1.5% and minimum TSS/Acid ratio (12.20) was recorded in GA<sub>3</sub>25 ppm + urea 1.5% indicates fruits were firm and less matured compared to other treatments. Foliar application of 20 or 25 ppm of GA<sub>3</sub> along with 1.5% urea will delays the senescence of tissues and makes albedo tissues compact, thus reduced creasing incidence with improvement in yield and quality characteristics of Nagpur mandarin fruits in delayed harvesting of one month.

Keywords: Creasing, Delayed harvesting, Gibberellic acid, Mrig bahar, Nagpur mandarin



## AV imagery deep learning technique for mandarin orange yield estimation

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Estimation of yield prior to harvest is of vital importance for the farmers, it helps in making assertive decisions for achieving impactful economic goals. Accurate estimation of fruit yields is important for both producers and agricultural cooperatives in timely decision-making. The traditional image processing techniques follow algorithms based on the spectral response among pixels as a unique feature to detect and count fruits. As image pixels are very sensitive to illumination changes under unstructured light conditions, a significant reduction in accuracy is observed when traditional image processing is implemented in field. In such situations, deep learning (DL) based computer vision technique is typical, can be used since it allows the computer to autonomously learn the most suitable feature without human intervention. To replace the manual method of yield estimation, UAV imagery deep learning method can be used as it allows the computer to learn automatically the features and identify objects without human intervention. The integration of unmanned aerial vehicles (UAVs) and advanced deep learning algorithms has surfaced as a comprehensive solution for the automation of fruit yield estimation. The state-of-the-art deep learning, object detection models SSD, Faster RCNN, YOLOv4, YOLOv5 and YOLOv7 were developed for fruit detection and performance evaluated for "harvest-ready" and "unripe" fruits from the tree image. During the flight, the UAV maintained a horizontal distance of 2.5 to 3 m (SE: ± 0.20 m) and vertical height of 1 to 1.25 m (SE: ± 0.25 m) from tree canopy and images were captured from both the right side as well as the left side. Total 800 high resolution images were collected from both the orchards farm for model development and validation. Yolov4 model has an accuracy of 85 % in detection of fruits

Keywords: UAV, Computer vision, Deep learning, Yield estimation



# Recent initiatives in citrus microbudded plant production under greenhouse through Jain Hi-Tech Nursery

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The citriculture industry plays an important role among fruit crops world-wide. In India, citrus crops cover one million hectare area and annual production touched a new high of 14 million tonnes recently. Since 1994, Jain Irrigation Systems Ltd. (JISL), Jalgaon, Maharashtra, has been a pioneer in commercial tissue-culture planting material production in "Jain Plant factory" where 140 million tissue culture banana plants are produced annually. JISL has taken initiative since 2010 to produce quality planting material of sweet orange varieties through integrated hi-tech production systems in greenhouses on a very large scale. A facility for production of disease-free planting material of sweet orange and mandarin by adopting advanced techniques of nursery management to ensure quality and quantity of plants has been established. JISL is maintaining virus free mother stocks in controlled conditions, sourcing virus free scion wood, micro-budding, root trainer cups, soilless growing substrate, automated nutrition and irrigation management and advanced greenhouse climate control management. Ex vitro micro-budding technology is adopted for large-scale commercial production and nearly one million Jain sweet orange plants have been planted all over India under high density planting. JISL is the only company in India to have sweet orange mother trees under an insect-proof greenhouse for clean scion budwood. Indexing of mother trees and ready-to-sale plants is done routinely to ensure plants are free from HLB and known virus and virus-like pathogens prevalent in India. Rootstock seeds are obtained from specially developed mother plantations of Rangpur lime and Volkamer lemon. This paper deals with the detailed description of quality planting material production of sweet orange and mandarin.

**Keywords:** Citrus spp., Mother trees, Microbudding, Root-trainer cups, Soilless medium, Nursery management, Quality planting



# Studies on different nutrient levels and plant density in acid lime (*Citrus aurantifolia* Swingle)

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The experiment on "Studies on different nutrient levels and plant density in acid lime (Citrus aurantifolia Swingle)" was carried out at between 2017 to 2022 at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu under the All India Coordinated Research Project on Fruits (ICAR-AICRP-Fruits). This experiment includes three planting densities with (i) Recommended spacing (6mx6m = 277 plants/ha.), (ii) Reduced the spacing between plants by 20 per cent (6mx5m = 333 plants/ha.) & (iii) Reduced the spacing between plants by 60 per cent (6mx4m = 416 plants/ha.) and three different nutrient source combinations with (i)75 per cent RDF inorganic source + 25 per cent (Organic source-FYM), (ii) 50 per cent RDF inorganic source + 50 per cent (Organic sourcevermicompost) & (iii) 100 per cent RDF inorganic source only (Check). The acid lime variety PKM 1 used as the experimental material and evaluated for various growth, yield and quality parameters. In this experiment, the reduced spacing of 6mx5m (333 plants/ha) with 75 per cent RDF inorganic source + 25 per cent (Organic source-FYM) showed the enhanced performance of yield per tree (44.50 kg / tree), number of fruits per tree (882.70 / tree), enhanced fruit weight (48.91 gm), enhanced acidity content (6.34 %) and increased ascorbic acid content (33.92 mg/100 ml juice). The reduced spacing of 6mx5m (333 plants/ha) with 75 per cent RDF inorganic source + 25 per cent (Organic source-FYM) also influences the soil available nutrient content and leaf nutrient content. It recorded increased available soil N content of 292.88 kg/ha, available soil K<sub>2</sub>O content of 228.63 kg/ha, leaf nutrient N content of 2.55 %, leaf nutrient Ca content of 1.414 %, leaf nutrient Mg content of 0.39 ppm, increased leaf nutrient Mn content of 35.81 ppm, leaf nutrient Cu content of 6.94 ppm, leaf nutrient Zn content of 32.56 ppm and leaf nutrient B content of 0.22 ppm.

Keywords: Citrus, Acid lime, PKM 1, Spacing, Plant density, Inorganic nutrients, Organic nutrients



## Dynamics of soil organic carbon stock under citrus, cotton and forest systems in black cotton soils of the Nagpur district, Maharashtra, India

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Soil organic carbon is a key component of any terrestrial ecosystem and any variation in its quality and composition has important effects on many processes that occur within the ecosystem. The SOC contributes many important effects on physical, chemical and biological properties of soils for maintaining their productivity are well known. To sustain the quality and productivity of soils the knowledge of the SOC in terms of its amount and guality is essential. It is reported that the carbon stock of a soil is related with the quality of the substrate i.e., the clay minerals. It therefore becomes imperative to know the characteristics of the clay minerals as well as those of the organic matter for better understanding of OC status in a particular group of soils. The present study has been undertaken to assess the soil organic carbon sequestration in black soils under different land-use system. Three representative pedons from Nagpur district, Maharashtra, under horticulture (Orange), agriculture (cotton) and forest (Teak) with the time of cultivation ranging from 20 years to centuries were selected. All the soils are smectitic in natures and the percent smectite content was 83, 85 and 77 percent respectively. The study indicates that, shrink-swell soils can maintain a better SOC stock in the horticultural system (30.8Tg) than the soils under agricultural system (19.3Tg). The rate of change of SOC stock in different systems indicated that the carbon sequestration is more in horticultural system compared to other two systems. Low SOC stock in black soils under agricultural system indicates low inputs and more exhaustion due to increased agricultural activity.

Keywords: Carbon sequestration, Citrus, Cotton, Land-use systems, SOC Stock, swell-shrink soils



Advancing Citriculture for Agro-economic Prosperity

### **Poster Presentation**

### TS-2-P-01

## Effect of plant nutrition on fruit set, retention, and yield of mandarin in Dhankuta, Nepal

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The study investigated the effects of foliar applications of nitrogen, potassium, calcium including boron and zinc; alone and their combinations; on fruit set, growth, and yield of mandarin; the experiments conducting in the Dhankuta district of Nepal during 2019 and 2020. The results revealed that severe flowers/fruitlets drop occurred to a range of 97.7% to 92.7% during bloom fruit set period as the highest set (7.3%) occurred at foliar spray of NK + micro-nutrients compared to the control treatment (2.3%). Accordingly, compared to the control treatments, spray application of NK+ micro-nutrients, and NPK soil + micro-nutrients increased the higher fruit set by 151.7 and 41.3% respectively during post-bloom from 28<sup>th</sup> March until 1<sup>st</sup> May. In regard to fruit drop, two treatments of foliar sprays: NPK + B + Zn + Ca; and NK + B + Zn + Ca had the lower fruit drops by 15.8 and 15.4 during June; and by 16.2 and 15.9% during pre-harvest period respectively, compared to the control treatment. The highest fruit diameter (58.9 mm) and weight (63.4 g) were found at the treatment NPK + B + Zn + Ca among the treatments; likewise, the corresponding the highest number of fruits (1790 nos) and total fruit yield (101.81 kg) per tree were also observed at the same treatment.

Keywords: Abscission, Foliar spray, Fruit Set, Mandarin, Plant nutrient



## Mechanical pruning machine: Suitable for rejuvenation of old Nagpur mandarin orchards for improving yield and quality in central India

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Nagpur mandarin (Citrus reticulata Blanco) is an important fruit crop among the different fruit crops grown in India. In India, the average productivity of Mandarin is 10.00 MT per hectare. In Maharashtra state, it is mainly grown in Vidarbha region. The total cultivated area of Nagpur Mandarin in Maharashtra state is 1.47 lakh ha. In Maharashtra state, the average productivity of mandarin is 9.5 MT per ha and it is less compared to the National productivity. The less average productivity in Maharashtra state is due to various reason such as lack of good management practices, declining of orchards, lack of irrigation facilities, incidence of insect pest and diseases, lack of implementation of improved technologies viz, high density planting, use of pruning technology, use of drip irrigation and fertigation etc. Therefore, the productivity of this crop can be enhanced with pruning technology coupled with use of irrigation and nutrition management. First time in Central India, demonstrations of mechanical pruning in old orchards of Nagpur mandarin (6mX6m spacing) were given in Nagpur and Amravati district at 140 acre during 2012-13 and 2013-14 and it is continue till date. From this study it is revealed that pruning treatment was found most useful for Nagpur mandarin during December just after harvesting of ambia bahar fruits. Pruning intensity may be decided as per the orchard conditions. Medium type of pruning (removing 90-100cm branches from top) found most suitable for Nagpur mandarin. Pruning helps to rejuvenate the orchard and increase the productive life of mandarin orchards. Fruit size is increased due to pruning as fruit bears in plant canopy. No. of branches and foliage was increased due to pruning and hence improve the guality of fruits. Pruning helps in increasing the fruit yield ranging from 18 to 55 t ha<sup>-1</sup>. Mechanical tree pruner is useful for commercial and timely pruning for improving quality production of and increase the economic life of Nagpur mandarin.

Keywords: Mandarin orchards, High density planting, Pruning machine, Fertigation, Mechanical tree pruner



# Effects of pruning intensity and soil beneficial microbes on fruit morphological characteristics and yield of Assam lemon under foothills of Arunachal Pradesh

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Assam lemon, a significant citrus fruit, belongs to the Rutaceae family which is a native cultivar in North-Eastern India, grown in South India under Seville, Pat Nimboo, and Nepali oblong or Sivakasi names. Assam lemon's large size and juice make it versatile in culinary, nutritional, industrial, and medicinal applications. Lemon juice is also a good source of citric acid, making pharmaceutical preparations, soft drinks, and processed cheese emulsifiers. Fruits vary in size, shape, and color, with smooth surfaces and rinds. In view of the role of biofertilizers in optimum fruit morphology and yield, an experiment was carried out to evaluate the effects of pruning intensity and soil beneficial microbes on fruit morphological characteristics and yield of Assam lemon in Factorial Randomized Block Design with two factors which includes three levels of pruning viz., 25%, 50% and 0% of terminal shoots and five levels of biofertilizers viz., PGPR (Pseudomonas fluorescens) @ 90 g/plant, Azotobacter @ 15 g/plant, Trichoderma @ 90 g/plant, and the combination of PGPR (Pseudomonas fluorescens) @ 90 g/plant, Azotobacter @ 15 g/plant, Trichoderma @ 90 g/plant and the interaction of pruning and biofertilizers with overall 15 treatment combinations and replicated thrice. In this experiment, results found that the maximum fruit length (90.72 mm), fruit width (59.85 mm), fruit volume (83.92 cm<sup>3</sup>), fruit weight (103.67 g), peel weight (44.88 g), maximum average number of seeds per fruit (0.25) and fruit yield (4.45 t/ha) were recorded significantly superior in the treatment combination of pruning 50% + biofertilizers (PGPR-Pseudomonas fluorescens @ 90 g/plant + Azotobacter @ 15 g/plant + Trichoderma @ 90 g/plant) among the other treatments. The study finds efficient pruning 50% length with biofertilizers improves Assam lemon fruit morphology and yield.

Keywords: Assam lemon, Biofertilizers, Fruit Morphology, Pruning, Yield



## Effect of different concentrations of seaweed extract, IBA and Phloroglucinol on air layering in grapefruit

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Grapefruit (Citrus paradisi) is a subtropical citrus fruit known for its slightly bitter and tangy flavor. It's a hybrid fruit that was originally thought to be a cross between a sweet orange and a pomelo, though its exact origins are not entirely clear. The fruit is known for its distinctive taste and vibrant, often pink, or red, flesh. The present investigation was carried out during the year 2022-2023 in the Department of Horticulture, Sam Higgin bottom University of Agriculture Technology & Sciences Prayagraj in August 2022 to February 2023. This experiment was conducted to evaluate the best treatment combination among IBA, Seaweed Extract and Phlorog lucinol concentration for producing the best guality and most presentable layers of Grapefruit. The experiment was laid out in Randomized Design having 12 treatments and replicated 3 times. From the present investigation it is concluded that effect of treatment T<sub>7</sub> i.e., IBA@2000ppm + Phloroglucinol@5000ppm was found to be best in terms of Success in rooting percentage, Number of days to root formation (initial rooting), Root length, Root thickness, Number of roots per layer, Survival percentage, Number of new sprouts per layered plant, Number of new leaves per layered plant and length of new shoot in Grapefruit. it is also concluded that effect of treatment T<sub>7</sub> i.e., IBA@2000ppm + Phloroglucinol@5000ppm was found to be best in terms of Success in rooting percentage, Number of days to root formation (initial rooting), Root length, Survival percentage, Number of new sprouts per layered plant, Number of new leaves per layered plant and Length of new shoots whereas in root thickness and number of roots per layer T<sub>a</sub> i.e., IBA@2000ppm + Phloroglucinol@7000ppm is best in Grapefruit.

Keywords: Citrus, Grapefruit, IBA, Phloroglucinol, Seaweed



## Seed germination studies on citrus rootstocks

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The study was carried out on eighteen different citrus rootstocks viz. Rough lemon (C. jambhiri Lush.), Rangpur Lime (C. limonia Osbeck.), Carrizo citrange [C. sinensis (L.) Osbeck. × P. trifoliata (L.) Raf.], Troyer citrange (C. sinensis × P. trifoliata), Sour orange (C. aurantium L.), Cleopatra mandarin (C. reshine), Karna khatta (C. karna), Volkameriana (C. volkameriana), Calamondin (Citrus mudurensis Lour.), Trifoliate orange (P. trifoliata), NRCC RS-1 to 6, CRH-47 and Smooth Flat Sevelle (SFS)at Banda University of Agriculture and Technology, Banda, Uttar Pradesh. Qualitative and quantitative variations were observed among different rootstocks. The characters of the seed were evaluated as per the Descriptor of citrus. The seeds of all the rootstocks showed polyembryony. The variations were recorded for seed shape (ovoid, semi-deltoid, clavate, spheroid, semi-spheroid and, cuneiform), seed surface (smooth and the wrinkled), seed colour (brown, light brown, yellowish, light yellow, white and cream) and seed cotyledon (white, cream and light green). Variability also recorded for seed length (9 - 16.10 mm), seed width (3.96 - 7.8 mm) and seed weight (0.08 - 0.76 g). The earliest seed germination occurred in Rough lemon while the last germination was obtained in Carrizo citrange. The NRCC RS-6 showed superiority in germination percentage with 90.90% followed by Rough lemon (87.60%) whereas, SFS had the lowest germination percentage (53.57%) showing its inferiority. The highest survivability (89.04%) was obtained in Rangpur lime followed by Rough lemon (86.82%) and the lowest survivability (29.74%) was observed in CRH-47. The information can be utilized by the researchers and nursery growers for commercial multiplication of citrus.

Keywords: Citrus spp., Rootstocks, Seed, Germination, Survivability



# Influence of time and methods of budding in 'Khasi Mandarin' (*Citrus reticulata* Blanco.) on different rootstocks in the foothills of Arunachal Pradesh

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Khasi Mandarin (Citrus reticulata Blanco.) is an excellent quality Mandarin grown in North-East India. However, its productivity is quite low as they are mainly of seedling origin. With this background, the present investigation was undertaken at College of Horticulture and Forestry, Pasighat, Arunachal Pradesh during the year 2021-22 with 30 treatments executed in Three Factor CRD (Completely Randomized Design) replicated thrice. Different rootstocks viz., Rough Lemon, Rangpur Lime, Khasi Mandarin, Volkamer Lemon and Pummelo and different methods of budding ('T' budding and Modified Chip budding) were used for budding in Khasi Mandarin in February, March and April. Maximum bud take (93.33 %) was observed in Modified Chip budding performed on Rough Lemon and Rangpur Lime during February. Budding success (93.33%), length (11.30 cm), girth (2.14 mm) and dry weight of sprout (2.95 g), number of leaves (7.97), Chlorophyll 'a', 'b' and total (0.34, 0.50 and 0.86 mg/g, respectively) were recorded maximum when Modified Chip budding was done on Rough Lemon in February. Fresh weight (5.22 g) was higher in Rough Lemon budded with both 'T' budding and Modified Chip budding during February. Minimum days required to sprout (32.00) and maximum root diameter (8.23 mm) were observed in Khasi Mandarin with Modified Chip budding done in February. Leaf perimeter (18.7 cm), Root length (32.37 cm), fresh weight (22.13 g) and dry weight (16.22 g) were maximum in Volkamer Lemon with 'T' budding done during February. Thus, Modified Chip budding is the best method of budding to perform during February in Khasi Mandarin using Rough Lemon as rootstock with respect to bud take, budding success and growth of sprout.

Keywords: Khasi Mandarin, Budding, 'T' budding, Modified Chip budding, Rootstock, Time



## Performance of commercial citrus species under humid sub-tropical region of northeast India

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This experiment was conducted on 17 citrus varieties comprising mandarin, sweet orange, lemon, lime, pummelo and grapefruit budded on rough lemon rootstock. The evaluation was performed under both raised bed and conventional flat systems planted at 5m x 3m spacing during 2018-22. Overall, a significant variation among different varieties was observed. Among mandarins, maximum plant height (5.53 m) was recorded in Khasi mandarin seedling while maximum canopy volume (12.8 m<sup>3</sup>) was observed in Khasi mandarin grafted on raised bed. Similarly, in pummelos, the maximum plant height (4.43 m) and canopy volume (15.8 m<sup>3</sup>) was noted under raised bed in US Pummelo-145. Contrastingly, among sweet oranges, the maximum plant height (4.50 m) and canopy volume (33.6 m<sup>3</sup>) was noted in Cutter Valencia under flat system which also recorded the maximum stock girth (50.8 cm). A significant variation in fruit yield and quality performance among speies and varieties was observed. In mandarins, fruit weight was observed to be maximum in Khasi mandarin Seedless grafted (134.67 g) while TSS (8.57°B) and Vitamin C content (39.33 mg/100 g pulp) was noted maximum in Nagpur mandarin (STG) on raised bed. However, fruit yield of mandarin in flat system was higher than raised bed as noted in Nagpur mandarin-STG (22.56 t/ha). Regarding sweet oranges, maximum average fruit weight (218.3 g), fruit length (72.0 mm), fruit diameter (73.33 cm) and lowest seed content (2.33) was noted in Cutter Valencia whereas juice content was maximum in Pineapple on raised bed (52.0%). Similarly, maximum TSS (9.93°B) and yield (31.26 t/ha) was recorded in Sweet orange Mosambi (TM-33) followed by Cutter Valencia (28.34 t/ha) on raised bed. Notably, pummel and grapefruit performance on both planting systems was comparable with Flame Grapefruit registering the lowest seeds per fruit (3.00) and maximum juice content (40.0%); however, US Pummelo-145 registered the maximum TSS (8.97°B). Among the acidic types, Citron mutant registered the maximum fruit weight (217.67 g), peel thickness (4.00 mm) and seeds/fruit (40.3) while NRCC Acid lime-7(28.9 t/ha) and NRCC Acid lime-8 (30.5 t/ha) recorded maximum yield on raised bed. Study indicated that Sweet orange Mosambi (TM-33), Cutter Valencia, US Pummelo-145 and NRCC Acid lime-8 depicted excellent growth and fruit yield performance under the plains of north-eastern region.

Keywords: Citrus, Raised Bed, Flat Bed, Northeast, Yield



Advancing Citriculture for Agro-economic Prosperity

### TS-2-P-08

## Effect of pruning to improve yield and fruit quality of 'Kinnow' mandarin plants under high density plantation

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Due to rapid increase in population and limited available resources, world trend is moving towards high density plantation for proper utilization of land and resources. The present experiment was planned to investigate the effects of pruning on plant yield and fruit quality of 'Kinnow' mandarin grown under high density plantation. Plants were pruned by three different ways i.e. (i) pruning of one side's branches, (ii) pruning of both sides' branches and (iii) pruning of top branches. Overall results revealed that all the pruning treatments were effective but the plants pruned from both sides performed better in terms of improved yield (greater number of fruits and higher total fruit yield per plant) and fruit quality (increased fruit size, higher juice weight, lower peel weight, lower juice TA, greater TSS: TA ratio, and higher total phenolic content (TPC) and total antioxidants). Keeping in view the above results, it is concluded that 'Kinnow' plants grown under high density plantation should be pruned from both side of canopy to obtain better yield and fruit quality.

Keywords: Citrus, Pruning, Total phenolic content, Total antioxidants



## Citrus rootstock tolerance to *Phytophthora* root rot

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Rootstocks play an important role in intensive citrus cultivation, especially in terms of disease resistance. Twelve diverse root stocks (NRC-1, NRC-2, NRC-3, NRC-4, NRC-5, NRC-6, CRH-12, CRH-47, Alemow, Sour orange, Rangpur lime (Akola), and Rough lemon) were evaluated for tolerance or susceptibility to *Phytophthora nicotianae* under sick pot conditions. Ten months old seedlings of respective root stocks were transplanted in plastic pots and after settings in pots inoculated with *P. nicotianae* suspension and evaluated after six months. Disease incidence, feeder root rot rating, leaf fall percentage and seedling height were used as parameters to measure the tolerance or susceptibility of the rootstocks to the tested pathogen. Significant differences occurred between the root stocks, but none of the rootstocks found immune in respect to Phytophthora root rot. However, comparatively, Alemow (*Citrus macrophylla*) was found tolerant to *P. nicotianae* as recorded minimum disease incidence (3.33%), least feeder root rot rating (0.60) and lowest leaf fall (3.07%) while Rough lemon (*C. jambheri*) showed susceptible to *P. nicotianae* as recorded maximum root rot incidence (27.78%) with highest feeder root rot rating (3.10) and leaf fall (21.80%). Tolerant rootstocks can offer excellent means of disease control against *Phytophthora* root.

Keywords: Citrus root stocks, *Phytophthora nicotianae*, Root rot, Tolerance



## Enhanced acid lime production through microbes-mediated rhizosphere hybridization

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Altering functionality of rhizosphere microbiome of any crop by either tailoring the rhizosphere through inoculation of microbial consortium or hybridizing two contrasting crop-based rhizospheres, are some innovative way forward approaches to sustain improved soil health mediated quality citrus production. In this background, we carried out an experiment to study the effect of rhizosphere hybridization on yield of acid lime (cv. NRCC acid lime-7) at experimental orchard of Indian Council of Agricultural Research- Central Citrus Research Institute, Nagpur, Maharashtra, India. A total of eight treatments consisting of combination vermicompost (10 kg or 20 kg/plant) and rhizosphere soil of acid lime (Citrus aurantifolia Swingle) and Nagpur mandarin (Citrus reticulata Blanco) were formulated as rhizosphere hybridization, along with microbial consortium (Bacillus pseudomycoide Nakamura, MF113272; Acinetobacter radioresistens Nishimura, MF113273 ; Micrococcus yunnanensis Cohn, MF113274; Aspergillus flavus Link, MF113270, and Paenibacillus alvei Cheshire and Chevne, MF113275) and tested through RBD experimental design during 2018-20. Treatment containing microbial consortium along with vermicompost (10 kg or 20 kg/ plant) expressed the best response in terms of soil microbial population (54.60 x  $10^{3}$  cfu/g soil) compared to either hybridized soil treatment (49.74 x  $10^{3}$  cfu/g soil) or non-hybridized rhizosphere (43.06 x  $10^{3}$  cfu/g soil) or even with chemical fertilizer treatment (36.90 x  $10^{3}$ cfu/g soil), ensuring a consequently higher plant available nutrients pool and concentration of different nutrients in index leaves, a trendsetter for elevated crop response. Increased leaf nutrient accumulation and response on vegetative growth parameters (plant height, trunk girth, tree spread, all expressed as canopy volume) were observed strongly inter-related, thereby, leading to elevation in fruit retention, and fruit size dimension coupled with reduced fruit drop. These physiological events culminated in higher fruit yield (43.78 kg/plant) with a higher percentage of A- grade size fruits (15%). Such microbes mediated rhizosphere transformation also influenced the quality index of acid lime fruits. These results are strongly depictive of the value-added response of microbial consortium plus vermicompost followed by hybridized rhizosphere in combination with vermicompost with regard to guality production of acid lime coupled with rhizosphere health.

Keywords: Acid lime, Rhizosphere hybridization, Microbial consortium, Vermicompost



## Evaluate the different mandarin cultivars under Bundelkhand conditions

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A study was conducted to evaluate the different Mandarin (Citrus reticulata) cultivars under Bundelkhand conditions at Banda University of Agriculture and Technology, Banda, namely Kinnow, Daisy and W. Murcott. Kinnow originated as hybrid between king and willow leaf Mandarin (Citrus nobilis × C. deliciosa) the skin colour of kinnow is golden yellow which is a major asset of this fruit marketing it matures in January to February and Daisy is cross between Fortune and Fremont and the skin colour of daisy is reddish orange and glossy appearance and it matures within first week to third week of November. W. Murcott is cross between Murcott and unknown parent skin colour is orange coloured and easy to peel skin It is mid-season variety, maturing in January. Maximum fruit weight (229.50), Fruit diameter (74.54 mm) and fruit length (64.53 mm) were observed in Daisy followed by Kinnow (175.45g, 72.78 mm and 58.87 mm respectively) and minimum fruit weight (169.50), Fruit diameter (71.54 mm) and fruit length (56.53 mm) observed in W Murcott. Maximum number of fruit per tree was observed in kinnow (403 fruit /tree) followed by W. Murcott (360fruit/tree) and minimum observed in Daisy (325 fruit/tree) but highest yield per tree was maximum observed in Daisy followed by Kinnow and minimum observed in W. Murcott (72kg, 69kg, 61kg respectively kg /tree) physico-chemical properties indicated maximum value of TSS kinnow(9.77°Brix) followed by Daisy(9.65°Brix) and minimum observed in W. Murcott. (9.12°Brix) The maximum acidity found in W. Murcott (0.82%) followed by Kinnow(0.71%) and the minimum was observed in Daisy(0.54%). The average no of seed per fruit was maximum observed Kinnow (18.34) followed by Daisy (17.95) and minimum seed observed in W. Murcott (14.0).

Keyword: Mandarin, Bundelkhand region, TSS, Acidity, Peel colour



## Response of acid lime (*Citrus aurantifolia* Swingle) to varied nutritional regimes under high density planting in diverse Indian states: Andhra Pradesh, Tamil Nadu and Maharashtra

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An experiment was carried out within the ICAR-All India Co-ordinated Research Project on Fruits at three research centres, with the aim of investigating the optimal nutrient requirements under high density planting for enhancing the growth and yield of acid lime cv. Balaji in Andhra Pradesh, PKM-1 in Tamil Nadu and Phule Sharbati in Maharashtra. The primary objective of this study was to determine how productivity could be increased by manipulating the number of plants per unit area and adjusting nutrient levels using different nitrogen (N) sources. The specific agricultural practices and preferences of the respective regions were taken into account during the study. The experimental design employed a factorial randomized block design, replicated thrice, with three different planting densities (referred to as S1, S2, and S3) and three nutrition levels (referred to as L1, L2, and L3). The planting densities are as follows: S1 with a spacing of 6x6 meters, accommodating 277 plants per hectare; S2 with a spacing of 6x5 meters, accommodating 333 plants per hectare and S3 with a spacing of 6x4 meters, accommodating 416 plants per hectare. The nutrition levels were categorized as L1 with 75% nitrogen from inorganic sources, 25% nitrogen from organic sources (FYM), and full inorganic P & K fertilization, excluding the contribution from FYM; L2 with 50% nitrogen from inorganic sources, 50% nitrogen from organic sources (vermicompost), and full inorganic P & K fertilization, including the contribution from vermicompost; and L3 with 100% recommended dose of fertilizer (RDF) inorganic sources only, serving as the control. The findings of the experiment revealed that the HDP (S3: 6x4 m) of acid lime led to increased productivity when compared to the conventional planting system at all research centres. Notably, there was a significant interaction between nutrients and high density planting at Tirupati, with the application of 50 kg vermicompost and 800 g N, 250 g P2O5 and 400 g K2O per plant per year through inorganic fertilizers under high density planting at 6 x 4 m spacing (416 plants/ha) and hence recommended for higher yield (21.68 t/ha) and net monetary returns(1.92). Whereas, plantation of acid lime plants at 6 x 5 m spacing (333 plant/ha) along with the application of 30 kg FYM and 450 g N, 140 g P2O5, and 150 g K through inorganic fertilizers are recommended for higher yield (14.57t/ha) and net monetary returns(1.95) at Periyakulam centre. However, these interaction effects were not significant at Rahuri.

#### Keywords: HDP, Nutrition, Acid lime



## Leaf nutritional status of Nagpur mandarin orchards of Katol *tehsil*, Nagpur district

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The present exploration was undertaken to study "Leaf Nutritional Status of Nagpur Mandarin orchards" of Katol tehsil, Nagpur District". Seven locations of Katol tahsil viz., Ladgaon, Dhiwarwadi, Fetri, Lamdhan, Kalkuhi, Parsodi and Amnergondhi (rithi) were selected to evaluate leaf nutritional status of Nagpur mandarin. In all fifteen orchards were selected from selected locations. The leaf samples were collected from all fifteen mandarin orchards to study nutritional status of mandarin. The samples are collected from both bearing and non-bearing terminals (5-7 months old) from randomly selected 10 trees from each orchard. The initial analysis of soil revealed that, soil reaction of study area exhibited slightly acidic to slightly alkaline in nature. In all locations, there was no much variation in electrical conductivity of soil and these soils were non-saline in nature. Soils were medium to high for organic carbon content and slightly calcareous to calcareous for calcium carbonate content. The available nitrogen was ranged from (250.88 to 398.68 kg ha<sup>-1</sup>) in surface, available phosphorous was observed in range (11.85 to 26.36 kg ha<sup>-1</sup>) in surface indicating that, soils are low to medium in category for both available nitrogen and phosphorous. Available potassium ranged from (300.49 to 483.13 kg ha<sup>-1</sup>) in surface indicating high to very high availability of potassium in soil. Available Sulphur ranged from (10.25 to 15.24 kg ha<sup>-1</sup>) in surface indicates its low to moderate availability in soil. Leaf nutrient like total nitrogen ranged from 1.82 to 2.64 %, total phosphorous ranged from 0.25 to 0.43 % and total potassium ranged from 0.90 to 1.63 %. The lowest total nitrogen present in the leaves of mandarin orchards is found in the location 1 Ladgaon and highest total nitrogen 2.64 % in the location 6 Dhiwarwadi. The lowest total phosphorous 0.25% was present in the location 2 Ladgaon and highest 0.43 % total phosphorous in the leaves of location 3 Ladgaon. The lowest total K 0.90 % present in the leaves of location 2 Ladgaon and highest total K 1.63 % present in the location 11 Kalkuhi of Nagpur mandarin orchards of Katol tahsil. Leaf micronutrient Fe, Mn, Cu and Zn ranged from 135.20 to 158.01 ppm, 32.20 to 58.50 ppm, 13.90 to 55.80 ppm and 20.00 to 35.40 ppm in leaves of orchards. Total N, P and K were moderate to high in leaves of orchards. From the above results, it can be concluded that soils of Nagpur mandarin are slightly alkaline and good fertility status for growing healthy Nagpur mandarin orchards.

Keywords: Mandarin orchards, Katol tehsil, Leaf nutrition, Major nutrients, Micro nutrients



## Fruit Distortion: A ruinous disorder for Nagpur mandarin

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Nagpur mandarin is one of the best mandarins in the world. Production of this fruit crop in the Central and Western parts of India is increasing every year. Maharashtra is the country's largest producer and exporter of oranges. The area under orange cultivation in the state is about 1.21 lakh hectares and the total production exceeds 7 lakh tons annually. A distortion is being seen in the orange fruit from last few years, which the local people call wai-bar. Since last few years, this distortion is increasing and due to this, farmers suffer a heavy loss. To minimize the incidence of fruit distortion in Nagpur mandarin experiment was carried out at farmer's field at Ruikhed of Akola district. Keeping in view the earlier study, 7 modules were prepared which includes soil application of NPK, Micronutrient, Humic acid, Jivamrut, Arka Microbial Consortia, MAU Consortia Biomix, Foliar spray of Potassium sulphate, Zinc Sulphate, Micronutrients (Fe, Mn, Zn, Cu) With integrated pest management. Module 7 is treated as Control (Farmers Practices). Trees treated with Module 4 (At the time of stress release 400 g N + 200 g P + 200 g K + 200 g Plant<sup>-1</sup>MAU Consortia biomix + 1000 ppm Humic acid, After 1 month of 1<sup>st</sup> application (February), 400 g N + 200 g P + 200 g K, After 1 month of 2<sup>nd</sup> application of 400 g N + Spray of 1% 0:52:34 (N:P:K) + 20g Plant<sup>-1</sup>Zn+Fe+Mg (Soil application), At pea stage Foliar spray of NAA 10 PPM + Neem oil 5 %, June – July Spray of Nimbodi ark 5% + 2,4-D 10 ppm, August – September Spray of Ethion + 1 % Potassium nitrate KNO<sub>3</sub>, 7 doses of Jivamrut 10L Plant<sup>-1</sup> was applied at 15 days interval ) Shows the minimum incidence of distorted fruits followed by Module 3 (At the time of stress release 400 g N + 200 g P + 200 g K + 200 g Plant-1 MAU Consortia biomix + 1000 ppm Humic acid, After 1 month of 1st application 400 g N + 200 g P + 200 g K, After 1 month of 2nd application, 400 g N + Spray of 1% 0:52:34 (N:P:K) + 20g Plant-1, Zn+Fe+Mg, At pea Stage Foliar spray of NAA 10 PPM + Neem oil 5 %, June – July Spray of Nimbodi ark 5% + 2,4-D 10 ppm, August – September, Spray of Ethion + 1 % Potassium nitrate KNO3, 7 doses of Jivamrut 10L Plant<sup>-1</sup> was applied at 15 days interval). Plants treated with Module 4 and Module 3 reduced the losses by 75% and 60% respectively.

Keyword: Waibar, Nagpur mandarin, Plant nutrition, Integrated nutrient management.



## Rooting media's effect on acid lime on germination and growth of its root and shoot (*Citrus aurantifolia* Swingle.)

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The present study was carried out in 2020–2021 at the Horticultural Research Centre of the Sardar Vallabhbhai Patel University of Agriculture & Technology in Modipuram, Meerut. Two citrus cultivars (Balaji, NRCC-7) were subjected to an experiment to examine the effects of various growing media compositions made up of soil, sand, FYM, cocopeat, leaf mould, and vermicompost. There were 12 different treatments in total, each replicated four times, and the experiment was set up using a factorial randomised block design (FRBD). Two plants are used in each treatment. A total of 96 plants were chosen for the investigation. The observations were recorded on days to germination, germination percentage, final survival percentage, number of shoot/seedling, average shoot length, number of leaves/shoot, total number of leaves/seedling, length of largest shoot, height of seedling, girth of seedling stem, number of roots/seedling, dry weight of shoot, fresh weight of shoot, fresh weight of root, dry weight of root. The findings showed that the NRCC-7 variety with growing media composition T5 (Soil, Cocopeat, and Vermicompost) had the highest germination percentage (92.005), final survival percentage (95.580), number of shoots per seedling (12.750), average shoot length (15.475 cm), number of leaves per seedling (14.548), total number of leaves per seedling (64.750), number of roots per seedling (5.250), and fresh weight of root (5.210 gm). In accordance with additional observations, Balaji's growing media composition T5 (Soil + Cocopeat + Vermicompost) was determined to have maximum shoot length (15.475 cm), length of longest shoot (24.950 cm), height of seedling (33.623 cm), girth of seedling stem (0.253 cm), and fresh weight of shoot (15.063 gm). The number of days to germination is greatest in Balaji with T1 (soil + sand + FYM). With growing media T6 (soil + sand + FYM + Azotobactor), Balaji has the heaviest dry shoot weight (9,015). Balaji with T4 (soil, cocopeat, and FYM) has the root with the highest dry weight (1.993). The present study revealed that the NRCC-7 variety with growing media composition T<sub>5</sub> (soil, cocopeat, and vermicompost) showed significantly higher growth in the maximum number of parameters studied.

Keywords: Rooting media, Acid lime, Germination, Vermicompost, Factorial randomised block design



## Recent advances in propagation, planting system and micro-irrigation of citrus

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Citrus is a most important crop among the fruits crops belong to the family Rutaceae. Citrus has been grown as a major fruit crop in more than 140 countries of the world. Citriculture is a significant agricultural practice worldwide. Citrus production has been considerably impacted by biotic and abiotic stressors in recent years like that high incidence of insect-pests (Citrus psylla, aphids), many diseases (Citrus canker, Phytophthora, Citrus greening etc.), Viruses and virus like organisms (Tristeza, Exocortis, Psorosis, viroids, etc.), citrus abatement as well as bad management of orchard. Several new technologies have been developed and adopted around the world to overcome above issues. Shoot tip grafting and micro budding are the most viable options for the generation of virus-free planting material. Citrus productivity was increased by using high-density planting, proper training and pruning. The micro-irrigation system is also used to optimise the usage of nutrients and water. Additionally, precision citriculture aims are to improve fruit quality, chemical use effectiveness, energy conservation, environment protection and balanced eco-system. Regardless of the availability of soil, water, and fertilizer resources, one of the primary problems of modern citrus fruit production is micro-irrigation systems. Different micro-irrigation techniques have proven to be superior to the more common flood irrigation, with micro-jets having minimal advantage over the other techniques. This review presents and discusses the main aspects of these advances, such as advances in propagation techniques, scion and rootstock selection, genetic characterization and certification, tree size standards, cultural practices such as irrigation and fertilisation for the production of citrus propagation materials and nursery plants, and nursery management tools.

Keywords: Citrus, High density planting, Precision citriculture, Micro-irrigation



# Effect of growth regulators on fruit splitting, yield and quality attributes of Daisy mandarin

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The purpose of the current study was to investigate the impact of foliar application of growth regulators on fruit splitting, yield and quality attributes of Daisy mandarin budded on 'Rough lemon' (*Citrus jambhiri* Lush) rootstock. The study was carried out in 2021 at Punjab Agricultural University Fruit Research Station, Jallowal-Lesriwal, Jalandhar and the Laboratory of the Department of Fruit Science. Different concentrations of growth regulators including Gibbrellic acid (GA<sub>3</sub>) at 10ppm, 20ppm and 30ppm and Naphthalene acetic acid (NAA) at 10ppm, 20ppm and 30ppm, along with control were applied at fruit set, 30 DAFS and 60 DAFS. The experiment was laid out in a Randomized Block Design with three replications. The main objectives of the experiment were to minimize fruit splitting, improve productivity and enhance fruit quality. The results showed that foliar application of GA<sub>3</sub> @10ppm significantly reduced the fruit splitting percentage, followed by NAA @ 20ppm as compared to control. The foliar application of GA<sub>3</sub> at 10ppm significantly increased fruit size, fruit retention, fruit yield, total phenolic content and antioxidant activity in the fruit juice as compared to control. In conclusion, foliar application of growth regulators particularly 10 ppm GA<sub>3</sub> and 20 ppm NAA showed promising results in reducing fruit splitting, increasing productivity and improving fruit quality in Daisy mandarin.

Keywords: Antioxidant, Citrus, Cracking, GA<sub>3</sub>, Growth regulators, NAA



# Effect of different sources of nutrient on biological properties of soil in acid lime orchard

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An experiment was carried at Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola to study the effect of different combinations of bio-organics on soil nutrient status of acid lime. The experiment was laid out in Split Plot Design with three replications. There were ten treatment combinations comprising two bio-fertilizer levels. Significantly highest soil microbial population, soil microbial biomass carbon, CO<sub>2</sub> evolution and dehydrogenase activity were recorded in treatment B<sub>1</sub>. The treatment M<sub>1</sub> was found highest microbial population during both the seasons of experiment, whereas lowest fungi and *Actinomycetes* were observed in treatment M<sub>3</sub> and bacteria in M<sub>4</sub> during first season, while during second season fungi was noticed in M<sub>4</sub> and bacteria and *Actinomycetes* in treatment M<sub>5</sub>. The treatment combination B<sub>1</sub>M<sub>1</sub> were recorded highest microbial population which was found at par with treatment combination B<sub>1</sub>M<sub>2</sub> whereas, lowest obtained in treatment combination B<sub>2</sub>M<sub>5</sub>. The treatment M<sub>2</sub> was found maximum soil microbial biomass carbon, CO<sub>2</sub> evolution and dehydrogenase activity and minimum in M<sub>5</sub>during both the seasons of experiment. The soil biological properties were significantly improved with application of M<sub>2</sub> (7.5 kg VC + 2.5 kg PC + 10 kg NC per plant per year).Whereas higher soil microbial population B<sub>1</sub>M<sub>1</sub>

Keywords: Bio-fertilizer, Organic manure, Soil microbial



#### Effect of different bio-organics on growth and yield of acid lime

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An experiment was carried at Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola to study the effect of different combinations of bio-organics on soil nutrient status of acid lime. The experiment was laid out in Split Plot Design with three replications. There were ten treatment combinations comprising two bio-fertilizer levels. Significantly maximum incremental growth was recorded in treatment  $B_1$  and minimum in  $B_2$ . Themaximum incremental growth was observed in treatment  $M_5$ . The maximum yield and yield contributing parameters were recorded in treatment  $M_5$  while, minimum were recorded in treatment  $M_3$ . In interaction effect of bio-fertilizers and sources of nutrients. The treatment combination of  $B_1M_5$  was recorded maximum fruit yield kg plant<sup>-1</sup>) and t ha<sup>-1</sup> which was found at par with treatment combination of  $B_1M_2$ . Whereas, minimum fruit yield was recorded in treatment combination of  $B_2M_3$ . On the basis of finding reported in present investigation. The interaction effect of bio-fertilizers and different sources of nutrients was found significant effect on incremental growth and fruit yield of acid lime.

Keywords- Bio-fertilizer, Organic manure, Growth, Yield acid lime



#### Management of fruit drop in Nagpur mandarin using different growth substances

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Mandarin is one of the important fruit of central India, considering major issues, the fruit drop identified as a most burning issues specially in *ambia bahar*. The result indicated, that the treatment  $T_7$  comprised of spraying of CPPU 10ppm + Urea 1% at pea stage followed by three sprays of N-ACTA 10 PPM + Brassinoides 4 PPM + Folic acid 40 PPM each during the month of viz. July-Aug-Sept recorded maximum yield parameter ie. 679.33,number of fruits /tree having fruit weight 156.33 kg/tree(43.46 t/h) with an average weight of 184.83g. with juicy fruits (48.33%). The higher number of fruits harvested in the treatment  $T_7$  is resorted to checking of physiological fruit drop (recorded least fruit drop ie. 41.02 %) at different stages, initially at pea stage which mostly occurs due to competition amongst the fruitlets later peak drop which is reported in the recent years during period Aug-Sept mostly due to adverse climatic conditions (continuous rains, vis-à-vis water stagnation). The experiment was conducted at the Experimental farm of AICRP on fruits, Dr. PDKV, Akola during the year 2021-22 using different growth substances, different concentrations and combinations with other SOP of the crop having total 10 treatments randomised thrice in RBD. The results also indicated that the absence of any measures to check physiological drop due to the adverse climatic conditions can cause as much as 73.30 percent fruit drop making meagre yield levels.

Keywords: Fruit drop, Mandarin, CPPU, Brassinoides, Folic acid, N-ACTA, Growth substances



#### Effect of plant growth substances on germination of acid lime seedlings

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The present investigation is entitledon "Effect of plant growth substances on germination of acid lime seedlings (*Citrus aurantifolia* swingle). cv. PDKV Lime was conducted at (AICRP) on Fruits, (Citrus) Dr. PDKV, Akola during 2020-21 and 2021-22. The experiment was laid out in a Randomized Block Design with three replications and fourteen treatments. Initially, seeds were pre-soaked in the plant growth regulator solution and kept in muslin cloth impregnated with the same plant growth regulator solution for 12 hours in the plant growth regulator solution of respective treatments. Which was followed by foliar spraying of growth regulator solution viz.GA<sub>3</sub>, brassinolide, and 6-BA comprising of different combinations and concentrations at 90 and 120 days after sowing and compared with a check (GA<sub>3</sub> - 50 ppm + Urea 1%) and control (only water soaking). The results revealed that treatment comprised of GA<sub>3</sub> 50 ppm + 6 BA - 25 ppm + Brassinolide 2 ppm was found effective maximum germination percentage (94.50%), seedlings vigor index-I (1414.94), seedlings vigor index-II (34.12), Bartletts rate index (0.501), emergence rate index (44.25), polyembryony percentage (65.50) and number of seedlings per seed (2.33). The treatment recorded minimum days for the germination (28.17). while minimum values were observed in T<sub>14</sub> control.

Keywords: Acid lime, PGRs, Germination, Nursery seedlings



### Studies on effect of different plant growth substances on growth of acid lime seedlings

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Acid lime seeds often lose viability and vitality very soon while seed coat acts as barriers because it interferes with early germination due to the presence of certain inhibitory substances. Further raising optimum buddable-sized seedlings is also a time-consuming job. in order to overcome this problem the present study was framed to get the seedlings ready within the shortest possible time, and growth will be accelerated which enhances guick germination and growth of acid lime seedlings. The present investigation is entitled on "Effect of plant growth substances on growth of acid lime seedlings. cv. PDKV Lime was conducted at (AICRP) on Fruits, (Citrus) Dr. PDKV, Akola during 2020-21 and 2021-22. The experiment was laid out in a Randomized Block Design with three replications and fourteen treatments. Initially, seeds were pre-soaked in the plant growth regulator solution and kept in muslin cloth impregnated with the same plant growth regulator solution for 12 hours in the plant growth regulator solution of respective treatments. Which was followed by foliar spraying of growth regulator solution viz. GA<sub>3</sub>, brassinolide, and 6-BAcomprising of different combinations and concentrations at 90 and 120 days after sowing and compared with check (GA<sub>3</sub> - 50 ppm + Urea 1%) and control (only water soaking). The results revealed that treatment comprised of GA<sub>3</sub> 50 ppm+6 BA -25 ppm + Brassinolide -2 ppm was found effective maximum height of seedlings (94.81 cm), No. of branches (6.58), No. of leaves (93.22), a diameter of seedlings (1.12 cm), Leaf area (56.27 cm2), Chlorophyll content (67.06 mg/g), and absolute growth rate (1.05 cm/day) while minimum values were observed in  $T_{14}$  control.

Keywords: Acid-lime, Growth, Nursery, PGRs



# Innovative and cost effective method for rapid production of disease free planting material of acid Lime

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Availability of genuine & disease free planting material is one of the problems of Citrus Industry and hurdle for the proliferation of Citrus Industry. Although envisages great scope for the production of disease free planting material, technical knowhow, higher unit cost of production, initial investments towards capital and laboriousness leads to poor adoption level by the nursery man. A cheaper and rapid technique for the multiplication disease free planting material of acid lime developed at All India Coordinated Research Project Fruits, Dr. PDKV, Akola. The technique comprised of containers developed, out of local waste material of size 60 cm depth (height) 300 cm width and length 8 to 10 m. The arrangements of irrigation to the modified containers were made with micro-sprinklers, foggers & drip irrigation. A fumigation assembly provided to sterilize soil frequently. The plants raised on modified containers under Net houses were compared with different multiplication techniques comprised of viz. T<sub>1</sub>: Outdoor raised beds of size 2m length, 1m breadth, 15cm height (Local practice), T<sub>2</sub>. Nursery under shade net on raised beds of size 2m length, 1m breadth, 15cm height, T<sub>3</sub>, Nursery under shade net in Containers / plastic trays of size 56×36×11cm, T<sub>4</sub>: Nursery under shade net in : Modified containers prepared out of locally available material (asbestos sheet) of size 7.5m×1m×0.6m, T<sub>5</sub>: Nursery under shade net in Polythene bags of size 22.5×50cm. Pooled data from 2015 to 2019 (four year pooled mean) indicated seedlings raised in modified container (T<sub>4</sub>) exhibited highest growth parameter viz. plant height (68.53 cm), no of leaves/seedling (76.84), girth of seedling (2.68 cm), fresh wt of root: (26.58 g), root volume (40.56 cc). Seedlings raised on modified nursery took minimum days for attainment of sailable quality (213.19 days), with maximum C:B ratio (3.92) and transplanting survival (95.91%) next to and at par with seedlings raised in polythene bags and plastic trays. Thus modified containerized nursery was found to be effective method which had reported early & rapid growth parameter with economic production of planting material of acid lime with this nursery can be raised twice a year.

Keywords: Acid lime seedling, Cost effective, Disease free, Innovative



### Enhanced tree retention of fruits by applying growth regulators improved yield of mandarin

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Mrug bahar is most remunerative bahar of mandarin compare to the Ambia bahar. However many times regulation of the Mrug bahar becomes difficult as the bahar mostly regulated by imposing the water stress and many times unseasonal rains in April- May may break stress and even if flowers it may shed due to high temp. So as unassured rains and climatic conditions may result less flowering or fruit drop, making off season. Thus, necessities the retention of valuable fruit and finally count as on season. In view of above the experiment was carried out for the checking of mrug bahar fruit drop in mandarin. An experiment conducted at AICRP on Fruits, Dr.PDKV, Akola during period from year 2020-2023 laid out in Randomized Block Design comprised 15 treatments of different new generation growth regulators and promoters alone and different combinations as well as concentrations. Growth substances were applied through foliar sprays compared with earlier recommendation and also check (no treatment). Effect of growth regulators and promoters in combination helped in checking fruit drop (which was reflected in final produce, and confirmed in treatment GA15 ppm + brassinolides 2 ppm applied twice i.e at the initiation of flowering and at pea size fruit stage which has recorded maximum pooled yield parameters (96.91 kg/tree for 711.50 fruits having average wt 150.88 g) which is due to least fruit drop 16.36 %) vis -a -vis enhanced fruit retention and it was at par with treatment GA15 ppm + CPPU 15 ppm (applied at initiation of flowering and pea size fruit stage) The guality parameters were not influenced by the treatments when compared.

Keywords: Brassinolides, CPPU,GA, Fruit drop, Fruit retention, Growth regulator, Mandarin



Advancing Citriculture for Agro-economic Prosperity

#### TS-2-P-25

### Effect of different chemical for abandon of unwanted *ambia bahar* to regularize flowering of *mrug bahar* in mandarin

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Mrug and ambia bahar in mandarin are common in Central India. Ambia bahar is considered as the natural bahar mostly governed by water stress as well as low temp prevailed during month of December is is most assured. It may flower many times even if any measures for flowering regulation not taken. However, many times regulation of the Mrug bahar becomes difficult as there may be unwanted flowering in Ambia bahar which may hinder subsequent Mrug bahar results partial or no flowering. So also, Mrug bahar mostly regulated by imposing the water stress and unseasonal rains in April- May months may break stress and even if flowers it may shed due to high temp. Thus necessities the abandonment of Ambia flowering and there by regularize the flowering in Mrug bahar. In view of above the experiments was carried out for the checking of Ambia bahar and regularize the flowering in Mrug bahar. The experiment was laid out in Randomized Block Design comprised nine treatments (replicated thrice) of different potent flower/ fruit thinner used different concentrations applied through foliar sprays so to assess safest and effective for the set objective. The results indicated that treatment NAA 300 ppm has recorded maximum induced fruit drop (76.44%) owing to which recorded minimum yield parameters (3.33 kg/tree for 22 fruits) in ambia bahar. However absence of treatment (control) recorded minimum fruit drop (43.63%) and highest yield parameters (24.65 kg/tree for 159.50 fruits/tree) in ambia bahar 2022. Measures towards abandonment of ambia bahar 2022 reflected in fruiting and production of mrug bahar 2022-23. The results indicated that treatment 2,4-D 50 ppm has recorded maximum yield parameters 52.63 kg/tree for 368.67 fruits) in mrug bahar and was at par with treatment NAA 300 ppm and Ethaphon 1000 ppm. However the absence of treatment (control) recorded least yield in mrug bahar 2022-23. Quality parameters were found not influenced, irrespective of the treatments when compared.

Keywords: Abandon, Ambia flowering, Ethaphon, GA, Mrug flowering, NAA



#### Effect of transplanting age of seedling on growth yield and quality of acid lime

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Juvenile age of orchard is of the hurdle for the popularization of the fruit crop. Acid lime crop has 4 to 5 years age of juvenile period, during which the farmers has to take care of the unfruitful orchard using all the resources. With the coming up of the aged and large sized material in a large polythene bags from the private nurseries and farmer fancied their chances for early establishment of the orchard. There is always dilemma, exactly what should be age of transplanting for the successful and productive orchard? Thus, the issue needs to be addressed on serious note. Following which, an experiment on "Effect of transplanting age of seedling on growth yield and quality of acid lime" carried out at AICRP (Fruits) Dr. PDKV, Akola during period from 2019-20 to 2022-23. The treatments included transplanting of one, two and three years seedlings of acid lime cv PDKV lime in the field and were replicated seven times in RBD. The results indicated 100% survival with transplanting of one year aged seedlings, where as 85.75 % in treatment three year aged seedling after four years of experimentation. The maximum plant height (2.08 m) and canopy volume (6.0m<sup>3</sup>) was reported in treatment with two year aged seedlings. The bearing and fruit yield was reported in all the aged seedlings, however three year aged seedling started bearing itself in the first year of transplanting and recorded maximum no of fruits i.e. 241.50 weighing 7.40 kg per tree during its fourth year of bearing.

Keywords: Acid lime, Transplanting age



#### Effect of different mulches on growth, yield and quality of acid lime

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The present investigation was carried out to explore the Effect of different mulches on Growth, Yield and Quality of acid lime and conducted at Acid lime garden, College of Horticulture, Dr. PDKV, Akola. The experiment was laid out in Randomized Block Design with eight treatments and four replications during *mrig bahar* 2021-2022. Result obtained in the present investigation revealed that, polythene mulches and organic mulches influenced the growth, yield and quality of acid lime fruits. The black polythene mulch showed significantly more length of shoot (14.07 cm), number of new shoots per branch (6.39), leaf area (15.05 cm<sup>2</sup>) followed by dry grass mulch. Black polythene mulch also record highest number of fruits tree<sup>-1</sup> (683.62), weight of fruits tree<sup>-1</sup> (36.09 Kg), yield ha<sup>-1</sup> (99.96 q), diameter of fruit (3.97 cm), average weight of fruit (52.80 g), average volume of fruit (31.69 cm<sup>3</sup>), juice percent (52.46 %), TSS (8.43 <sup>o</sup>Brix), ascorbic acid (30.32 mg/100 ml), soil temperature (41.17 <sup>o</sup>C) and soil moisture (22.88 %) followed by dry grass mulch. Significantly minimum fruit drop (85.14 %), peel percent (17.93 %) and weed count (3) was observed in black polythene mulch followed by dry grass mulch. Among the polythene mulches, black polythene mulch and among the organic mulches, dry grass mulch gave significant increase in all characters.

Keywords: Kagzi lime, Mulches, Yield, Quality



# Nature and extent of rough skin disorder in Nagpur mandarin (*Citrus reticulata* Blanco) and its management

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Rough skin fruit disorder is an important physiological fruit disorder affecting quality production and tonnage in Nagpur mandarin. The extent of disorder occurs in first 2-3 years of bearing when number of fruits is less (100-200/plant) in the young orchards with almost 60 % fruits assuming rough skin. In some old and orchards when the number of fruits is limited to 1-200 fruits or when the fruits are borne in single clusters on the shoots near scaffold branches the fruits tend to assume rough skin. At harvest rough skinned fruits are found to be large in size (154.25-204.25g) with improper fruit shape (length/breadth ratio is 0.89-1.03), and more thickened rind (3.02-3.38 mm) compared to normal fruits. Core diameter was more (20.59-23.61 mm) and also noted the reduced juice per cent (40.47-43.20%) and TSS (6.90-7.87%). A field experiment was carried out at ICAR-CCRI, Nagpur, Maharashtra during 2020 and 2021to correct the disorder and improve the fruit yield and quality characteristics of Nagpur mandarin (Citrus reticulata Blanco) in Ambe bahar (Spring blossom crop) crop through foliar application of 2,4-D 15 ppm+ urea 1.5%, GA<sub>3</sub>15 ppm + urea 1.5%, amrut pani, and soil application of recommended dose of fertilizer (600:300:300g of NPK/plant) with and without potassium. The minimum per cent of disorder incidence (5.80 %) was recorded in RDF without potassium followed by GA<sub>3</sub> 15 ppm + urea 1.5% (7.22%) over the control (13.04%). The treatment GA<sub>3</sub> 15 ppm + urea 1.5% found to record the maximum yield per plant (88.97 kg/plant), total estimated yield per hectare (24.64 t/ha), fruit weight (169.50g), fruit volume (167.50cm<sup>3</sup>), fruit breadth (73.02 mm), acidity (0.69%) and vitamin C (46.74 mg/100 ml of juice) over the control. The treatment 2,4-D 15 ppm + urea 1.5% found to record the maximum juice per cent (47.64%) and TSS (10.92%), and *amrutpani* recorded the maximum TSS/Acid ratio (17.57). It was concluded that foliar application of plant growth regulators like 2,4-D 15 ppm+ urea 1.5% and GA<sub>3</sub>15 ppm + urea 1.5%, and reducing soil application of potash fertilizers in high potassium available soils reduced the rough skin disorder and improved the physico-chemical characteristics of fruits at harvesting.

Keywords: 2,4-D, GA<sub>3</sub>, Nagpur mandarin, Rough skin physiological disorder, Soil available potassium



Advancing Citriculture for Agro-economic Prosperity

#### TS-2-P-29

# Evaluation of citrus (*Citrus aurantifolia* Swingle) cultivars through rooting of cuttings for mass propagation

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The present study on performance of different varieties of acid lime (Citrus aurantifolia Swingle) cultivars viz. Kagzi lime, Sai Sharbati, Balaji, Vikram and Pramalini using a methodology involving hardwood cuttings, 500ppm IBA and cocopeat as growing media under shade net condition has been evaluated for mass propagation employing Completely Randomised Design (CRD). The results were significantly affectedin all parameters. The lowest number of days taken for sprout initiation was recorded in cv. Balaji (8.5 days) and highest was in Vikram (13.4 days), respectively. The highest and lowest sprout per cent was recorded in cv. Vikram (91.66%) and cv. Kagzi lime (48.61%). The number of sprouts at 30 days after planting was highest in pramalini (11.7) and lowest of it was in Kagzi lime (7.4) and the number of sprouts was decreased at 60 and 90 days. The highest number of leaves at 30 days was in pramalini (8.45) and lowest was in Kagzi (3.6) and number of leaves increased upto 60 days and then decreased later. Shoot length was increased up to 90 days and the highest shoot length was in cv. Vikram (13.10cm) and lowest was in cv. Balaji (7.19cm). The highest and lowest fresh and dry weight was recorded in cv. Saisarbati (8.54gm and 6.29gm) and cv. Pramalini (7.19gm and 3.67gm), respectively. The highest and lowest number of primary roots was recorded in cv. Vikram (4.2) and Sai sarbati (2.6), highest and lowest average root length was recorded in cv. Vikram (18.70cm) and cv. Kagzi lime (12.26cm), highest and lowest rooting per cent was recorded in cv. Vikram (88.88%) and Kagzi lime (45.83), respectively at 90 days after planting. There was considerable variation in responses which was found to be dependent on genotype. The results are helpful in future root stock studies as well as mass multiplication of planting material on commercial scale.

Keywords: Acid lime, Cuttings, Propagation, IBA, Cocopeat, Shade net



#### Foliar Application Role of Potassium Nitrate and Salicylic Acid for Quality Improvement of Acid Lime (*Citrus aurantifolia* Swingle) cv. Kagzi Lime

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An experiment was conducted in order to see the effects of potassium nitrate and salicylic acid on citrus crop i.e. acid lime during the year 2019-20 at research farm, College of Horticulture, Mandsaur, Madhya Pradesh. The experiment was conducted in Factorial Randomized Block Design with three replications. Acid lime cv. Kagzi lime was sprayed twice during the experiment, the first spray was done at flower initiation and second spray during the fruit development. Maximum number of flowers per plant (1177.50 and 1073.38), maximum fruit setting (59.43% and 64.29%), maximum fruit retention (66.00% and 63.22%) and minimum fruit drop (34% and 36.78%) was examined under the 1.5% spray of potassium nitrate along with 4 ml/L salicylic acid. The juice content was also significantly the highest in 1.5% spray of potassium nitrate (49.72%) and 4 ml/L salicylic acid (47.81%). Highest TSS value 7.48 °B and 7.36 °B, maximum ascorbic acid content i.e. 31.04 mg/100ml and 30.03 mg/100ml were also found among the spray of potassium nitrate 1.5% along and 4 ml/L salicylic acid respectively. Highest NPK content in leaf (2.68%, 0.21% and 1.90%) was observed under 1.5% potassium nitrate and (2.50%, 0.19% and 1.77%) under salicylic acid of 4 ml/L.

Keywords: Acid lime, Quality, Spray



# Assessment of exogenous application of micronutrients and PGR combinations on fruit retention and size of Nagpur mandarin (*Citrus reticulata* Blanco) in district Chhindwara Madhya Pradesh

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Pre-harvest fruit drop is major reason of low yield, this drop of fruit at various stages of fruit development is due to malnutrition, water stress, excessive insect pest attack and most important is the hormonal imbalance. Tree drops its fruit when the concentration of auxins decreases and the concentration of abscissic acid (ABA) increases as the endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs. The excessive fruit drop can be controlled by the exogenous application of plant growth regulators and micronutrients. The exogenous application of PGRs and micronutrients can provide significant economic advantages to mandarin growers when used properly as these have proven effective in stimulating a number of desired responses such as increase in fruit retention and size. Exogenous applications of 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA<sub>3</sub>) with combination of Urea and Monopotassium phosphate control fruit drop at various stages during fruit growth and development. Hence, the OFT was conducted under JNKVV, Krishi Vigyan Kendra, Chhindwara to study the effectiveness of micronutrients and PGR combinations with foliar application at the farmers field including control (Farmers practice). Application of the treatments was done one month after fruit set and in the months of October and November. Application of monopotassium phosphate and urea in combination with 2, 4-D and GA<sub>3</sub> resulted in higher rate of per cent increase in fruit retention and size as compared to farmers practice (Control). Phytophthora root rot and gummosis are the most important soil borne diseases of Nagpur mandarin causing mortality, slow decline and yield loss of mature trees Phytophthora root rot and gummosis are the most important soil borne diseases of Nagpur mandarin causing mortality, slow decline and yield loss of mature trees.

Keywords: Nagpur mandarin, Retention, Size, GA<sub>3,</sub>2,4-D, Monopotassium phosphate, Urea



# Standardization of nutrients level for sweet orange (*Citrus sinensis* L. Osbek) under western Maharashtra conditions

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The new thinking about the soil operation technologies demanded for the nonstop improvement of the productivity. An excessive amount of chemical fertilizer charges more money as well as decreases fruit yield and quality whereas less application of nutrients can cause deficiency in plant nutrients and lowers the fruit production. Hence, standardization of nutrients level is necessary to meet the proper demand of nutrients by the plant without any excessive loss of fertilizer. A field experiment was carried out at AICRP on Fruit crops under Mahatma Phule Krishi Vidyapeeth, Rahuri during 2021-2022 with a view to study the "Standardization of nutrients level in sweet orange (*Citrus sinensis* L. Osbeak) under Western Maharashtra conditions." The experiment followed Factorial Randomized Block Design (FRBD) with three levels and 27 (+1 control) treatment combinations of N, P and K viz. three nitrogen level N<sub>1</sub>(600g/plant), N<sub>2</sub> (800g/plant), N<sub>3</sub> (1000 g/plant), three phosphorus level P<sub>1</sub> (200g/plant), P<sub>2</sub> (300 g/plant), P<sub>3</sub> (400 g/plant) and three potassium levels K<sub>1</sub> (400 g/plant), K<sub>2</sub> (600 g/plant), K<sub>3</sub> (800g/plant). Among all treatment combinations, N<sub>3</sub>P<sub>2</sub>K<sub>3</sub> recorded highest yield parameters and N<sub>2</sub>P<sub>2</sub>K<sub>3</sub> recorded high TSS, high ascorbic acid content and low titratable acidity in sweet orange under Western Maharashtra conditions.

Keywords: Sweet Orange, Nitrogen, Phosphorus, Potassium, Nutrients level standardization.



# Effect of foliar spray of bio-stimulants and micronutrients on growth, flowering and fruiting of coorg Mandarin

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A field experiment was conducted at College of Horticulture, Mudigere during 2019-20. To study the Effect of Foliar Spray of Bio-Stimulants and Micronutrients on Growth, Flowering and Fruiting of Coorg Mandarin. The experiment was laid out in Randomized complete block design (RCBD) with eleven treatments replicated thrice. Treatments comprised of different levels of bio-stimulants and micronutrients, sprayed at three different stages such as vegetative, flowering and fruit set. Results of the experiment revealed that, Sea Weed Extract - Biovita at 0.5 % exhibited superior performance than the other treatments with respect to morphological parameters viz., plant height (1.97 m), stem girth (6.30 cm), plant spread (1.59 m in N-S and 1.51 m in E-W), canopy volume (6.23 m<sup>3</sup>), number of new shoots per plant (44.26), length of newly emerged shoots (20.50 cm) and leaf area (11.33 cm<sup>2</sup>), followed by Citrus special at 0.5 % and the lowest was recorded with control. Flowering parameters like days taken forfirst flower emergence (28.67 days), days taken for 50 per cent flowering (34.00 days), number of flowers per cluster (6.03), number of flower clusters per plant (56.33), flowering intensity (33.69) were maximum with biovita at 0.5 per cent and minimum was recorded with control. The present study confirmed that the use of bio-stimulant is an eco-friendly technique to enhance crop production. Thus, it may be recommended that the Coorg mandarin plants can be sprayed with biovita at 0.5 percent to get maximum growth, flowers and fruits which may ensure growers to get a maximum net return.

Keywords: Canopy volume, Coorg Mandarin, Bio-stimulants, Flowering intensity



#### Influence of foliar spray of bio-stimulants and micronutrients on growth, leaf zinc and boron nutrient status of Coorg mandarin (*Citrus reticulata* Blanco.) under Hill zone of Karnataka

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A field experiment was conducted at College of Horticulture, Mudigere during 2019-20. The experiment was laid out in Randomized complete block design (RCBD) with eleven treatments replicated thrice. Treatments comprised of different levels of bio-stimulants and micronutrients, sprayed at three different stages such as vegetative, flowering and fruit set. Sea Weed Extract - Biovita at 0.5 % exhibited superior performance than the other treatments with respect to morphological parameters viz., plant height (1.97 m), stem girth (6.30 cm), plant spread (1.59 m in N-S and 1.51 m in E-W), canopy volume (6.23 m<sup>3</sup>), number of new shoots per plant (44.26), length of newly emerged shoots (20.50 cm) and leaf area (11.33 cm<sup>2</sup>), followed by Citrus special at 0.5 % and the lowest was recorded with control. Results of the experiment revealed that, zinc content in the leaf was positively increased with foliar spray of micronutrients. In the present investigation the plants supplied with Zinc (0.5%), increased the zinc status in leaf (29.53 ppm), followed by Boron at 0.1 per cent and the least was recorded with control. Biovita at 0.5 % exhibited superior performance than the other treatments with respect to morphological parameters.

Keywords: Growth, Biovita canopy volume, Leaf analysis, Zinc analysis, Boron analysis



# Grate a worth of foliar application of growth substances on vigor and bud success of Citrus cv. Coorg mandarin

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Now a day's citrus cultivars are not get genuine vigour plants, for that to augment the vigour and healthy, an experiment was conducted in poly house, Department of Fruit Science, College of Horticulture, Mudigere, Chikkmagluru district of Karnataka India during 2017-18, to enhance the vigour and bud success by using foliar application of nutrients and growth substances. It was laid out complete randomized block design with three replication and 12 treatments. Among the different foliar spray treatments, highest bud sprout height (16.00, 21.83 and 33.33 cm), number of leaves (11.67, 17.80 and 23.67), buddling height (31.64, 38.46 and 43.93 cm), bud vigour index I (1525.28, 2081.05 and 3177.35 cm), bud vigour index II (30162, 366.39 and 4190.71 cm) was recorded in citrus special at 0.2 per cent concentration. While highest bud diameter (4.30 mm), budding diameter (7.60, 7.48 and 8.56 mm) was recorded in treatment GA<sub>3</sub> at 100 ppm in 30,60 and 90 days after bud imitation. Further highest bud success percentage (98.00 per cent), bud survivability (100 per cent) was recorded in treatment *panchagavya* 5 per cent concentration. Foliar spray with citrus special 0.2 per cent concentration helps in increasing the vigour of Coorg mandarin buds.

Keywords: Budlings, Citrus special, Panchagavya, Bud vigour index, Bud success



### Investigation on viability of Rangpur lime seeds and its effect on germination, vigour and root characters for bud -take of Coorg mandarin

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An investigation was carried out during 2017-18 in poly tunnel, Department of Fruit Science, College of Horticulture, Mudigere, Chikkamagaluru district of Karnataka, to study on viability on germination, growth of Rangpur lime rootstock and bud-take in Coorg mandarin. The seeds of Rangpur lime were extracted from fruit and kept under ambient conditon. Among the different treatments, The seeds sown on day of extraction (16.07) took minimum number of days for initiation of germination, whereas nine days after extraction took minimum number of days to attain 50 per cent and completion of germination (19.00 & 28.00), highest germination percentage (96.67 per cent) and germination vigour index (0.15 days) was observed in six days after extraction of seeds, highest rootstock primary root length (24.00 cm), number of secondary roots (43.67) was observed in nine days after extraction of seeds, whereas minimum observation parameters were observed in twenty seven days after extracted seeds. The germination percentage ranged from 96.67 to 66.67. The highest bud success percentage (95.23 per cent), bud survival percentage (96.67 per cent) was recorded in nine days old seeds. Rangpur lime seeds being recalcitrant, loose its viability quickly. The seeds could be stored at ambient condition for eighteen days after extraction without much variation in germination and growth parameters. However by use of seeds fifteen days after extraction reduced in growth and bud parameters. The seeds sowed at nine days after extraction showed highest bud success and bud survival percentage.

Keywords: Seed storage period, Germination percentage, Bud success, Bud survivability



#### Integration of micro irrigation and fertigation on productivity, profitability of acid lime with quality and water use efficiency in semi- arid climatic conditions of central India

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The present study was conducted to assess the effect of micro-irrigation based on evaporation depletion with reduced doses of NPK applied through fertigation on fruit yield, profitability, quality and water use efficiency of acid lime at Dr. PDKV, Akola during 2013-15. The experiment was laid out in FRBD comprised of three levels of micro-irrigation (I<sub>1</sub>-100, I<sub>2</sub>-90 and I<sub>3</sub>-80 % Evp) and three levels of fertigation (F<sub>1</sub>-100, F<sub>2</sub>-80 and F<sub>3</sub>-60 % RDF) in nine treatment combinations replicated thrice. The results revealed that the highest total number of fruits per plant and fruit per hectare (annual harvest) was found significantly higher in micro-irrigation level @ 100% at Evp. (I<sub>1</sub>) and fertigation at 100% RDF (F<sub>1</sub>) individually which were at par with micro irrigation at 90 % Evp. (I<sub>2</sub>) and fertigation at 80 % RDF (F<sub>2</sub>). However, in *Hasta bahar* the significantly highest fruit yield per tree and per hectare was obtained with micro irrigation at 90 % Evp. (I<sub>2</sub>) and fertigation at 100 % RDF (F<sub>1</sub>) which was at par with I<sub>3</sub> and F<sub>2</sub>. The treatment combination of I<sub>3</sub>F<sub>1</sub> produced significantly highest fruit per tree and per hectare followed by I<sub>2</sub>F<sub>1</sub> and I<sub>2</sub>F<sub>2</sub> which were at par with each other. The higher B:C ratio was obtained with micro irrigation at 90 % Evp. and fertigation at 80 % RDF and in treatment combination of I<sub>3</sub>F<sub>2</sub> and I<sub>2</sub>F<sub>2</sub> levels. The significantly higher fruit quality (TSS), fruit yield efficiency and water use efficiency was observed with micro irrigation at 80 % Evp. and fertigation at 100 % RDF followed by micro irrigation at 90 % and fertigation at 80 % RDF which was at par with each other. Hence, it can be concluded that the application of irrigation level at 80% Evp and fertigation at 80 % recommended dose of fertilizers was found beneficial in enhancing productivity, profitability and quality of acid lime along with higher water use and fruit yield efficiency in semi-arid climatic conditions of Central India.

Keywords: Acid lime, Fruit yield, Fertigation, Micro-irrigation, Quality



#### Covering of stock length with waste fertilizer bag strip or black or transparent poly tape, a cost-effective alternative for producing quality citrus grafts with higher benefits in central India

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The study was conducted to assess the effect of the stock length with waste fertilizer bag strip or with black or transparent poly tape on control of side shoot growth and its effect on growth of scion at citrus secondary nursery with profitability during 2021-23 at Regional Fruit Research station, Katol dist. Nagpur, Maharashtra, India. The experiment was laid out in RBD with five treatments replicated four times. The treatments consisted of Use of light weight 1" PVC pipe (9 to 10" length), use of white poly tape/ waste fertilizer bag strip, use of black poly tape (100 micron), use of transparent poly tape (100 micron) and conventional method without any cover by manual removal of side shoot. The pooled results indicated that the significantly maximum plant height, highest no. of leaves and higher stem diameter was recorded with the use of black poly tape (100 micron) for covering the stock length  $(T_3)$ followed by covering stock with transparent poly tape of 100 micron (T4) which was at par with each other. The lowest plant height, no. of leaves and stem diameter was observed with the conventional method without any cover by manual removal of side shoot (T<sub>s</sub>). However, the mean data revealed that higher B:C ratio was obtained with the use of white poly tape/ waste fertilizer bag strip (T<sub>2</sub>) followed by use of black poly tape of 100 micron and use of transparent poly tape of 100 micron. The lower B:C ratio was obtained in conventional method without any cover by manual removal of side shoot (T<sub>5</sub>). Hence, it can be concluded that covering the stock length with waste fertilizer bag strip or with black or transparent poly tape is a cost-effective alternative to the traditional way of recurrent removal of reemerging side shoots for producing quality citrus grafts with higher benefits in central India.

Keywords: Black/transparent poly tape, B:C ratio, Citrus, Growth, Secondary nursery



### Reciprocal graft mediated growth and physiochemical behaviour of contrasting citrus rootstocks under water deficit and rehydration

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Drought stress poses a significant challenge in fruit production, adversely affecting crop yield and quality. To address this challenge, one strategy is the utilization of drought-tolerant rootstocks in fruit cultivation. This study explores the growth response of contrasting citrus rootstocks, namely Citrus jambhiri Lush cv. JattiKhatti (JK) and X639 (C. reshni Hort. ex Tan. x Ponciroustrifoliata), to water deficit conditions and subsequent rehydration through reciprocal grafting. In the case of self-grafted JK, we observed the most significant declines in scion growth traits, the highest wilting score, and drought injury index. It also exhibited a higher increase in leaf proline content compared to self-grafted X639. However, when subjected to reciprocal grafting, the JK/X639 combination showed a lesser decline in growth, the lowest wilting score, and drought injury index when exposed to water deficit, similar to normal moisture conditions. Moreover, plant combinations with X639 as the rootstock demonstrated a notable recovery in scion growth after rehydration. In the context of oxidative stress, JK/JK and JK/X639, when compared within the group, displayed the highest increase in TBARS content, along with the lowest catalase and glutathione reductase activities after experiencing water deficit. In contrast, X639, whether used as a scion or rootstock, effectively mitigated lipid peroxidation compared to self-grafted JK. Both auto-grafted X639 and hetero-grafted X639/JK showed a significant decline in TBARS content, catalase, and glutathione reductase activities following rehydration.X639 rootstock prioritized growth under water scarcity conditions, attributed to elevated relative water content, membrane stability index, and a strong antioxidant system, regardless of whether it was used for self or reciprocal grafting. Conversely, JK focused on survival, as indicated by leaf shedding and reduced leaf area. These findings provide valuable insights into the responses of citrus rootstocks to water stress, offering practical implications for citrus cultivation in regions with varying water availability.

Keywords: Antioxidant, chlorophyll, oxidative stress, phenol, proline, scion-rootstock combinations



#### Selection and evaluation of root stocks for propagation studies in citrus

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Rootstocks greatly influence varietal behavior as it ensures tolerance to abiotic stress conditions, as well as the provision of minerals and water for the total plant and consequently impact crop yield and fruit quality. At present rough lemon and Rangpur lime are most popular roots stocks in citrus industry in Maharashtra. The available rootstocks are having less seeds due which more number of fruits are required for production of nursery plants. Also more labours are required for seed extraction. Similarly compact skin fruits may give tight jacket fruits which have export potential and good keeping quality. However, no such variety is available and hence to cope-up the demand of farmers of the region, the study on root stock selection and evaluation was initiated to find out the suitable genotypes of rough lemon for cultivation in the region. The rough lemon plants were planted during the year 2010-11 and some plants were planted during 2007. From these plants selection, identification of rough lemon fruits were undertaken from Satpuda Botanic Garden and Centre of Excellence for Citrus, Bharat Nagar College of Agriculture, Nagpur during the year 2018-19 and 2021-22. In all, fruits of 7 promising genotypes of rough lemon, Karna Khatta lemon were selected and Jambhiri as a check. The seeds were sown in nursery during 2022-23 for further study. It is revealed that, among the different genotypes of rough lemon significantly maximum number of fruits (516), fruit weight (315.41 g), fruit diameter (88.80) fruit rind thickness (7.24 mm), number of seeds (41.60) and germination (92.00%) were recorded in Karna Khatta. The genotype NGRL5 recorded fruit weight (306.22) which was at par with Karna Khatta. The minimum number of fruits (235) and fruit weight (235.11g) were recorded in NGRL4. The Genotype NGRL-6 recorded maximum fruit length (103.3mm). Whereas, maximum number of segments fruit-1 (12.40) were observed in genotype NGRL2. The present investigation may help as a new root stock for citrus. Also there may be a positive impact of stock scion relationship for growth, flowering, fruiting and fruit quality.

Keywords: Citrus root stock, Abiotic stress, Seed extraction, Karna Khatta lemon



#### Performance of acid lime varieties on raised bed system in central India

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Acid lime (Citrus aurantifolia Swingle) is the third most important citrus species in India after mandarin and sweet orange. The soil and climatic condition of semi-arid regions in Vidarbha offers ample scope for acid lime cultivation under both irrigated and rain-fed conditions. The performance of ten acid lime varieties viz. Pramalini, Chakradhar, PKM –1, Vikram, Phule Sharbati, Sai Sharbati, PDKV bahar lime, Balaji, NRCC-7, and NRCC-8 budded on rough lemon rootstock were planted at 6×3mspacing on raised bed during 2015. Seedlings of acid lime have been planted as control on same pattern. The maximum plant height (4.36m), canopy volume (55.24m<sup>3</sup>), and stock and scion girth (45.54cm and 38.38cm, respectively) was recorded in PKM-1(Jaidevi) followed by Pramalini .The maximum average fruit weight (80.10 g) was also observed in PKM-1(Jaidevi). Phule Sharbati recorded maximum vitamin C content (30.11mg/100g)) whereas highest acidity and juice content were recorded in PKM-1(Jaidevi) (4.35% and 48.90% respectively) followed by Vikram. The minimum no of seeds recorded in Balaji (5.03 seeds/fruit). The highest fruit yield was recorded in PKM-1 i.e.10.06 t/ha followed by Pramalini after seventh year of planting. The available soil macronutrients N, P and K showed significant variation among all the varieties maximum N Phule Sharabati (220.36 kg/ha). Among the acid lime, minimum population of citrus psylla recorded in Pramalini, the lowest leaf miner infestation was recorded on Balaji (12.66%). Thrips, lemon butterfly and blackfly infestation were below the ETL on different cultivar. All the clones were found to be susceptible to citrus canker, NRCC-7 showed minimum disease leaves i.e., 34.04%. This study indicated that acid lime variety PKM-1 budded on Rough lemon planted on raised bed was found to be promising with respect to fruit yield and quality in central India.

Keywords: Acid lime, Rootstock, Rough lemon, Raised bed



#### Performance of exotic sweet orange varieties under black clay soils of Central India

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Rootstocks play an important role in the agronomic performance of the citrus tree. The present experiment was initiated at Research farm of ICAR-Central Citrus Research Institute, Nagpur during 2014 with two rootstock combination under black clay soil. The performance of exotic sweet orange varieties budded on different rootstocks i.e. Hamlin on Cleopatra mandarin, Hamlin on C.volkameriana, Pera on C.volkameriana, Pera on Limacrovo, Natal on C.volkameriana, Natal on Limacrovo, Valencia on C.volkameriana, Westin on Limacrovo, Westin on Swingle citrumelo, Mosambi on Rough lemon, Mosambi on Rangpur lime, Washington Navel on Rough lemon, Lanlate on Rough lemon, Newhall on Rough lemon, Valencina Olinda on Rough lemon were evaluated. The growth data pertaining to plant height (3.96m) and canopy volume (32.82m<sup>3</sup>) recorded maximum in Hamlin on C. volkameriana. The maximum fruit weight (387.49g) was recorded in Lanelate on Rough lemon, while highest juice content (58.25%) was recorded in Natal on C. volkameriana. Whereas, maximum TSS content (9.83%) was recorded in Westin on Swinglecitrumelo. The average maximum fruit yield (14.65t/ha) was recorded in Natal on C. volkameriana folloed by Natal on Limocrova. The granulation did not observed in the combination with Natal on C. volkameriana, Valencia on C. volkameriana, Westin on Swingle citrumelo, Westin on Limocrova. Among soil nutrient, maximum soil N was recorded in Westin on Limocrova (242.79 kg/ha) while soil P was maximum in Newhall on Rough Lemon (42.83 kg/ha) and soil K content was maximum (1537.67 kg/ha) in Valencia on Olinda on rough lemon. The insect pests incidence, there is no any significant difference was observed in citrus psylla infestation, CLM infestation was lowest on Hamlin on Cleopatra (31.80%), thrips infestation observed below the ETL and blackfly and mites infestation crossed the ETL level. The maximum disease incidence not found in the experiment. This study indicated that exotic cvs. Natal on C. volkameriana has showed promising with respect to fruit yield and quality under black clay soil.

Keywords: Exotic, Sweet orange, Combinations, Rootstocks, Volkameriana, Natal

### Thematic Area -3

### Current Approaches in Citrus Health Management, Insect-Pests & Disease Surveillance and Diagnostic Approaches



Advancing Citriculture for Agro-economic Prosperity

#### **Oral Presentation**

#### TS-3-O-01

### Chinese citrus fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) invasion in the mid-mountain citrus orchards in Nepal and its successful management: A review

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The Chinese citrus fly, *Bactrocera minax* (Enderlein) (Tephritidae: Diptera), is a serious frugivore pest of citrus particularly of tight skin kinds of fruits that has caused significant fruit losses ranging from 5 to 100 % in the mid-mountain citrus orchards. It is reported geographically extended exclusively in Nepal, India (Sikkim and West Bengal), Bhutan, and China in world. In December 1984, the Chinese citrus fly, *B. minax*(earlier misidentified as *B. tsuneonis*), was reported for the first time from an infested sweet orange fruit obtained from a citrus orchard in Sindhuplchock district, Nepal. Its damages reported in the sweet orange fruits in the eastern mid-mountain citrus orchards in Dhankuta district during 2007–2008. Later, during 2014–2015, this pest found attacking on the sweet orange fruits in the central mid-mountain citrus orchards in Sindhuli district, Nepal. At present, most of Nepal's mid-mountain citrus-growing districts have been found infested of *B. minax* in the orchards was initiated for the first time in Nepal. Farmer community based organized spot applications of lethal protein hydrolysate bait in the productive sweet orange trees along with the orchard sanitation measures fetched a successful fruit fly management in orchards that curbed the *B. minax* incurred sweet orange losses from 56.7% in 2017 (prior to AWCP) to 10.9% in AWCP, 2018, and then down to 4.5% due to next approach of AWCP in 2019.

Keywords: AWCP, Bactrocera minax, Citrus, Invasion, Management



# Selective autophagy and CRISPR/Cas Used to engineer resistant cultivar against viral disease in citrus

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Autophagy is a cellular-degradation mechanism that has been in antiviral defense in plants. Despite the increased interest in "green autophagy," we still have significant gaps in our understanding of selective autophagy pathways in Citrus. Selective autophagy as a pathogen-fighting weapon and how pathogens have evolved techniques to fight or undermine the immunity mediated by selective autophagy. The selective autophagy molecular machinery must enable efficient cargo recognition and sequestration within autophagosomes. Autophagic cargo receptors that precisely bind the cargo material and the autophagosomal membrane can mediate cargo specificity. The emerging characteristics of selective receptors/adaptors and their interactions with both cargoes and Autophagy-related gene 8s (ATG8s) are highlighted, as are the evolving functions of selective autophagy in both biotic and abiotic stress tolerance. In citrus, the gene involved in autophagy and its mechanism are largely unknown. Within the sweet orange (Citrus sinensis), there are 35 autophagy-related gene (ATGs) ver. Identify. Activating autophagy by suppressing GAPC genes improved plant resistance to viral infection, but silencing the autophagy-related genes ATG5 and ATG7 decreased plant resistance to the DNA viruses CLCuMuV, Tomato yellow leaf curl virus, and Tomato yellow leaf curl China virus. Selective autophagy was used to target and degrade the infection of citrus leaf blotch virus (CLBV; genus Citrivirus, family Betaflexiviridae). We also identified that CLBV MP interacted directly with NbATG8C1 and NbATG8i, the isoforms of autophagy-related protein 8 (ATG8), which are key factors that typically bind cargo receptors for selective autophagy. This results show a novel example in which autophagy specifically targets a viral MP to prevent the intercellular spread of the virus in plants. The incorporation of various resistance genes through breeding programmes, along with gene editing tools such as CRISPR/Cas technologies, holds great promise in developing virus-resistant plants.

Keywords: ATG gene, Citrus, CRISPR/cas, Plant virus, Selective autophagy



# Cultural and bio-chemical characterization of bacterial canker disease caused by *Xanthomonas axonopodis*pv. *citri* in acid lime

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The *Xanthomonas axonopodis* pv. *citri* causing bacterial canker of acid lime was isolated from different acid lime growing locations of Ahmednagar district of Maharashtra State and were used for these tests. Biochemical tests and Cultural characteristics *viz.*, colony shape, margin, elevations and pigmentation help to identification of bacteria. In biochemical tests Gram staining, Potassium hydroxide (KOH) test, Catalase test, Starch hydrolysis test were performed to characterize the *Xanthomonas axonopodis* pv. *citri*. Different ten test isolates were studied using Nutrient Agar as basal culture medium. In that most of isolates showed yellow pigmentation and all the test isolates showed filiform colony shape, convex elevation and entire colony margin. Morphologically all the test isolates were found single rod shaped. In biochemical test *Xanthomonas axonopodis* pv. *citri* isolate were found to Gram negative staining test and observed positive to Catalase test (H<sub>2</sub>O<sub>2</sub>), Potassium hydroxide test (KOH) and Starch hydrolysis test. The results of all morphological, biochemical and cultural tests were confirmed that *Xanthomonas axonopodis* pv. *citri* a Gram-negative bacterium.

Keywords: Acid lime, Bacterial canker, Biochemicaltests, Cultural characters, Xanthomonas axonopodis pv. citri



### A RT-RPA assay for rapid and robust detection of citrus yellow vein clearing virus infecting Kinnow mandarin

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Citrus yellow vein clearing virus (CYVCV), Genus (*Mandarivirus*), family (*Alphaflexiviridae*) is an emerging,graft transmissible plant pathogen with the potential to cause significant economic losses to citrus industries. Requirement of fast, reliable, efficient & economical CYVCV indexing assay is a prerequisite for production of healthy planting material. An isothermal based RT-RPA assay for rapid, sensitive and robust detection of CYVCV infecting Kinnow mandarin, has been developed, optimised and validated. The developed RT-RPA assay could detect the CYVCV up to a dilution of 10<sup>-7</sup> with the prepared templates of both RNA and crude saps at an isothermal temperature 40° C in 25 min and showed higher sensitivity in detection of CYVCV infection in field samples as compared to the conventional RT-PCR.Developed RT-RPA assay showed high specificity without any cross-reaction with other citrus pathogens *viz*.citrus yellow mosaic virus, Indian citrus ringspot virus and citrus tristeza virus. RT-RPA using crude leaf sap as template is quite simple, robust, highly sensitive and cost effective. This assay can be utilized in laboratories with insufficient basic conditions or plant protection and quarantine stationas an indexing tool for the production of clean planting materials and certification programmes of Kinnow mandarin.

Keywords: Kinnow mandarin, Yellow vein clearing virus, RT-RPA assay, Plant protection, Healthy planting material



#### Management of citrus gummosis by using chitosan copper nanoparticles

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Citrus is the most valuable fruit in international trade and one of the top ten economically significant crops in terms of total global fruit yield. Citrus yield loss is caused by a variety of biotic and abiotic causes. Biotic agents such as viruses, bacteria, and fungi all have a role in yield loss, with fungi alone accounting for 30% of citrus production losses. Gummosis is caused mostly by *Phytophthora* species, the most prevalent and major of which is *Phytophthora nicotianae* var. *parasitica*. Chemical fungicides are widely used but they are hazardous to soil, the environment, and human health. Development of resistance in pathogens also occurred due to regular and excessive use of chemical fungicides. Hence there is a need for novel and ecofriendly approaches to tackle these demerits. Nanotechnology has the potential to develop an alternative to chemical fungicides and hence it was explored to mitigate losses incurred due to gummosis. The present study, evaluated the *in-vitroandin-vivo* antifungal efficacy of biologically synthesized chitosan copper nanoparticles (CHCuNP) by using cell-free extract of T.asperellum and P. fluorescence against citrus gummosis. Both NPs showed spherical and irregulars hapes, respectively, and size ranges between 30-70 nm. The maximum percent of inhibition (100%) was found at 100 ppm for both NPs against P. parasitica. Nanoemulsion Gel (NEG) of both NPs resulted in a decrease in lesion size andan increase in disease suppression efficiencyi.e. 5.88 to 4.50 cm<sup>2</sup>(23.46%) and 3.41 to 2.90 cm<sup>2</sup>(14.95%) after 30 days of treatment. Thus, the biogenic synthesized CHCuNPs can be used as nanofungicides and it is also cost-effective, required in fewer amounts and eco-friendly for the control of citrus gummosis.

Keywords: Antifungal, Citrus gummosis, Inhibition, Nano-emulsion Gel.



# First report of *Colletotrichum siamense* causing fruit drop in Kinnow mandarin (*Citrus nobilis x Citrus deliciosa*) in Punjab, India

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Fruit drop is a major bottleneck in Kinnow mandarin (Citrus nobilis × Citrus deliciosa) causing considerable reduction in quality and yield of the fruits. The symptoms appear as small, circular, light brown lesions around stem-end of the fruit. The affected area around the fruit rots and fruit drops prematurely. The twigs of the infected trees exhibit die-back symptoms and show numerous black dots like fruiting bodies of the fungus. Different species of Colletotrichum are reported to cause fruit drop in citrus worldwide viz., C. gleosporioides, C. truncatum, C. fructicola, C. acutatumetc. The citrus post-bloom fruit drop pathogen was originally identified as C. gloeosporioides, but was later found to be C. acutatum. In recent studies of Colletotrichum species-complexes using multi-locus analyses, at least 15 species of Colletotrichum have been reported to be associated with citrus fruit drop. The present study was, therefore, undertaken to characterize the pathogen associated with fruit drop in Kinnow mandarin. Molecular characterization of 33 isolates of the fungus obtained from different Kinnow growing orchards was done by amplification of ITS region, ACT and TUB2 genes. The results revealed that all the isolates were of *Colletotrichum*, whereas amplification of species-specific primer CgInt/ITS4 confirmed that all the isolates belong to Colletotrichum gloeosporioides complex and no amplicon was observed in any of the isolates with primer pair CaInt2/ITS4 (C. acutatum). The phylogenetic analysis of two isolates C-4 and C-19, selected on the basis of high aggressiveness and growth rate proved that Colletotrichum siamense(belonging to gloeosporioidescomplex) is associated with fruit drop in Kinnow mandarin. Based on morphological characteristics, a phylogenetic analysis and pathogenicity assay, the causal agent was identified as Colletotrichum siamense. This is the first report of C. siamense causing fruit drop of Kinnow in Punjab, India.

**Keywords:**Citrus, *Colletotrichum gloeosporioides*, *C.siamense*, Fruit drop, Kinnow mandarin, Molecular characterization



### Integrated management of fruit fly (*Bactrocera* spp.) in *Citrus reticulate* Blanco in eastern Himalaya

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Integrated management of fruit flies (Bactrocera spp.) wasevaluated in an experiment which infest Mandarin Orange orchards in multiple locations of Eastern Himalayan region. The experiment consisted of 3 treatments and a control, each replicated 3 times across 50 selected plants per treatment. The study was replicated in multiple sites: Daruwa, Lapsibotey, Tansing, Mungpoo and Munsong. The control groups were placed in Reshep, Lapsibotey, and Tansing. The treatments were T<sub>1</sub>: Methyl Eugenol+Neem Oil, T<sub>2</sub>: Tapas Fruit Fly Lure+ArtemisiaOiland T<sub>3</sub>: IPM Fruit Fly Trap+Citronella Oil. Two parameters were assessed, mainly the number of fallen fruits due to fruit fly infestationand the number of trapped flies.Data was recorded daily from March 2020 to October 2022. The findings revealed significant differences among the treatments in terms of fruit fly management. Treatment T<sub>1</sub> (Methyl Eugenol+Neem Oil) resulted in the lowest number of fallen fruits, indicating effective fruit fly control. T<sub>2</sub> (Tapas Fruit Fly Lure+Artemisia Oil) also exhibited promising results, with a moderate reduction in the number of fallen fruits. T<sub>3</sub> (IPM Fruit Fly Trap+Citronella Oil) showed variable outcomes across the replicates. Furthermore, the number of fruit flies trapped was monitored during the experiment period. T<sub>1</sub> (Methyl Eugenol+Neem Oil) and T<sub>2</sub> (Tapas Fruit Fly Lure+Artemisia Oil) treatments demonstrated maximum number of fly trapped per day which led to significant reduction in fruit drop compared to the control. T<sub>3</sub> (IPM Fruit Fly Trap+Citronella Oil) exhibited limited success in trapping fruit flies consistently. The major conclusion of the study suggests that both T<sub>1</sub> and T<sub>2</sub> have the potential to effectively manage fruit flies in Mandarin Orange orchards. These treatments significantly reduced fruit infestation and maintained fruit quality. T<sub>3</sub> did not yield consistent results in fruit fly control. The treatments offer viable alternatives to synthetic pesticides, reducing chemical inputs and promoting sustainable farming practices.

Keywords: Mandarin Orange, Fruit fly infestation, Integrated management, Eastern Himalaya.



# Potassium phosphonate, a fungicide used to treat *phytophthora* foot rot and gummosis of mandarin

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The most important commercial Citrus in India is the mandarin orange (Citrus reticulata), followed by the sweet oranges and acid limes. Nagpur Mandarin, Kinnow, Coorg and Khasi mandarin is a famous ecotype and varieties of mandarin grown in the Central, North and North East parts of India. It plays a vital role in thefruit economy of the country. Phytophthora species have been shown to cause some serious soilborne diseases of Citrus, including damping off of seedlings in nursery, root and crown rot, foot rot/gummosis and brown rot of fruits. The majority of mandarinroot stocks grown in country is susceptible, or at best moderately susceptible, and necessitates frequent fungicide applications to avoid heavy yield losses. Four years of field trials in three different locations (Akola, Ludhiana and Tinsukia) investigated the effect of different conventional and non-conventional chemical integration with bioagents on *Phytophthora* root rot and gummosis. The results showed that foliar spraying of potassium phosphonate (a Potassium salt of phosphonic acid) at 3 g/liter water was superior in terms of average reduction in lesion oozing (28.39%), minimum feeder root rot index (2.17), increase in canopy volume (11.15%), and higher fruit yield (65.89 kg/per tree). Potassium phosphonate is known to induce defense responses in mandarin as well as to have direct toxic effects on Oomycetes, which in turn inhibits the development of root rot and gummosis. The use of potassium phosphonate in mandarin crops has been promoted as a feasible method as part of the integrated disease management strategy for Phytophthora root rot and gummosis management.

Keywords: Gummosis, Mandarin, Potassium phosphonate, Phytophthora



#### Integrated approach for management approaches for citrus greening disease

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Citrus greening is the most devastating citrus disease in the world, threatening the sustainability in major citrus-growing countries. As globalization has intensified, citrus greening has spread from Asia to Africa and America. Since there are now no effective management methods in habitation, the goal of the current research activity is to increase production and prolong the lifespan of affected trees. The field trial was conducted across the country at six different All India Research Coordinated Project Centre. The vector citrus psylla has been control at the time of new flush emergence by spray of systemic insecticide Imidacloprid 17.8 SL @ 0.5 ml /l. Citrus greening disease severity was reduced by 26.49, 28.59, 34.03, 28.0, 33.99 and 28.36% at locations in Akola, Ludhiana, Periyakulam, Rahuri, Tinsukiaan, and Tirupati respectively, with higher yield (51.66, 55.75, 23.87, 42.98, 47.77, and 26.92 kg/tree respectively) with two sprays of Tetracycline hydrochloride @ 0.6 g/l of water at 45-day interval from October to December, as well as soil application of 50% more than recommended dose of Phosphorus and 15 days later zinc sulphate (200 g/plant), and ferrous sulphate (200 g/plant). The use of antibiotic and nutritional treatments has been suggested to manage the disease.

Keywords: Citrus greening, Antibiotic, Nutrition, Management



### Cataloguing the seasonal pathogen and pest diversity in citrus orchards of Meghalaya

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Khasi Mandarin orchards surveyed in RiBhoi and South Jaintia Hill districts showed that most of the orchards are more than 15-16 years old and some were more than 25 years old. The orchards were of seedling origin and have not been maintained well with adequate fertilizer and irrigation during the lean period. Proper schedule of pruning and application of Bordeaux paint was not practiced. Intercropping is a common practice followed in all the orchards with exhaustive crops like ginger, sweet potato and pineapple. Amongst the orchards surveyed, 20% of the orchards were in near-decline stage with reduced number and size of fruit bearing. The common diseases observed in the orchards were felt disease (15.38% incidence), citrus scab (5.26% incidence) and sooty mould (17.52% incidence). Routine observations of insect pests on citrus orchard in horticultural farm as well as some selected orchards in farmer's field of Meghalaya have been recorded. Results showed that leaf miner, aphids, black flies, hoppers, coleopteran beetles, lemon butterfly, mealybugs, cowbug, scale insect, damage symptoms of citrus trunk borer were found citrus orchard. Among these, leaf miner infestation was maximum followed by trunk borer infestation. Among the natural enemies, coccinellid beetles were more common in citrus orchard. Pathogen and pest build up over the years with changing climate scenario may aggravate the citrus decline status in the state if appropriate remedial measures are not taken up.

Keywords: Citrus, Decline, Incidence, Pathogens, Pests



#### TS-3-0-11

#### Changing pest scenario in acid lime growing regions of Tamil Nadu, India

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Citrus is commercially grown throughout India and occupies a place of prime importance among the major fruits of India, which ranks third after mango and banana. Acid lime is widely grown in the southern region of India. In Tamil Nadu, acid lime occupies an area of 11,889 hectares with the production of 167085 tonnes annually with the productivity of 14.05 tonnes per hectare. However, in recent years, acid lime yield is reliant on environmental, agronomical and biotic factors. Pests are causing severe menace in acid lime cultivation. Hence, it is necessary to observe the absolute occurrence of pest complex and their importance in respect to changing scenario of climatic factors on their biology, behavior and ecology. Thus, roving surveys were carried out during 2022-2023 in different acid lime growing regions of Tamil Nadu to record the various insect pests. The incidence of citrus leaf miner, rust mite on fruit, mealy bug, citrus butterfly and mealy bug were observed during survey. The level of mealy bug damage (2.75%) was found to be low compared to average of past years (3.86 %). The citrus leafminer (15.48%) and citrus butterfly (15.28%) incidence were medium damage rating scale however, the citrus butterfly, was to be in slightly increasing trend. The damage of citrus rust mite on fruits was recorded maximum with severe rating scale (26.45%), which needs to be monitored regularly and managed to prevent market value economic loss. The occurrence of new pest was not observed and there was no change in minor pests becoming major under changing weather scenario. This will help in finding out the susceptible nature of the pest population and nature of damage infestation and disease transmission for deciding suitable pest management strategies.

Keywords: Acid lime, Insect Pests, Monitoring, Management



# Sustainable management of sweet orange gummosis and fruit drop: A formidable challenge to sweet orange industry in Marathwada

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The results revealed that all the six bioagents tested in vitro applying dual culture technique against Phytophthora nicotianae, Phytophthora citrophthora and Phytophthora palmivora significantly inhibited the mycelial growth of the test pathogen over untreated control. However, bioagent, Trichoderma harzianum recorded minimum mean colony diameter (7.73 cm 2) and highest inhibition (87.85%) of mycelial growth of P. nicotianae over untreated control followed by the bioagent T. asperellum, T. koningii which recorded mean colony diameter of 9.95 cm2, 14.15 cm2 and mean mycelial inhibition of 84.36%, 77.76%, respectively. Evaluation of different botanicals by Poisoned Food Technique showed that all plant extracts tested in vitro were found significantly effective in reducing the percentage mycelial growth of *P. nicotianae*, *P. citrophthora* and *P. palmivora* over untreated control. However, plant extract (@ 5, 10 and 15%) of Garlic, recorded lowest mean colony diameter (47.45 mm) and highest mean mycelial growth inhibition (47.26%) followed by Neem, Onion which recorded the mycelial growth of 55.20 mm, 60.85 mm, and the mean mycelial growth inhibition of 38.65%, 32.38%, respectively. Results revealed that all the nine fungicides tested in vitro applying Poisoned Food Technique against P. nicotianae, P. citrophthora and P. palmivora significantly inhibited the mycelial growth of the test pathogen over untreated control. However, fungicide, Cymoxynil 8% + Mancozeb 64% (Curzate M-8) recorded minimum mean colony diameter (16.12 mm) and maximum mean inhibition (82.09%) of mycelial growth of the test pathogen over untreated control (mean colony diameter 90.00 mm and mean inhibition 0.00) followed by the fungicide Metalaxyl-M 4% + Mancozeb 64%, Metyram, which recorded mean colony diameter of 20.16 mm, 24.16 mm and mean mycelial inhibition of 77.59%, 73.14%, respectively.

Keywords: Sweet Orange, Gummosis, *Phytophthora* spp.



# The temporal and spatial patterns of bacterial diversity in mandarin tissues infected with citrus greening disease

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Identifying temporal and spatial patterns of bacterial diversity in Citrus tissues provided an unified framework for understanding their differential diversity in infected and healthy tissues. This study compared the bacterial diversity and their relationships in samples of healthy and greening disease affected mandarin orange tree in lower Pulney hills of Tamilnadu, India. The leaf and root samples of both healthy and greening disease affected mandarin tree were analysed for their bacteriome composition in V5-V7 region of 16S gene. Amplification and sequencing were carried out through illumina platform. Alpha diversity analysis in healthy and infected leaves of mandarin orange and roots revealed that, genus richness was higher in healthy leaves than in infected leaves. In root sample, bacteria were more diverse than those in healthy and infected leaves. B diversity analysis indicated that bacteria in roots exhibited a significant compositional distinction to leaf samples. The most abundant phylum was proteobacteria in both infected and healthy leaves (99%) but they showed less dominance in roots (75%); among the proteobacteria, gamma proteobacteria occupied major fraction of 97%. In healthy leaf sample, firmicutes (0.3%) were more abundant than infected leaves. Root endophytic community showed significant diversity in phyla viz., Proteobacteria (75%), Myxomycoccata (1%), Bacteroidota (1%), Armatimonadota (1%), Firmicutes (0.3%), Actinobacteria (20%) and Acidobacteria (1%). Genus abundance study revealed the abundance of *Ensifer* (phylum – Proteobacteria) in healthy leaves than symptomatic leaves and roots. Similar trend was observed with the genus Paenibacillus(phylum -Firmicutes). The abundance of the genus Streptomyces, Rhizobium, Sphingomonas and Methylobacterium were higher in roots than in healthy leaves and still lower in infected leaves. In sum, these data improved our understanding of diversity patterns and provided new insights into the relative abundance of bacterial genus in healthy and infected tissues of mandarin orange.

Keywords : Bacterial diversity, Citrus Greening Disease, Leaves, Mandarin Orange, Relative Abundance, Roots



# Dieback and gummosis incidence caused by *Lasiodiplodia theobromae* affecting acid lime (*Citrus aurantifolia*) cultivation in Tamil Nadu

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In the Citrus group, acid lime is widely grown in India next to mandarin and sweet orange both under tropical and subtropical climatic conditions. The area under acid lime in Tamil Nadu is 11,889ha which is increasing every year with an annual production of 16,7084.96MT during 2021 - 22. Though the production is increasing, there are many constraints which contribute to the low performance of the crop reducing the income of the farmers. One among the constraints being the incidence of dieback and gummosis caused by Lasiodiplodia theobromae. This is evident from the roving survey conducted from 2021 to 2022 in Dindigul, Salem, Pudukkottai, Tirunelveli, Theni and Thenkasi districts of Tamil Nadu were the area under acid lime is more. The average incidence recorded in these regions was 21.92 % of twig blight/die back, 4.47 % of stem end rot and 8.86 % of gummosis caused by *L. theobromae*. The fungus was isolated from the samples collected from affected tissueswhich produced greyish black coloured mycelium and was further confirmed by the production of dark pycnidia and bicelled ellipsoidal spores. In the fixed plot at Horticultural College and Research Institute, Periyakulam, the plants showed incidence upto 25 % in the 10 year old plants which expressed gummosis and breaking of branches at the site of infection. Management was done in the experimental field by pruning the affected branches with some healthy tissues and spraying 0.1% carbendazim followed by two sprays of 0.3% copper oxychloride at 15 days interval with copious irrigation during the dry period resulted in the recovery of the plants.

Keywords: Acid lime, Dieback, Gummosis, Lasiodiplodia theobromae



# Analysis of variable number of tandem repeats (VNTR) of '*Candidatus* Liberibacter asiaticus' using CLABASIA\_01645 locus

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*Candidatus* Liberibacter asiaticus (CLas) is a Gram negative bacterium, known as etiological agent of Huanglongbing (HLB)/ citrus greening, devastating disease with significant global economic implications on citrus production worldwide.CLas is the most widespread and destructive pathogens of citrus. CLas killed the millions of citrus plants globally. It is transmitted by the *Diphorina citri* most commonly known as Asian citrus psyllid, which is prevalent in citrus-growing regions worldwide.Present work emphasis on the determination Variable Number Tandem Repeats (VNTRs) present in the CLABASIA\_01645 locus from the various strains found across different regions of ten countries worldwide. Four distinct classes were identified based on tandem repeat number (TRN): Class I (TRN  $\leq$  5), Class II (TRN > 5  $\leq$  10), Class III (TRN > 10  $\leq$  15), and Class IV (TRN > 15).The finding of study revealed that Class II is more dominated among the all other three classes. It also shows that, Indian population of *Candidatus* Liberibacter asiaticus exhibits greater diversity compared to populations reported in countries such as Thailand, China, and Bhutan.The CLIBASIA\_01645 locus of the 'CLas' was found to be useful to detect and characterize the genomic variability of the bacterial population. Currant work is the first to attempt to catalogue variations among 'CLas' populations worldwide.

Keywords: Citrus, Huanglongbing, Candidatus Liberibacter asiaticus, VNTR, Class



#### Nematode problems and their management in citrus: Progress from India

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Plant parasitic nematodes are one of the major constraints in citrus producing areas in the country by causing significant yield losses. Citrus crop being perennial in nature harbours and encourages the multiplication and build up of nematode population round the year. Several ecto, endo and semi-endo parasites have been reported to attack roots from citrus growing regions. Among, different groups of plant parasitic nematodes reported to be associated with citrus roots, few species *viz., Tylenchulus semipenetrans, Pratylenchus coffeae, Hoplolaimus indicus, Helicotylenchusdihystera, Tylenchorhynchus sp., Rotylenchulus* sp. were proved to be pathogenic to citrus. However, Citrus nematode, *Tylenchulus semipenetrans* and Asian citrus root knot nematode, *Meloidogyne indica* are reported to infect and cause severe damage to different citrus species in different citrus growing regions in the country. The yield losses caused by *T. semipenetrans* are estimated to be in the range of 6.8-17.5% annually. Systemic research has not been undertaken for economic loss incurred by *M. indica.* Integration of botanicals with biological control agents (*Pseudomonas fluorescens, Purpureocillium lilacinum*) or lower dosage of chemical insecticides (Carbofuran), interculture of onion, garlic and marigold in the citrus orchards reduce the nematode population. Use of rootstock, Trifoliate orange (*Poncirus trifoliata*) and its hybrids, found resistant to *T. semipenetrans*.

Keywords: Citrus, Plant parasitic nematode, Tylenchulus semipenetrans, Meloidogyne indica, Management



#### Meloidogyne indica Whitehead, 1968: an Emerging Nematode Pest of Citrus in India

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Citrus have been found to be attacked by Root knot nematodes, *Meloidogyne javanica* (Mani, 1986), *Meloidogyne incognita* and *Meloidogyne indica* (Whitehead, 1968) in India. In recent years, citrus root knot nematode, *M. indica* is emerged as serious problem in some citrus growing regions of the country. This nematode infects citrus species such as Acid lime/Kagzi lime (*Citrus aurantifolia*) and sweet orange (*C. sinensis*). Recently, Acid lime orchards in North Gujarat, particularly in Kherva, Jagudan and surrounding villages in Mehsana and Banaskantha districts were observed to be heavily infected by *M. indica*. Systemic research has not been undertaken for economic loss incurred by this nematode species. Infected trees exhibited stunting, yellowing, twig blight leading to citrus decline symptoms. On uprooting such infected plants, numerous small to very big size galls/knots were noticed on the roots, resulting in very poorly developed root system. As root knot nematodes are among the most economically harmful nematodes, they may have the potential to significantly affect citrus production if they are established in other citrus producing areas of the country. Moreover, the distribution of *M. indica* on citrus in the country is unknown.

Keywords: Plant parasitic nematode, Citrus, Acid lime, Root knot nematode, Meloidogyne indica



#### Citrus blackfly management: An integrated approach

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Citrus blackfly (Aleurocanthus woglumi Ashby), a pernicious sucking insect, poses a significant threat to citrus cultivation by targeting young flushes, extracting sap, and excreting abundant honeydew, thereby fostering the growth of the troublesome sooty mold. This study sheds light on the multifaceted issues surrounding citrus blackfly infestations in Maharashtra's citrus orchards and underscores the need for management strategies from the perils posed by blackfly. An unprecedented surge in the citrus blackfly population, surpassing the economic injury level observed due to heavy rainfall. This surge led to the rapid proliferation of sooty mold (Capnodium sp.), locally known as 'Kolshi,' which encased entire citrus plants. Active infestation periods are observed from February to April, June to August, and October to December, with an Economic Threshold Level of 5-10 nymphs per leaf. Organic interventions encompass measures such as preventing close planting and averting waterlogging conditions, alongside refraining from planting guava, sapota, mango, and pomegranate near citrus orchards. Foliar application of neem oil @10 ml/l or azadirachitin 1% @3 ml/l or neem soap or pongamia soap @5 gm/l of water coinciding with adult emergence reduces the pest infestation. Avoiding applying insecticides in the late winter to early spring will help preserve beneficial bioagents. Chemical management alternatives involve the foliar application of insecticides such as imidacloprid (17.8% SL) 0.5 ml/l or thiamethoxam 0.3g/l or dimethoate (30EC) 2 ml/l of water at adult emergence and 50% egg hatching stage coinciding with I<sup>st</sup> Fortnight of April, II<sup>nd</sup> Fortnight of July and I<sup>st</sup> Fortnight of December in winter season. Furthermore, the use of starch @ 2% followed by copper oxychloride (COC) @ 0.3% application effectively eliminates the black fungal layer caused by sooty mold on affected trees.

Keywords: Bioagent, Citrus blackfly, Economic injury level, Integrated pest management



Advancing Citriculture for Agro-economic Prosperity

#### **Poster Presentation**

#### TS-3-P-01

### A review and updated information on *Colletotrichum* species associated with citrus trees

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The diversity of citrus as a commercial fruit resulted to become a proper host for various fungal pathogens, endophytes, and saprobes. So investigation, research and identification of fungal diseases of this fruit trees is a very critical and key step for its diseases management. The *Colletotrichum* species are among the list of the eight to 10 most economically important fungal plant pathogens in the world and are responsible for numerous diseases in Citrus and other crops. In the case of Citrus, *Colletotrichum* species are associated as saprobes, pathogens, latent pathogens, epiphytes, and also endophytes. It may switch to important pathogenic lifestyles while their hosts are faced with any stress or post-harvest conditions. Species of *Colletotrichum* can cause some citrus important diseases including anthracnose, twigs wither-tip, dieback, citrus fruit drop, and postharvest anthracnose. Multilocus genetic information showed that *Colletotrichum* species associated with citrus species mostly related to *C. gloeosporioides* complexes. In Iran, *C. fructicola, C. karstii, C. gloeosporioides, C. siamense, C. novae zelandiae* and *C. nymphaea* have been reported on Citrus plant hosts. Based on updated information, at least 25 Colletotrichum species have been identified on Citrus around the world. For plant breeding programs and finding the host range of *Colletotrichum* species, accurate identification is very important.

Keywords: Anthracnose, Citrus, Leaf spot, Whiter-tip



# Prediction of Asian citrus psyllid, *Diaphorina citri* Kuwayama on Kinnow mandarin using weather parameters in Punjab, India

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Field experiments were carried out to study the population dynamics of Asian citrus psyllid, Diaphorina citri Kuwayama on Kinnow Mandarin for twelve years (2007-08 to 2019-20) in relation to different weather parameters and a population prediction model was developed and evaluated for citrus psylla in Punjab, India. Adult psyllids were observed throughout the year but the population was very low during November-January in all the years. Two population peaks were observed- first peak during March and second during September months. Highest population (138.3 nymphs/25 twigs) was observed during second fortnight of September. Psyllid population showed significant and positive correlation with maximum and minimum temperature, vapour pressure, wind speed, sunshine hours, total rainfall, evaporation and negative correlation with relative humidity and number of rainy days, indicating that weather parameters played a significant role in population dynamics of *D. citri*. The validation of the model carried out using various indices viz, Root mean square error (RMSE), Coefficient of determination, Nash-Sctuliffe efficiency, Mean Bias Error, suggested that, the model predicted the population of citrus psylla quite satisfactorily (index of agreement > 0.74). Based on rationale of weather based prediction of pest, forecasting of citrus psylla can be made available to the citrus growers in Punjab, India. Moreover, the use of different weather parameters allows to examine the sensitivity of the predictions, and to improve the communication with the farming community and decision-makers; a key aspect in integrated pest management.

Keywords: Citrus pests, Correlation, Diaphorina citri, Prediction model, Weather parameters



### Studies on bioremediation potential of HAD enzyme from *Candidatus* Liberibacter asiaticus

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Organophosphate compounds (OPs) are a group of synthetic chemicals used extensively in agriculture, industry, and household products, in the form of insecticides, herbicides, and fungicides. These compounds are highly toxic and cause various acute and chronic health effects in humans and animals. Bioremediation is a promising approach to address this issue, where the enzyme can be utilised to degrade and detoxify OPs. Candidatus Liberibacter asiaticus, a gram-negative bacterium, are mobile causative pathogen of citrus greening disease. The bacteria had great exposure to several pesticides for several years and have been adapted to several toxic chemicals by different mechanisms. To unravel the research gap of significant enzymes associated with detoxifying harmful compounds, we have considered one of the potent enzymes in our study. This enzyme belonging to HAD superfamily from Candidatus Liberibacter asiaticus was characterised by biochemical and biophysical techniques. The biochemical assay with pNPP as the substrate revealed that the enzyme is an alkaline phosphatase with Km 7mM and showed optimum activity at pH 6 and at 25°C. To get further insights into its threedimensional secondary structural element arrangement and to explore its applications, the protein was modelled and validated using online servers/tools. Docking results showed the binding of organophosphate molecules to the probable active site residues of the enzyme, along with polar interactions with several other residues. The preliminary results reveal that this novel HAD enzyme can be employed as a bioremediation tool, in detoxification of pesticides in solution.

Keywords: HAD, Candidatus Liberibacter asiaticus, Organophosphates, Characterisation, Bioremediation



# Characterization of salvage pathway enzyme ATIC from *Candidatus* Liberibacter asiaticus and screening of potential inhibitors for the management of HLB disease

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Citrus Huanglongbing(HLB), is one of the most devastating, fast-spreading diseases in *citruscrops* leading to major economic losses worldwide. Candidatus Liberibacter asiaticus (CLa) is a phloem-limited, gram-negative bacterium that severely affects fruit quality and crop health by patchy chlorosis of leaves, blotchy mottle and yellowing of leaves. Despite multiple attempts by the scientific community, effective drugs or management strategieshavenot been discovered yet to control HLB disease. 5aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) is a bifunctional enzyme of the de-novo purine biosynthesis pathway catalyzing the last two penultimate steps i.e. AICAR transformylase involving the transfer of formyl group from its co-factor 10-f-THF and the IMP cyclohydrolase, the final cyclization of FAICAR forming IMP. ATIC is vital for the survival or growth of bacteria and is a promising target for drug development to control HLB disease in citrus. In this study, cloning and recombinant expression of ATIC from CLa have been performed in the prokaryotic expression system followed by biophysical characterization and bioinformatics studies. Further, screening of potential inhibitor molecules was performed against ATIC using the insilico approach. We have identified three potential inhibitors namely azobenzene sulphonic acid, Diosbulbin A and Lepidine D with binding affnity -7.8, -7.6 and -7.3 respectively. An important therapeutic application of ATIC enzyme as a wound-healing agent on the CHO cell line has also been explored in our study. Present research workprovides a strong foundation for the future development of potential drug candidates for the effective management of HLB disease in *citrus* and a novel therapeutic application of CLa ATIC.

Keywords: Citrus Huanglongbing, ATIC, Purine biosynthesis, Drug development



# Eco-friendly approach for the management of citrus butterfly, *Papilio demoleus* L. on acid lime (*Citrus aurantifolia*)

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Citrus butterfly, Papilio demoleus is an economically important pest on citrus crops. The larvae of this pest devour large quantity of foliage both under nursery and open field condition. The acid lime is a major citrus crop grown in Karnataka, which is severely affected by larvae of citrus butterfly throughout the year. It is important to produce the acid lime fruits with less pesticide residues since the fruits are having lot of export demand. The *in-situ* vermiculturing involves the conversion of organic residues into useful vermicompost by the action of earthworms near the root zone of the crop. The field experiment involving in-situ vermiculturing at varying rates of earthworms considerably reduced the incidence of the citrus butterfly on acid lime. The in-situ vermiculturing with earthworms @200/plant + FYM @30 kg/plant + crop residue mulching and earthworms @100/plant + FYM @30 kg/plant + crop residue mulching was significantly reduced the citrus butterfly (3.58 to 5.08 larvae/plant). The release of earthworms at 75 and 50 per plant along with FYM and crop residue mulching was found statistically on par in reducing the incidence of the pest. The application of inorganic fertilizers recorded high level of incidence of citrus butterfly on acid lime (12.08). The slow and balanced release of nutrients from vermicompost helps plants to uptake required nutrient continuously for their growth without nutrients deficiency. The organic fertilizers were also found to enhance accumulation of phenolic compounds in plants which may not attract larvae for feeding. The uptake of these components by plants alters activities within plant system making the plant tolerant against pest attack. The increased thickness and high degree of lignification of epidermal cells, altered levels of sugar concentrations in apoplast, changed levels of phenolic and secondary plant substances may have interfered with colonization of citrus butterfly.

Keywords: Acid lime, Citrus butterfly, Earthworms, In-situ vermiculturing, Tolerance



#### Population dynamics of major sucking insect pests of acid lime, Citrus aurantifolia

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The sucking insect pests are becoming serious menace for cultivation of acid lime, Citrus aurantifolia. It is important to know about the seasonal fluctuation in population of these insect pests under changing climatic conditions. A fixed plot survey was carried out to study the population dynamics of major sucking insect pests of acid lime at College of Agriculture, Vijayapura, Karnataka, India during November 2020 to June 2022 on Kagzi lime. The observation on incidence of pests wasrecorded on 30 randomly selected plants of three replications. The sucking insect pests viz., psyllid, Diaphorina citri Kuwayama, blackfly, Aleurocanthus woglumi Ashby, mealybug, Planococcuscitri (Risso) and aphid, Aphis craccivora Koch were recorded to hamper acid lime production. The peak activity of psyllid was observed during March to May (5.61 to 9.19 psyllid/ terminal shoot) while minimum incidence was recorded during November to January. The blackfly reached maximum activity during April and May (8.15 to 12.50 blackfly/ leaf). The activity of blackfly was noticed to be nil during August to November. The mealybug attained peak activity (2.09 to 2.96 colonies/shoot) during February to March, whileaphidsreached maximum density from February to April (9.29 to 15.00 aphids/10cm shoot). The correlation analysis indicated that the maximum and minimum temperatures influenced positively (r = 0.342 to 0.855<sup>\*\*</sup>), while relative humidity influenced negatively (r = 0.215 to 0.841<sup>\*\*</sup>) on the activity of sucking insect pests on acid lime. This information forms basis for predicting the possible outbreak of these pests, in addition it can be used for developing forecasting model and suggesting timely intervention measures for management of sucking insect pests on acid lime.

Keywords: Blackfly, Correlation, Mealybug, Population dynamics, Psyllid, Weather parameter



#### Severity of citrus canker disease in major acid lime growing provinces in India

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A roving survey was carried out from 2017-18 to 2021-22 in major acid lime-growing areas of Vidarbha and the Western region of Maharashtra (Central India), Andhra Pradesh, and Tamil Nadu State (South India) to assess the severity of citrus canker disease incited by *Xanthomonas citri* subsp.*citri* (synonym *X. axonopodis* pv. *citri*).occurring under natural conditions. From the results obtained, it was clear that leaves were more susceptible to the infection by the citrus canker disease, as more disease severity was recorded in leaves than stem and fruit, irrespective of location. Among the states surveyed, maximum disease intensity (31.79%) on leaf and fruit was recorded in Akola district of the Vidarbha region, followed by Ahmednagar district (30.51%) of Western Maharashtra.The lowest intensity (9.76%) was recorded in the Andhra Pradesh state's Nellore district. The intensity of canker disease in Tamil Nadu state was 26.04% on average, with the highest recorded in Dindigul, Theni, Tiruchy, and Tirunelveli districts. During the study period, a slight increasein the constant pressure trend of citrus canker was noticed in the study area.

Keywords: Acid lime, Canker, Roving survey, Severity



### Status of *Phytophthora* root rot and greening diseases of citrus causing mandarin decline in India

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The mandarin orange (Citrus reticulata Blanco) is the most significant commercial citrus in India. Mandarins are sweeter and less acidic than other citrus fruits, and they are easier to peel and segment separation. A few ecotypes of mandarin, such as Nagpur mandarin, Khasi mandarin, and hybrid Kinnow, have high quality and export potential. Survey and surveillance of diseases such as Phytophthora root rot/gummosis and greening associated with Nagpur mandarin, Khasi mandarin, and Kinnowwere conducted in a systematic manner in growing areas of different age groups, with an emphasis on the occurrence of new and emerging diseases. Approximately 30 plants were selected for observation in each orchard from 2017-18 to 2021-22 in the Vidarbha region of Maharashtra, Punjab, and Assam state. The most widespread and serious diseases prevalent in the study area were *Phytophthora* root rot and gummosis caused by Phytophthora nicotianae. Phytophthora root rot and gummosis across all age groups ranged from 2.40 to 4.90%, 11.42 to 12.37%, and 8.32 to 12.85% in all districts of Maharashtra's Vidarbha area, Punjab, and Assam state, respectively. Citrus greening severity caused by Candidatus Liberibacterasiaticus was assessed and observed to the tune of 0.94 to 5.47%, 5.82 to 7.17%, and 16.42 to 38.51% in the Vidarbha region of Maharashtra, Punjab, and Assam state, respectively. Amravati in the Vidarbha area, Hoshiarpur in Punjab, and Margherita and Tinsukia in Assam state had the highest disease incidence of *Phytophthora* root rot/gummosis and greening among the districts surveyed. According to the current survey data, mandarin susceptibility was confirmed in all regions surveyed.

Keywords: Nagpur mandarin, Phytophthora root rot, Greening, Disease, Status, India



# Mass production of *Aschersonia aleyrodis*, a entomopathogenic fungi of citrus black fly by using agricultural foodstuffs

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Twelve different foodstuffs were examined to determine the utility of locally accessible food grains and substrates for mass-scale culture of the entomopathogenic fungus, *Aschersonia aleyrodis,* in terms of conidial production. These foodstuffsperformed differently in terms of the number of days required for the initiation of growth. Sorghum was significantly more effective than other grain media in initiating growth, with a minimum 16.67 days and maximum of 25.0 days. The sporulation of the fungus differed significantly among various foodstuffs. Among all the foodstuffs, sorghum grains produced the most spores (3.43 x  $10^7$  conidia/gm), followed by wheat (3.30 x  $10^7$  conidia/gm), sunhemp (3.27 x  $10^7$  conidia/gm), and Maize ( $3.17 \times 10^7$  conidia/gm). Bajra grain and soybean were on par with each other by recording 2.97 x  $10^7$  and 2.93 x  $10^7$  conidia/gm, respectively. Rice, barley, fox millet and Pigeon pea were found to be the least preferred grains for sporulation of *A.aleyrodis*. The lower conidial production was noticed in groundnut kernels ( $1.02 \times 107$  conidia/gm). Thus, it was determined that crushed sorghum, wheat and sunhemp grains were the most favourable food media for *A. aleyrodis*'s faster and higher conidial production.

Keywords: Aschersonia aleyrodis, Mass production, Food stuffs, Sporulation



# *In vitro* bio-efficacy of different antibiotics and bioagents against bacterial canker *(Xanthomonas axonopodis* pv. *citri)* of Acid lime

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Four antibiotics in combination with antibacterial chemicals sandtwobioagents viz., *Pseudomonas fluorescence* and *Bacillus subtilis*, were evaluated *in vitro* against *Xanthomonas axonopodis* pv. *citri* (Hasse). Among the different antibiotics in combination with antibacterial chemicalsrevealed that Streptocycline (100 ppm) + Copper oxychloride (0.25%) shows maximum inhibition zone (10mm) followed by Streptocycline (100 ppm) + Copper hydroxide (0.2%) i.e.9.6mm whereas least inhibition formed by Kasugamycin (0.15%) (8.6mm). *In vitro* study of bioagents by Dual culture method revealed that *Pseudomonas fluorescence* shows maximum inhibition zone (97.2%) followed by *Bacillus subtilis*(92%). *In vivo* evaluation of antibiotics in combination with antibacterial chemicalsand bioagents revealed that the minimum disease intensity of 19.53% was recorded by Streptocycline (100 ppm) + Copper oxychloride (0.25%) with 40.71% disease control followed by Streptocycline (100 ppm) + Copper hydroxide (0.5%) 19.63% with 39.46% disease control.

Keywords:Xanthomonas axonopodis, Pseudomonas, Bacillus subtilis canker, Acid lime



# Management of stem-end rotof sweet orange caused by Colletotrichum gloeosporioides

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Among the most important fruit crops in the State of Maharashtra, the sweet orange (*Citrus sinensis* L. Osbeck) which holds a distinctive position. It is third largest crop industry in India after mango and banana. Sweet orange is known to be affected by several fungal diseases. Recently, the pre-harvest fruit drop caused by *Colletotrichumgloeosporioides* has become severe which affects the marketable fruit yields of sweet orange in Maharashtra state. Preharvest application of fungicides showed significant reduction in per cent fruit drop over untreated control. After first spray the fungicidal treatment of Thiophanate Methyl @ 0.7g/lit) had the lowest mean per cent fruit drop of 6.67% followed by the treatment of Carbendazim + Mancozeb 75%WPi.e.7.22 %. Whereas after second spray it was showing an average of 5.50 per cent fruit drops in treatment with Thiophanate Methyl @ 0.7g/lit) and found at par with treatment of Carbendazim + Mancozeb 75%WP with a mean fruit drop of 5.95 %. However, after third spray, the treatment of Thiophanate Methyl @ 0.7g/lit was found significantly superior over rest of the treatments and recorded the least mean fruit drop of 4.33 %with maximum per cent disease control of 53.73 per cent and maximum fruit yield of 45.44 kg/plant and B: C ratio 1: 2.30.

Keywords:Citrus sinensis, Colletotrichum gloeosporioides, Novel fungicides, Thiophanate methyl, SER, Sweet orange



#### Management of sucking pests in citrus

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A field study was carried out during 2016-17 and 2017-18 at Rahuri and Tirupati location to evaluate the effective insecticides for the management of sucking pests of citrus. Pooled data of two years of experimentation revealed that at 14 days after second spray the treatment with neem formulation 10000 ppm @ 5ml/L followed by spinosad (0.015%) recorded lowest population of aphids (3.45) and psylla (1.55) at Rahuri whereas at Tirupati the spray treatment of neem formulation 10000 ppm @ 5 ml/L f. b. thiamethoxam 25WG (0.008%) recorded minimum population of aphids (1.98), psylla (1.78) and black flies (0.70). However, the lowest percent infestation of thrips (5.29%) was recorded in treatment neem formulation 10000 ppm @ 5 ml/L followed by spinosad (0.015%). Significantly higher marketable yield (19.00 t/ha.; 24.96 t/ha) and B:C ratio of 1: 2.67 and 1: 2.49 was realized in neem formulation 10000 ppm @ 5 ml/L followed by spinosad (0.015%) at Rahuri and Tirupati, respectively and it was at par with neem formulation 10,000 ppm @ 5 ml/Litref.b.thiamethoxam 25 WG (0.008%).

Keywords: Aphid, Black fly, Psylla, Spinosad, Thrips, Thiamethoxam, Yield



# Microbiocidal activity of volatile essential oils against *Colletotrichum gloeosporioides* causative agent of Citrus twig blight

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The use of non-toxic alternative microbiocidal agents is gaining acceptance these days. One such alternative is to use antifungal essential oils, which are more environmentally friendly and largely accepted by the general civic. Twig blight disease, caused by *Colletotrichum gloeosporioides*, is the most recurrent disease of citrus species in the Vidarbha region of Maharashtra state, India, and causes canopy decline and fruit reduction in orchards. In the current investigation, eight essential oils from various plant origins were tested for their ability to inhibit the development of *C. gloeosporioides* using the volatile phase effect at 0.5 and 1.0% concentrations.Results revealed that all of the essential oils tested were significantly superior to the control in inhibiting *C.gloeosporioides* pathogen growth over the untreated control (90.00 mm) and were found to be decrease with the higher concentrations of the essential oils tested. At the measured concentrations, lemongrass, citronella, garlic, and tea tree oil had the highest activity (0.00 mm growth and 100% growth inhibition) against *C. gloeosporioides*. This demonstrates the volatile nature of these essential oils. Next best was Eucalyptus oil, which had the highest activity (37.22 and 63.70%) against *C. gloeosporioides* at 0.5 and 1% concentrations. Karanj oil was found to be less effective at both concentrations.

Keywords: Citrus, Essential oil, Colletotrichum gloeosporioides, Microbiocidalactivity



### Compatibility, cross infection, and laboratory evaluation of *Aschersonia aleyrodis* (Webber) against citrus blackfly *Aleurocanthus woglumi* (Ashby)

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*Aschersonia aleyrodis*(Webber) 'red whitefly fungus' is a fungal pathogen of nymphs of black flay, *Aleurocanthus woglumi*(Ashby) and whitefly, *Dialeurodes Citri* (Ashmead) on citrus plant. It is used as a promising biological control agent. Samples of the fungus *Aschersonia* isolated from naturally infected Citrus Blackfly collected from citrus orchard in Morshi Tahsil was identified as *A. aleyrodis* based on morphological and cultural characteristics.In present study, was conducted during 2018-2019 at the Department of Entomology, PGI, Dr. PDKV, Akola. Attempts have been made to check thecompatibility against popular insecticides among the tested insecticides, Dimethoate 30 % EC was observed compatible with *A. aleyrodis* as it shows less inhibition at half and recommended dose of insecticide. In Cross infectivity study *A. aleyrodis* was found nonpathogenic to citrus aphid and mealy bug. Whereas efficacy of *A. aleyrodis* for the management of citrus blackfly under laboratory condition. Illustrates LC<sub>50</sub> values of 2<sup>nd</sup> instar nymph was calculated 1.1x 10<sup>8</sup> spore/ml and LT<sub>50</sub> values, 8.14 to 13.77 days were derived for 2<sup>nd</sup> instar nymph of citrus blackfly at respective concentration.

**Keywords:***Aschersonia aleyrodis, Aleurocanthu swoglumi,* Biological control agent, Entomopathogenic fungus, Compatibility



# Genetic diversity of *Colletotrichum gloeosporioides*, causing pre-harvest stem end rot fruit drop of Mandarin in the vidarbha region of Maharashtra

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Diseased fruit samples of Nagpur mandarin exhibiting typical symptoms of pre-harvest stem end rot were randomly collected from the different citrus growing areas of Vidarbha region. The fungus was subsequently purified, and by proving Koch's postulates, *Colletotrichum gloeosporioides* was identified as pathogenic and responsible for Nagpur mandarin fruit drop of in ambiabahar. The molecular technique Random Amplified Polymorphic DNA (RAPD) was used to detect the genetic variation among the nine selected *C. gloeosporioides* isolates. To examine molecular variance, a total of 20 primers from the OPD, OPN and S series group were used. Using the RAPD marker, a genetic variation was found among nine isolates of *C. gloeosporioides* based on a simple matching coefficient. The numbers of bands generated, were primer and isolate dependant and ranged from minimum 6 to maximum 23 amplicons. RAPD which showed <50 per cent genetic variability among all *C. gloeosporioides* isolates based on dendrogram constructed. High genetic similarity was observed 72% in Cg20 and Cg24, however least genetic similarity was observed 52% between Cg1 and Cg10 isolates.

Keywords: C. gloeosporioides, Nagpur mandarin, Genetic variability, RAPD



# Transcriptome profiling reveals the mechanism of HLB tolerance in Pumelo found in Manipur, northeast India

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Huanglongbing is a dreadful citrus pandemic worldwide. Almost all citrus grown today are susceptible to HLB for which there is now no treatment. Using RNA-seq data, gene expression profile tests were conducted in this investigation on the HLB-tolerant *C. maxima*. The transcriptome profile revealed 3453 DEGs in the HLB infected *C. maxima* when compared with the healthy control plants. Out of these 3453 DEGs, 1388 were up-regulated and 2065 were down-regulated. Importantly, the most significant differentially expressed proteins include receptor like kinases, Glutathione S transferase, disease resistance proteins which are responsible for immune response to infection. Furthermore, PERK3 and other WRKYs protein activation may improve the tolerance of *C. maxima* to HLB. This study sheds light on the host reaction of an HLB-tolerant citrus cultivar to HLB. Future citrus breeding for HLB tolerance or resistance may be analyzed from *C.maxima*.

Keywords: Pumelo, Huanglongbing, Transcriptome profile, Tolerance



#### Bioefficacy of acaricides for the management of mites in acid lime

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Acid lime, Citrus aurantifolia (Swingle), belonging to the family Rutaceae, is an important fruit crop cultivated in vast area since the fruits are rich in vitamin C as well as minerals. The fruits are used for pickles, jellies, squashes, citric acid and pectin etc. The acid lime production in the country is found to decline due to damage caused by insect and mite pests. More than 50 mite species have been reported as serious pests on citrus in India. The change in climatic factors, favours the growth and development of mites, as a result mites are emerging as serious pest on acid lime in Tamil Nadu. Hence, the field trials were conducted to test the bioefficacy of ddifferent acaricides at Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India consecutively from 2019-2021. The trials were laid out in RBD, replicated four times for each treatment. The acaricides, Spiromesifen 240 SC (0.009%), Fenazaguin 10 EC (0.01%), Propargite 57 EC (0.057%) and Dicofol 20 EC @0.04%) were imposed during active period of the pest twice at 15 days interval. The observations on population counts of mites before and 3, 7 and 14 days after treatment and infested fruits (%) before harvest were recoded. The results on citrus leaf mite population indicated that the effect of new acaricides on citrus leaf mite on 3 DAT, there was no significant difference in the population recorded, which was ranged from 2.91 to 3.56/ leaf as against the standard check dicofol (6.52/leaf). However, all the acaricides were superior to that of control, which recorded 15.47 mites/leaves. The acaricidal effect of newer molecule on 7 DAT showed significant difference from each other. Propargite showed its superior efficacy against leafmites (1.23 mites/leaf) as against 5.40/leaf in dicofol and 14.73/leaf in control. During the second week after imposing the treatment *i.e* 14 DAT, the acaricidal effect of propargite 57EC was found to be an effective 0.33 mites/leaf as against 14.03 mites/leaf in control. Propargite showed its stable and gradual effect on reducing citrus leaf mite population from 3 DAT to 14 DAT. Though the treatment effects were significant from each other and all the acaricides showed less incidence of mites in fruits when compared to control (26.08%), Propargite (4.04%), stood first in its efficacy and recorded maximum of citrus fruit yield from 24.75 to 25 kg/ha during the three years of consecutive experiments. However, citrus whole fruit analysis by HPLC indicated that the residue level of spiromesifen (0.095 mg/kg) is lesser than that of propargite (0.135 mg/kg). The experimental results revealed that acaricide propargite 57 EC (0.057%) was found to be effective for the management of mite incidence in acid lime.

Keywords: Acid lime, Mites, Acaricides, Management



# Molecular detection of *Candidatus* Liberibacter asiaticus, the bacterium associated with citrus huanglongbing (greening) disease of Sweet Orange

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Huanglongbing (HLB or citrus greening or yellow shoot disease) is a devastating disease of citrus caused by nonculturable, fastidious phloem limited bacterium, *Candidatus* Liberibacter asiaticus and threatens the citrus industry in Asian countries. The putative causal agent of the disease is transmitted through insect vector or grafting with diseased budwood. The polymerase chain reaction (PCR) diagnosis is a more reliable and sensitive diagnostic tool for detecting greening bacterium than other conventional approaches like electron microscopy, DNA-DNA hybridization and immunofluorescence (IF) for detection of citrus greening. Results reveal that sodium sulphite method of DNA isolation provided higher yield and better quality DNA than other methods. To confirm the reliability of PCR, the greening bacterium was also detected in graft-inoculated plants, which showed typical greening symptoms. Results show amplification of 450 bp in PCR suggesting sampling in March is more suitable for PCR detection of greening bacterium. The methods validated in this study will be very useful for regulatory response, effective management of infected trees, and development of a *Candidatus* Liberibacter asiaticus free nursery system.

**Keywords:** Citrus greening, Huanglongbing (HLB), Molecular detection, Polymerase chain reaction (PCR), Phloem-limited bacterium



### Potential response of citrus rootstocks under natural field and protected- screened conditions against *Phytophthora nicotianae*

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Citrus cultivation imparts a vital role in global horticulture, but it faces significant threats from soil-borne pathogens, particularly Phytophthora species. Among these pathogens, P. nicotianae and P. citrophthora are the most prevalent. Gummosis is one of the most devastating and economically important diseases, decimating the backbone of the citrus industry by incurring 10–30% losses annually. The current study was carried out at Punjab Agricultural University, Ludhiana to examine the resistance/tolerance response of different citrus rootstocks against P. nicotianae under natural field as well as protected conditions. For the experiment, 14 different citrus rootstocks were artificially screened against *P. nicotianae*under protected conditions. Additionally, evaluations of each of these genotypes were conducted in the field.Moreover, all these genotypes were also subjected to evaluation under the field conditions. The morphological characteristics, viz., plant height, plant spread, leaf lamina length: width ratio, leaf thickness, and petiole wing length: width ratio, were also evaluated. Thestudy revealed that rootstocks including Rough lemon, Sohmyndog, Abohar RL, Florida RL, South Africa, Schaub, Brazilian RL and Limmonaria Rugosodawere found to be susceptible to P. nicotianae under protected conditions as well as open conditions. Whereas the rootstocks viz. Australian RL, Carrizo and Limmonaria Assam exhibited tolerance towards P. nicotianae under both conditions. Being eco-friendly in behaviour, the use of tolerant rootstock is one of the best approaches to combating the problem of *P. nicotianae*.

Keywords: Artificial screening, Citrus, Morphological, P. nicotianae, Rootstocks, Tolerance



#### Citrus greening disease: status and symptoms in Tamil Nadu

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Citrus greening disease (CGD) is one of the most devastating diseases in all Citrus spp. It produces very complex, variable symptoms and poses difficulty in disease diagnosis all the Citrus varieties. The disease leads to substantial economic loss. It is caused by fastidious phloem limited bacteria, CandidatusLiberibacterasiaticus (CLas) and spread by the heat tolerant vector, Asian Citrus Psyllid (ACP) Diaphorinacitri. This study is aiming to assess the extent of the CGD incidence (DI) and severity (DS) in southern districts of Tamil Nadu, India. The prevalence of disease and disease severity were recorded in three villages of Madurai district viz., Thiruvathoor, Palamedu and Kallupatti. In Dindigul district, it was observed in Thadiyankudisai and Oddanchathiram. Likewise, it was recorded in the villages of Pollachi, Poosaripalayam of Coimbatore district and in Sankarankovil of Tenkasi district. Among these surveyed areas, the highest disease incidence of 60.75 % and disease severity index of 64.38 were observed in Oddanchathiram, Dindigul district and the lowest disease incidence of 18.78 % and disease severity Index of 20.47 were recorded in Poosaripalayam, Coimbatore district. In the surveyed areas, the infected trees were observed to have the diagnostic leaf symptom of asymmetrical blotchy mottling. Besides chlorosis, distorted leaves and vein corking were also observed predominantly. Fruits were reduced in size, lopsided and with aborted seeds. It was also observed that the fields with more disease incidence and severity had reduced flowering and fruit set. The shoots showed pronounced yellowing and also expressed multiple micronutrient deficiency symptoms due to the deficiency of Zn, Fe and Mg. Weakening of root tissues and reduced secondary root formation were observed.

Keywords: ACP, CGD, Citrus, Disease incidence, Disease severity, Symptoms



#### Effect of foliar applications of Nano N and NAA on growth and yield of Mandarin

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A field experiment was framed on "Effectof foliar applications of nano N and NAA on growth and yield of mandarin (Citrus reticulata Blanco)" was conducted at Research Field of All India Coordinated Research Project on Fruits (Citrus), Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during Ambia season 2019 -2020. The objectives formulated as, to study the effect of foliar nano nitrogen and NAA on the quality of mandarin and to find suitable concentrations of nano nitrogen alone and in combination with NAA for maximum yield and better quality of mandarin. The experiment was laid out in a Randomized Block Design with three replications and eleven treatments. The different combinations and concentrations ofviz., Nano N, NAA, and Urea, were used in foliar sprays at pea size and 30 days after the first spray. The results of the present investigation indicated that treatment T<sub>5</sub> (nano N 20 ppm) recorded the maximum average fruit weight(155.88 g), fruit length (60.73 mm), and fruit breadth (70.02 mm) while least recorded in  $T_4$ . The treatment comprised of  $T_7$  (Urea 1 % + NAA 10 ppm) accounted for the maximum fruit juice percentage (47.20 %) which was at par with T<sub>10</sub> (nano N 20 ppm + NAA 10 ppm) while the minimum is observed in T<sub>1</sub>. However, treatment, T<sub>10</sub> (nano N 20 ppm + NAA 10 ppm) was most effective in checking the physiological fruit drop and recorded the least fruit drop (314.67) that reflected in yield attributes (867.50 no of fruits/tree, weighing 113.10 kg/tree, and 28.19 tonnes/ha). This treatment was at par with T<sub>a</sub> (nano N 15 ppm + NAA 10 ppm) and  $T_{11}$  (nano N 25 ppm + NAA 10 ppm). In Leaf nutrient statusthe maximum phosphorous and potassium content observed  $T_{10}$  (0.84%) and (1.53%).

Keywords: Mandarin, Foliar application, Yield, Quality



#### Reasons behind the fruit drop in Oranges and removal of orchards

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Maharashtra is the one of the largest producer of orange in the country. The Nagpur mandarin orange is one of the most important fruit crops of Maharashtra. The famous Nagpur orange (Santra) is grown in humid tropical Vidarbha region of Maharashtra where summer temperature reaches as high as 45-46°C. But orange cultivated area is plagued with various problems due to limiting growing conditions, limiting water resources and high incidence of pests and diseases warranting great care from planting till the plants come to bearing in order to sustain a productive life of a minimum of 15-20 years hence research objective was formulated to identify the reasons behind the fruit drop in oranges and removal of orchards as perceived by orange growers. The present study was conducted in Amravati and Nagpur districts of Maharashtra state where orange is grown on large scale. The ex-post-facto research design of social research was used with 300 respondents and analysed by frequency and percentage. The findings noted that in case of reasons behind fruit drop in oranges, majority of the respondents (97.33%) expressed reason that fruit drop due to abiotic factors like high temperature with water stress for longer duration. fruit drop due to the reason high humidity said by 94.33 per cent of the respondents and 88.00 per cent of them perceived reason behind fruit drop was due to Phytopthora (Dinkya) while major reasons behind removal of orchard as perceived by respondents were unsatisfactory market price to oranges (97.67%) and high cost of cultivation and interculture operations (86.04%). The findings revealed that lack of technical orchard management and recommended package of practices.

Keywords: Fruit drop, Reasons, Orange Growers, Farmers, Removal, Orchards



Advancing Citriculture for Agro-economic Prosperity

#### TS-3-P-23

# Toxicity of different insecticides against Asian citrus psylla (*Diaphorina citri* Kuwayama) in laboratory conditions

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The incidence of Asian citrus psyllids *Diaphorina citri* (Kuwayama) is appears throughout the year. Itact as vector of the devastating citrus disease, Huanglongbing (HLB) or citrus greening which is widely spread in citrus growing regions. The use of insecticide for control of citrus phylla is one of the prominent and effective methods to overcome their menace. However, recently very few insecticide have been found under label claim for this pest. The present investigation was conducted in order toknow the toxicity of some new chemistry insecticide against *D. citri*. The bioassay experiment was conducted during the year 2022-23 at Toxicology Laboratory, Department of Entomology, Dr. PDKV., Akola using Imidacloprid, Spirotetramat, Thiamethoxam, Abamectin and Fenpropathrin against *D. Citri*. The toxicity was assessed through uptake bioassay techniquewithfour replicates per insecticideby using fresh citrus twig.Numbers of dead and live psyllids were recorded After 48 hrs. The bioassay results demonstrated that, amongst the five tested insecticide Thiamethoxam was the most effective insecticide with lowest LC50 values. The relative toxicity of other insecticidewas in the range of 0.19 % to 0.61%.

Keywords: Abamectin, Bioassay, Citrus, Huanglongbing, Imidacloprid, Psylla, Thiamethoxam



# Evaluation and dose optimization of bio agents for the management of citrus nematode (*Tylenchulus semipenetrans*) in Acid lime (*Citrus auratiifolia*)

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Acid lime is one of the largest growing and ever demanding citrus groups of fruits cultivated in India. Tamil Nadu is one of the major producers of the acid lime fruit. The citrus root nematode *Tylenchulus* semipenetransis one of the limiting factor of the growth and production of citrus causing die back symptomes. A trial was taken up in five years old acid lime orchard with high infestation of citrus nematode, T. semipenetrans atlrur village in Perambalur district. The initial root and soil nematode population was estimated before the treatment (ranging from 442 – 462/200 gram of soil and 71 – 77 nematodes/1 gram of root). The treatments were imposed as per the technical programme with three replications. The biocontrol agents viz., Pseudomonas fluorescens, Trichoderma viride, VAM and TNAU consortium (Pfbv 22 + Bbv 57) developed by the dept. of Nematology were used at three different levels (20,30,40 g/tree), (for VAM 100,200,300g/tree) to control the citrus nematode (T. semipenetrans). The root and soil population wasrecorded, three, six, nine and twelve months after treatment and the fruit yield was recorded during the trial period (total). The Lowest root and soil nematode population was recorded in Consortia containing (Pfbv 22 + Bbv 57) @40 g/ tree, registering 58.8, 68.6, 77.73 and 63.84 per cent reduction over control in root population in three, six, nine and twelve months after treatments respectively and the same treatment recorded the lowest soil nematode population and highest fruit yield (814.3nos./ tree/during the one year trial period) and this was 40.6% yield increase when compared to untreated control.

Keywords: Acid lime, Biomanagement, Tylenchulus semipenetrans, Trichoderma viride, VAM



# Monitoring of oxidative stress and host defense response under *Phytophthora* attack in a resistant and susceptible citrus rootstock

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Citrus cultivation is highly constrained by *Phytophthora*. Upon pathogen recognition, plants generate rapid burst of reactive oxygen species (ROS) as a first line ofhost defense. Build up of ROS curtail the pathogen spread by regulating the expression of various enzymatic and non-enzymatic antioxidants.We monitored the oxidative stress and host defense in *Phytophthora* tolerant [Poncirus trifoliata (PT) and Sour orange (SO)] and sensitive [rough lemon (RL)] citrus rootstocks after *Phytophthora* inoculations. Oxidative stress build up was monitored at 10,20,30 days post inoculation (dpi) from the contents of H<sub>2</sub>O<sub>2</sub>, Malondialdehyde (MDA) and at the same intervals host defense was examined by quantifying phenyl ammonia lyase (PAL) and peroxidase (POX) enzymes. The electrolyte leakage, an indicator of cell death was recorded maximum in the susceptible rootstock, RL at 30-dpi, which proved its sensitivity to *Phytophthora*. Post pathogen inoculation, level of H<sub>2</sub>O<sub>2</sub>decreased slightly in tolerant rootstocks during initial days, which remained consistent at later stages. On the contrary, susceptible rootstock exhibited more accumulation ofH<sub>2</sub>O<sub>2</sub>in successive intervals. MDA contentwas lower in PT and SOat 20-dpiand was found higher in RL at 20-dpi and 30 dpi. Correlation studies determined a significant and positive association between  $H_2O_2$  and MDA (r=0.79, P< 0.01). This indicated that rootstocks with greater  $H_2O_2$ experienced greater oxidative stress. To cope up the ROS stress, the tolerant rootstock showed higher POD activity at 10 dpi while in RL its activity increased at 20 dpi indicating a delayed response. Hence, sensitivity of rough lemon to Phytophthorainfection might be due to its inability to develop defense response at initial infection stages. Lesser accumulation of H<sub>2</sub>O<sub>2</sub>content and high POD activity at initiation stage would be used as sensitive biochemical markers to screen citrus tolerance to Phytophthora.

**Keywords:** Citrus, H<sub>2</sub>O<sub>2</sub>, Oxidative burst, Peroxidase, Phenylalanine, *Phytophthora*, Plant defense.



### Diversity of citrus fungal endophytes and their biocontrol potential against *Phytophthora nicotianae*, the oomycete causing rootand foot rot disease

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Fungal endophytes have gained increasing attention due to their multifaceted roles in plant health, growth promotion, and stress tolerance. Fungal endophytes play a crucial role in shaping the health and resilience of plants, including those within the citrus family. In this study, we isolated and characterized 152 endophytic fungi from various *Citrus* species, including Sour orange, Smooth flat Seville (SFS), Nagpur mandarin, Pomelo, Trifoliate orange, Kinnow mandarin, Lemons and Acid lime. Remarkably, 151 of the isolates belonged to the phylum Ascomycota, while one isolate was belonged to the phylum Basidiomycota, highlighting the predominance of Ascomycota within this fungal community.

These endophytic fungi were further classified into 6 classes, 15 orders, and 37 genera, revealing a rich and diverse community residing within citrus plants. On the basis of colony color and surface structure, 60 morphotypes were recognized. To understand their potential for biocontrol, antagonistic assays were conducted against the notorious citrus pathogen, Phytophthora nicotianae. Among the 152 isolates, 26 exhibited promising antagonistic activity, with observable inhibition zones between their colonies and P. nicotianae after 7 to 14 days of incubation. Notably, Aspergillus terreus CFE-142 displayed the highest inhibition, while Colletotrichum gloeosporioides CFE-29 exhibited the lowest inhibition.DNA sequencing of internal transcribed spacer (ITS) regions elucidates the taxonomic diversity of these endophytes, revealing both known and novel species, further confirmed by Phylogenetic analyses of ITS region. We elucidated the mechanisms underlying the biocontrol capabilities of these endophytic fungi, which included antibiosis, nutrient and space competition, and the induction of defense responses. Moreover, we found that certain isolates, such as CFE-109, CFE-142, and CFE-157, produced potent secondary metabolites and organic compounds capable of inhibiting P. nicotianae growth, as confirmed by the poisonedfoodtechnique. Under greenhouse conditions, endophytes were visually efficient against P. nicotianae. Applications of mycelium and spores of the antagonists showed significant decrease in root rot rates and increase in plant weights of rough lemon seedlings inoculated with P. nicotianae. To assess the practical implications of our findings, pathogenicity tests were conducted on sweet orange fruits. While Chaetomium globosum isolates (CFE-109 and CFE-157) displayed minimal damage to the fruits, Aspergillus terreusCFE-142 caused very minute lesion primarily on the rind without inducing any fruit rot.Our research highlights the rich diversity of endophytic fungi within the Citrus family and their potential as biocontrol agents against the devastating plant pathogen P. nicotianae. Specifically, endophyticisolates like CFE-109, CFE-142, and CFE-157 showedgreat promise as effective biocontrolagents, offering new avenues for sustainable citrus crop management and disease control strategy.

Keywords: Fungal endophytes, Citrus species, Antagonistic activity, Biocontrol agent, Crop management



Advancing Citriculture for Agro-economic Prosperity

#### TS-3-P-27

### Comparative metagenomic insights of structure and diversity of healthy and HLB infected citrus microbiome

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Huanglongbing (HLB), also known as 'citrus greening' is one of the most devastating citrus diseases caused by phloem-limited proteobacterium Candidatus Liberibacter asiaticus (CLas). In this study, whole genome shotgun sequencing technique was employed for comparative metagenomic analysis of microbial communities in healthy and HLB- infected Khasi mandarin (Citrus reticulata) leaves which revealed the structure and diversity of the microbiome. Total number of reads generated was 244693402 and 251827322, for healthy and infected samples respectively. According to the taxonomic classification data, the Kaiju results showed that the infected samples were dominated with Proteobacteria, Actinobacteria and Bacteroidetes at the phylum level. The Proteobacterial population was found less while Actinobacteria and Firmicutes were more enriched in healthy samples. At the genus and species level, the infected samples were dominated with CLas which confirmed that the samples were HLB infected. When compared with the healthy samples, it was found that the Methylobacterium and Sphingomonas were more enriched in the infected sample. Shannon index of healthy and infected citrus samples were 22.46 and 22.03 respectively which determines the abundance of microbial population. This showed that CLas infection has reduced the alpha diversity of the population which has overall affected the structure of microbiome promoting the infection. Since the infected samples confirmed with the presence of CLas, further sequence data analysis was done. This lead to the identification of the whole genome sequence (WGS) of CLas strain AS-TNSK3 from the metagenomic data which is about 1.237 Mbp with G+C content of 36.5% and harbours both Type 1- and Type 2- like prophages with 1084 open reading frames (ORFs) and 45 RNA genes. The WGS of CLas strain AS-TNSK3 has been submitted to NCBI Genbank under the accession number JARPBB000000000.1 and is the first report from India. This provides the insights of whole genome of our CLas strain which might help us to understand the interaction between the host and CLas. This data of citrus leaf metagenome and of CLas whole genome will facilitate in understanding CLas biology and subsequently designing effective HLB disease management strategy.

Keywords: Citrus, Candidatus Liberibacter asiaticus, Huanglongbing, Genome, India



# Combating Huanglongbing pathogen, *Candidatus* Liberibacter asiaticus with indigenous rhizospheric and endophytic bacteria

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Phloem limited non-culturable bacteria Candidatus Liberibacter asiaticus (CLas) affects the worldwide citrus production by causing the most devastating disease Huanglongbing (HLB). As of today, there is no known cure or treatment to effectively control HLB, and current control methods are primarily based on the use of insecticides and antibiotics, where effectiveness is limited and may have negative impacts on beneficial and non-target organisms. Thus, there is an urgent need for the development of effective and sustainable treatment options to reduce or eliminate CLas from infected trees. In our present study, 195 microbial cultures (104 endophytic bacteria; 91 rhizobacteria) were isolated from HLB-escape Khasi mandarin (Citrus reticulata) trees from Northeast region of India. These isolates were screened for antimicrobial activity against a culturable surrogate of CLas, Agrobacterium tumefaceins strain EHA105. Among them, 2 endophytic isolates showed strong antagonistic activity. These isolates were characterized and identified as *Bacillus pumilus* strain KMME40 and *Bacillus safensis* strain KMME64 by 16SrRNA gene sequencing. An in-vitrocitrus half-leaf method was employed to test the efficacy of the potential endophytes against CLas. Application of Bacillus pumilus KMME40 and Bacillus safensis KMME64 at 10<sup>4</sup>-10<sup>5</sup> colony forming unit (cfuml<sup>-1</sup>) resulted in reduction in CLas copy number following 72 hrs of treatment by real-time PCR. These results may be further validated by introducing these endophytes in the CLas infected citrus plants under *in-vivo* controlled conditions. This can help in establishing a sustainable HLB control strategy through citrus endophytic microbiome restructuring using indigenous endophytes.

Keyword s: Candidatus Liberibacter asiaticus, Endophytes, Bacillus pumilus, Bacillus safensis



Advancing Citriculture for Agro-economic Prosperity

### TS-3-P-29

# Development of a nucleic acid-based multiplex diagnostics for simultaneous detection of major citrus pathogens

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Citrus is an economically important fruit crop globally and represents India's third largest fruit industry after banana and mango. The citrus crop is vulnerable to an array of biotic and abiotic factors that leads to low productivity and yield. The emerging incidence of most common pathogens such as CandidatusLiberibacter asiaticus(CLas), Citrus tristeza virus (CTV), Indian citrus ringspot virus (ICRSV), and Citrus yellow mosaic virus (CYMV), in citrus orchards is alarming for citrus industry. Presently, there is no control strategy available for these systemic pathogens viz., resistance-host, chemical, antimicrobial, or antiviral therapeutics. Thus, raising of pathogen-free seedlings is prerequisite to manage these pathogens. The production of quality pathogen-free seedlings needs rapid, sensitive, and robust diagnostics. Therefore, the present efforts were carried out to develop a simple, reliable, and sensitive nucleic acid-based multiplex PCR (mPCR) assay for the simultaneous detection of major citrus pathogens. The field survey was carried out to find out the samples having mixed infection of major citrus pathogens. The gene specific primer sets were designed and optimized to achieve the targeted amplification for each major citrus pathogen individually and simultaneously. The developed mPCR assay, detected four major pathogens viz., CLas, CTV, ICRSV, and CYMV by amplifying four different and specific targeted genomic regions, 16S rRNA, RNA binding protein (p23), and Coat protein (CP) genes respectively, in a single reaction tube (CLas: 236 bp, CTV: 627 bp, ICRSV: 309 bp, and CYMV: 811 bp). The mPCR assay was highly sensitive and could detect nucleic acid template ranging from 50 pg/µl to 0.40 ng/µl (for CLas: 0.9 ng/µl, for CTV: 0.6 ng/µl, for ICRSV: 0.5 ng/µl, for CYMV: 0.4 ng/µl). The mPCR assay was truly specific and not cross-reacted with other pathogens. The developed assay was also validated using different field samples and showed excellent diagnostic agreement (Kappa value = 0.884) with the simplex PCR (sPCR). The mPCR can detect four major citrus pathogens efficiently in a single tube, and therefore having potential to diagnose samples at large level in less time with affordable cost in citrus budwood programs for quarantine applications.

**Keywords:** Multiplex PCR (mPCR), *Candidatus* Liberibacter asiaticus, Citrus tristeza virus, Indian citrus ringspot virus, Citrus yellow mosaic virus



# Development of RT-PCR novel primers for the detection and molecular characterization of two mandariviruses infecting citrus cultivars in India

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Citrus, the most economically important fruit crop globally, faces the challenge of mandariviruses, including Indian citrus ringspot virus (ICRSV) and Citrus yellow vein clearing virus (CYVCV), which pose significant threats to the citrus industry. ICRSV and CYVCV attributed to the genus Mandarivirus and are transmitted through infected scion buds, sap extract, and vegetative propagation of virus-infected nursery plants. Therefore, accurate and reliable diagnostic tools are crucial to prevent the spread of these diseases. In this study, we have developed novel primer pairs targeting the conserved sequence of the mandarivirus coat protein gene. These primers enable the simultaneous detection of both viruses by RT-PCR, generating an amplified fragment of approximately 450 bp. The efficacy and specificity of the novel primers were evaluated using positive plant samples, including field citrus plants infected with either virus or a mixture of both, as well as other citrus pathogens. In silico analysis confirmed the high specificity of the novel primers for mandariviruses. Furthermore, the presence of ICRSV or CYVCV in these plants was confirmed using specific primer pairs for each virus. The amplified products were purified, sequenced, and deposited into GenBank. In our study, we observed the presence of ICRSV and CYVCV as individual infections or mixed infections in citrus-growing areas. The RT-PCR-based diagnosis revealed a higher incidence of ICRSV (61.22%) and CYVCV (24.48%) in symptomatic field samples. Molecular characterization of ICRSV and CYVCV was performed based on the coat protein (CP) and RdRP genes, respectively. The use of these novel RT-PCR primers for mandarivirus detection will be beneficial in citrus budwood certification programs.

Keywords: Citrus pathogens, RT-PCR, Degenerated primers, Mandariviruses



# Rapid detection of "*Candidatus* Liberibacter asiaticus", with recombinase polymerase isothermal amplification assay (HLB-RPA) based on the nrdBgene

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Citrus greening or Huanglongbing (HLB) is a highly destructive disease caused by 'Candidatus Liberibacter asiaticus', an alpha-proteobacterium, Gram negative, phloem-limited, and non-culturable bacteria. The disease has wiped out millions of citrus trees around the world and impacting the citrus industry. Presently, Polymerase Chain Reaction (PCR) and Real-Time PCR are considered the most reliable methods for detecting 'Ca. L. asiaticus'. However, these techniques are expensive, require advanced laboratories, are not user-friendly, and are not ideal for point of care detection of the pathogen. The current study introduced a diagnostic tool for detecting 'Ca. L. asiaticus' using a recombinase polymerase-based isothermal amplification combined with lateral flow assay (HLB-RPA-LFA) technique that is sensitive, reliable, guick, and affordable. Specific primers and probes were designed based on the nrdB gene of 'Ca. L. asiaticus' to standardize the assay. The assay was evaluated for reaction time and temperature, and appropriate HLB-RPA were reported at 38°C for 20-30 minutes. The assay was then refined for use with crude plant extracts. The effectiveness and sensitivity of the assay were evaluated on field-grown citrus cultivars, including mandarin, sweet orange cv. mosambi, and acid lime, that were either HLB-infected, HLB-doubtful, or healthy. The HLB-RPA-LFA was found to be both sensitive and specific, showing no cross-reactivity with other citrus pathogens. Thus, the nrdB genebased HLB-RPA method provides sensitive and reliable detection of CLas, making it suitable for use by mobile plant pathology laboratories, disease surveyors, and nurserymen for bud-wood certification and quarantine programs.

**Keywords:** Citrus greening, Huanglongbing, Diagnostic tools, Recombinase Polymerase Amplification (RPA), Isothermal amplification



# Identification and evaluation of potential inhibitor molecules against TcyA from *Candidatus* Liberibacter asiaticus

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Of the two putative amino acid binding periplasmic receptors of ABC transporter family in *Candidatus* Liberibacter asiaticus (CLas), cystine binding receptor (CLasTcyA) has been shown to mainly express in phloem of citrus plant and is a target for inhibitor development. The crystal structure of CLasTcyA in complex with substrates has been reported earlier. The present work reports the identification and evaluation of potential candidates for their inhibitory potential against CLasTcyA. Among many compounds, selected through virtual screening, and MD simulation, pimozide, clidinium, sulfasalazine and folic acid showed significantly higher affinities and stability in complex with CLasTcyA. The SPR studies with CLasTcyA revealed significantly higher binding affinities for pimozide and clidinium(Kd, 2.73 nM and 70 nM, respectively) as compared to cystine (Kd, 1.26 µM). The higher binding affinities could be attributed to significantly increased number of interactions in the binding pocket as evident from the crystal structures of CLasTcyA in complex with pimozide and clidinium as compared to cystine. The CLas TcyA possess relatively large binding pocket where bulkier inhibitors fit guite well. In planta studies, carried out to assess the effect of inhibitors on HLB infected Mosambi plants, showed significant reduction in CLastitre in plants treated with inhibitors as compared to control plants. The results showed that pimozide exhibited higher efficiency as compared to clidinium in reducing CLastitre in treated plants. Our results showed that the inhibitor development against critical proteins like CLasTcyA can be an important strategy in management of HLB.

**Keywords:** Huanglongbing, *Candidatus* Liberibacter asiaticus, X-ray crystallography, Surface plasmon resonance, TaqMan-qPCR



# Design and development of an efficacious controlled release system for Streptomycin/Oxytetracycline against citrus -related diseases

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Conventional agricultural practices heavily rely upon chemical pesticides and fertilizers but their overuse often results in minimal efficacy and environmental contamination. A growing concern about food safety and the environment has led to the development of controlled release formulations that utilize a variety of micro- and nanocarriers. Huanglongbing (HLB) is a devastating citrus disease, caused by phloemrestricted bacteria Candidatus Liberibacter asiaticus (CLas). It is currently recognized that this disease affects 40 different Asian, African, Oceanic, South, and North American nations, and it costs the US citrus sector over \$3.6 billion annually. Another disease of citrus called Citrus canker, caused by Xanthomonas citri ssp. citri(Xcc) is responsible for causing extensive damage and has no known treatment other than prophylactic measures to slow the growth of the infection. Copper-based compounds, a commonly used remedy, pose risks of its accumulation in soil and water and the emergence of copper-resistant strains. Alternative contact bactericides, like streptomycin and oxytetracycline, offer limited protection. Nanopesticides offers promise by offering a shielding to degradation, prolonged release profile, enhanced effectiveness, and cost-effectiveness. Here, we are encapsulating Streptomycin and/or Oxytetracycline in nanocarrier made up of phosphorylated Zein, a biodegradable corn protein; Tannic acid, an insecticidal phytochemical and Poly lactic acid which provides UV-shielding to the active ingredient. This study comprises a three-phase approach. Phase-I involves the synthesis and characterization of Streptomycin/Oxytetracycline loaded Phosphorylated Zein-Tannic acid-based Poly lactic acid nanospheres. Phase-II focuses on evaluating the efficacy of these nanospheres on plants affected by HLB and Citrus Canker. Phase-III extends the investigation to assess the toxicity profile of these systems on non-target entities, including the plant system, soil bacteria, earthworms, Daphnia magna, and human cell lines. In this study, we aim to develop a sustainable and effective method to combat citrus diseases while minimizing environmental impact, providing a promising avenue for the future of food and agriculture.

Keywords: Citrus, Huanglongbing, Nanopesticides, Sustainable Agriculture



# *Mallada desjardinsi* (Navas): Natural enemy to control sucking and foliage pest of citrus

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Citrus is the most important horticultural crop in our India. There are more than 250 major and minor insect pest on citrus in different climatic conditions. *Mallada desjardinsi* is a major Predator of citrus ecosystem. Mallada belongs to order Neuroptera and under the family Chrysopidae. The life period of mallada is 45-46 days. Mallada requires 30-32°C temperature and 67-87% relative humidity for its growth and production. Each female lays 200-300 eggs during its life span. It lays more eggs on fresh twigs of citrus plants than older ones. Mallada adult feed on pollen and nector of flowering plants like marigold.In laboratory condition, diet composition of Mallada adult is fructose + protinex (60:40) + vitamine E capsule. Mallada larvae require 24hrs frozen Corcyra cephalonica eggs for feeding. The larvae of malladadesjardinsi feed on sucking insect pest like black fly (*Aleurocanthus woglum*i), citrus psylla (*Diphorinacitri*), Aphid (*Aphis spp.*) and mealy bug (*Planococcuscitri*) and foliage pest Leaf miner (*Phyllocnistis citrella*)etc. Each larvae consumes 125-130 early instar larvae of blackfly or 115-120 nymphs of psylla for its development. Eggs 50 /tree and 30 larvae/tree twice in each flushing season coinciding with the pest incidence for the management of sucking and foliage pests.

**Keywords :** Aleurocanthus woglumi, Corcyra cephalonica, Diphorina citri, Fructose, Malladades jardinsi, Phyllocnistis citrella, Planococcus citri, Protinex



# *Trichoderma harzianum(NRCfBA-44)*: An effective bio-agent against the mitigation of *Phytophthora spp*. in Nagpur mandarin under Satpura plateau region of district Chhindwara Madhya Pradesh

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Mandarin (Citrus reticulata Blanco) is distinguished from other citrus species by the relatively loose skin of the fruits, the relative ease with which the segments can be separated and (in most cultivars) the green cotyledons. The world's famous Nagpur mandarin is suffering adversely due to *Phytophthora spp*. The Phytophthora fungi are present in almost all citrus orchards. Phytophthora spp. cause serious disease like root rot, crown rot, foot rot, gummosis and brown rot of fruits in citrus inflicting decline and yield losses. Under moist conditions, the fungi produce large numbers of motile zoospores, which are splashed onto the tree trunks. The species causing gummosis develops rapidly under moist, cool conditions. Hot summer weather slows disease spread and helps drying and healing of the lesions. Citrus decline is most wide spread severe problem in citrus orchards at dist. Chhindwara (M.P.). The infected tree shows decline symptom in combination with chlorosis of leaves, twigs dieback, discolored or poor colored fruits, wilting of tips and leaves withering. The infected twigs show gummosis, browning of the cortex, defoliation and desiccation. Symptoms are clearly seen on the above ground parts near the soil. Biological control has a great potential to manage the soilborn diseases *i.e Phytophthora spp*. In changing climate scenario the Trichoderma harzianum (NRCfBA-44) is proving to be very effective against Phytophthora effected declining orchardat Saunsar and Pandhurna region of dist. Chhindwara when applied along with FYM as soil application below of the plant canopy. Phytophthora root rot and gummosis are the most importantsoil borne diseases of Nagpur mandarin causing mortality, slow decline and yield loss of mature trees Phytophthora root rot and gummosis are the most importantsoil borne diseases of Nagpur mandarin causing mortality, slow decline and yield loss of mature trees.

Keywords: Nagpur mandarin, Decline, Trichoderma harzianum, Phytophthora species, FYM



# *In-vitro* management of three species of *Colletotrichum* infecting Mandarin orange (*Citrus reticulata* Blanco) of Darjeeling hills by *Bacillus subtilis*

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Darjeeling Mandarin fruit (*Citrus reticulata* Blanco) belonging to the family rutaceae is well known for its sweet flavor, pleasant aroma and abundant fruit juice. Recently, the production of fruit in the study area of Kalimpong district of Darjeeling Hills suffered huge losses due to the prevalence of diversefungalpathogens. The pathogens infecting the leaves and fruits of the plant. Infected mandarin leaf samples showing brown spot with yellow halo, chlorosis, leaf twisting and curling symptoms were collected from various fields of the present study area. The causal organisms were isolated, and morphological studies (based on colony, mycelium and conidial features) were done. Pathogenicity of the isolates were confirmed following Koch's postulates. Microscopic and molecular identification of all the pathogenic isolates were done by amplifying Internal transcribed spacer region (ITS1/ITS4) and D1/D2 region of 28S rDNA (NL1/NL4) using suitable primers. Upon BLAST analysis three different *Collectorichum* sp. were obtained namely *C. gloeosporioides, C. siamense* and *C. fructicola.* Isolation and screening of antagonistic bacteria was done from healthy Mandarin leaves following their antifungal activity in dual culture experiments. One of the bacteria, identification of the antagonist bacteria was done by partial 16S rRNA gene sequencing.

Keywords: Bacillus subtilis, Biocontrol, Colletotrichum species, Darjeeling Hills, Mandarin



# Exploring morphological disparities of *Pseudonemorphas versteegi* (Ritsema) in north eastern state of India

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Pseudonemophas versteegi (Ritsema) belongs to the beetle family Cerambycidae and is recognized as a distinctive pest within the realm of citrus cultivation, particularly affecting Khasi Mandarin trees in the Northeastern Region. Specimens were collected from various locations in the North Eastern Region (NER), including Assam, Meghalaya, and Nagaland. Morphometricanalysis were carried out on the 25 specimens which involves measurement of length and width of body, humeral width, pronotal width, abdominal length, length of different segments of antennae and length of head etc. The body length of male (31.77 -28.755mm) and female (30.442-27.391mm) is more in specimens collected from Nagaland as compare to Assam and Meghalaya. Regarding profemural length, a notable disparity was observed. In males from Nagaland, this measurement ranged from 8.486 to 7.227 mm, significantly surpassing those from Assam (7.020 - 5.083mm) and Meghalaya ranging from 6.683 to 4.238mm. Similarly, among females, the profemural length in Nagaland (7.511 - 6.999mm) was considerably larger than in Assam (6.201 - 5.152mm) and Meghalaya (7.072 to 3.139mm). The length of abdomen is more in male (15.692-13.344mm) and female (15.124-14.104mm) in specimens collected from Nagaland exhibited greater lengths compared to those from Assam and Meghalaya. Through meticulous examination, this research elucidates the distinct traits present in adult Pseudonemorphus versteegi (Ritsema) specimens, shedding light on their location based morphological distinctions.

**Keywords:** Cerambycidae, Humeral width,Profemural length, *Pseudonemorphus versteegi* (Ritsema), Morphometric analysis



# Unraveling citrus insect pest scenario through survey and surveillance in Nagpur

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Citrus fruits serve as the primary focus of commercial horticultural cultivation across Maharashtra state. Nagpur stands as a prominent hub for cultivating Nagpur Mandarin (Citrus reticulata) and Sweet Orange (Citrus sinensis). Roving and fixed plot surveys were carried out during 2021–23 in the Nagpur mandarin and Sweet orange major growing region of Saoner, Katol, Kalameshwar, Hingna and Narkhed locality to assess the insect pests scenario in Nagpur. About 11 distinct species of insect pests were monitored within the citrus ecosystem with their extent of infestation varying from negligible to very severe in orchards. The infestation of Citrus leaf miner (Phyllocnistis citrella) and Citrus mite (Phyllocoptruta oleivera, Scizotetranychus sp.) were more frequently observed in Nagpur, Kalameshwar, Narkhed and Katol area and their economic threshold value were as high as 13.38, and 16.41 per cent, respectively. In these areas, bark-eating caterpillar (Inderbela quadrinotata) infestation was higher (12%) in older orchards compared to younger, well-managed ones. During 2022-23, citrus blackfly (Aleurocanthus *woglumi*) crossed economic injury level due to heavy rainy season in Nagpur region due to which sooty mould grows wildly that leads to fungal manifestation (Capnodiumsp.) locally called as 'Kolshi', covering entire plant. The infestation of Citrus psylla (Diaphorina citri), lemon butterfly (Papilio demoleus), Citrus aphids(Aphis spp. and Toxopteraspp.), Citrus thrips, (Scirtothrips spp.), Fruit sucking moths (Eudocimaspp.) and Fruit fly (Bactrocera spp.) were moderate to low. The amalgamation of scientific and systematic pest surveillance is found to be the cornerstone for pest management which helped to avoid epidemic situations by monitoring and forecasting pest population.

Keywords: Citrus, Nagpur mandarin, Pest surveillance, Pest monitoring, Survey



# Deciphering sexual differences in *Pseudonemorphus versteegi* (Ritsema) (Coleoptera: Cerambycidae): Insights from the citrus trunk borer.

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Pseudonemorphus versteegi (Ritsema) (Coleoptera: Cerambycidae), commonly known as the citrus trunk borer, poses a significant threat to citrus crops across the northeastern region of India. Adult exhibit primarily white coloration with black spots covering their thorax and abdomen. They have eleven segmented filiform antennae and a black membranous junction between the abdominal sternites. The morphometric analysis of variations between 10 male adult specimens and 13 female adult specimen of the Citrus Trunk Borer collected from various locations in the North Eastern Region (NER), including Assam, Meghalaya, and Nagaland during year 2021-23. Various key external taxonomic traits, including body length, body width, antennal segmental length, abdominal sternite length in both male and female specimens were measured. Male and female beetles have enormous bodies that measure 16.91-36.31 mm in length, 8.50-13.87mm in width with no discernible gender differences. Pseudonemorphus versteegi (Ritsema) have exaggerated antennae that are usually sexually size-dimorphic. The antennae of the male significantly longer than that of the female. Male antennae have a length range of 24.4-79.55mm. Antennae of females range in length from 36.48 to 47.95mm. In both male and female, ventral surface of abdomen is shiny black, with five distinct sternites. Last abdominal sternite of female are longer than male. The sex of most adults can be distinguished by viewing the lengths of antennal segments compared to overall body length. This morphometric analysis provided valuable information for understanding the diversity and characteristics of this citrus trunk borer species.

**Keywords:** Citrus trunk borer, Filiform segments, Morphological characteristics, *Pseudonemorphus versteegi*, Sexual dimorphism



# The science of citrus blackfly management: An integrated approach

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Citrus blackfly (Aleurocanthus woglumi Ashby), a pernicious sucking pest, poses a significant threat to citrus cultivation by targeting young flushes, extracting sap, and excreting abundant honeydew, thereby fostering the growth of the troublesome sooty mold. This study sheds light on the multifaceted issues surrounding citrus blackfly infestations in Maharashtra's citrus orchards and underscores the need for management strategies from the perils posed by blackfly. In the year 2022-23, due to heavy rainfall, resulting in an unprecedented surge in the citrus blackfly population, surpassing the economic injury level. This surge led to the rapid proliferation of sooty mold (Capnodium sp.), locally known as 'Kolshi,' which encased entire citrus plants. Active infestation periods are observed from February to April, June to August, and October to December, with an Economic Threshold Level of 5-10 nymphs per leaf. Organic interventions encompass measures such as preventing close planting and averting waterlogging conditions, alongside refraining from planting guava, sapota, mango, and pomegranate near citrus orchards. Foliar application of neem oil @10 ml/l or azadirachitin 1% @ 3 ml/l or neem soap or pongamia soap @ 5 gm/l of water coinciding with adult emergence reduces the pest infestation. Avoiding applying insecticides in the late winter to early spring will help preserve beneficial bioagents. Chemical management alternatives involve the foliar application of insecticides such as like imidacloprid (17.8% SL) 0.5 ml/l or thiamethoxam 0.3g/l or dimethoate (30EC) 2 ml/l of water at adult emergence and 50% egg hatching stage coinciding with I<sup>st</sup> Fortnight of April, II<sup>nd</sup>Fortnight of July and I<sup>st</sup> Fortnight of December in winter season. Furthermore, the use of starch (at a concentration of 2%) followed by copper oxychloride (COC) application (at 0.3%) effectively eliminates the black fungal layer caused by sooty mold on affected trees.

Keywords: Bioagent, Citrus blackfly, Economic injury level, Integrated pest management



# Molecular characterization of fungal pathogens associated with citrus dieback, leaf blight and stem blight disease from southern Karnataka

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Lemon (Citrus lemon (L.)Burm. f.) is an important fruit crop cultivated worldwide, and is grown practically in every state in India. During a survey conducted in 2019-22, trees in a lemon orchard in southern Karnataka were found affected by leaf blight, stem blight and dieback disease. Approximately 15 to 20% of trees were affected as young shoots and branches showed progressive death from the apical region downward, leaves with necrotic lesions, and stems with necrotic lesions. Different samples were collected and diagnosed via morphological methods. The associated fungus was consistently isolated from the infected branches, stem and leaves on potato dextrose agar (PDA). Morphological, cultural and microscopic examination conducted and all relevant observations recorded to assist in identification. Based on the morphological and cultural features, the fungal pathogen was identified as *Diaporthesp*. (Dieback), Alternaria sp., Neopestalotiopsis sp. (leaf blight), Lasiodiplodia sp. (stem blight/canker). Further multi-locus sequencing was performed by using ITS-rDNA, TUB2 ( $\beta$ -tubulin), TEF-1 $\alpha$  (translational elongation Factor gene) were amplified, sequenced and combined phylogenetic analysis revealed that the identity of the associated fungal species confirmed viz., dieback disease associated with D. citrii, D. eres and D. phaseolorum; leaf spot/blight disease was associated with Alternariacitri, A. alternata and A. longissima, Neopestalotiopsisbraziliensis; and stem blight disease was associated with L. theobromae and L. pseudotheobromae respectively. Pathogenicity test was conducted on healthy 2-year-old lemon plants for each pathogen and conformed their association with their characteristic symptoms recorded from the fields. Development of disease symptoms recorded and the fungal pathogen was re-isolated from the inoculated lemon trees.

Keywords: Citrus, Leaf and stem blight, Dieback, Fungal pathogens, Molecular taxonomy



# Comparative genome analysis of Liberibacter spp. to identify prophages

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*Candidatus* Liberibacter asiaticus (CLas) is a gram-negative, phloem-limited, fastidious  $\alpha$ -Proteobacteria vectored by *Diaphorina citri* (*D. citri*) (psyllid), causing the disease citrus greening or Huanglongbing (HLB) in citrus. The study of CLas is constrained as the functional and structural characterization is restricted because of the lack of axenic culture of the bacteria. CLas genome contains prophage-like regions, homologous to phage genomes SC1 and SC2, and encodes for multiple virulence factors. Recent research has shown that SC1 carries lytic genes that are chromosomally integrated with CLas to form bacteriophages, which replicate phage particles within the host and may cause citrus phloem cells to undergo apoptosis by lytic burst of CLas. The SC2 genome, in contrast, produces excision plasmid prophages devoid of lytic genes. Additionally, it was reported that the repressor protein of the *Wolbachia* bacteria found in psyllid nosts rather than citrus plants. The present study aims to perform phylogenetic analysis of CLas and related bacteria to understand their evolutionary relationship. The comparative genome analysis of prophage genomes will be carried out to identify the lytic genes in different Liberibacter spp. and In Silico approaches to detect recombination events that might have enhanced the evolution rate.

Keywords: Candidatus Liberibacter asiaticus, Citrus greening, Diaphorina citri, Huanglongbing, Wolbachia



# A novel automated magnetic bead-based technology for the extraction of nucleic acid (DNA and RNA) from a wide range of plant samples

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Citrus is a globally important fruit crop as known for their nutritional and health benefits, accounting for the third largest fruit sector in India. It is vulnerable to different biotic andabiotic factors, resulting in reduced productivity and yield. The biotic factors which affect the health of the citrus are Bacterial (Candidatus Liberibacter Spp., Xanthomonas Spp.), Viral (Citrus tristeza virus (CTV), Indian citrus ringspot virus, and Citrus yellow mosaic virus, Citrus Phytoplasma, Citrus Viroid and fungal Pathogens. Among them, Candidatus Liberibacter asiaticus(CLas) and CTV areconsidered the most important and devastating pathogens, having wreaked havoc on the global citrus industry. Currently except "planting of disease -free trees" and "Quarantine" there is no treatment for these systemic pathogens. These systemic pathogens spreadfrom infected plant to healthy plant through vegetative propagation and insect vectors. To prevent or control the spread and damage caused by these diverse systemic pathogens, lots of molecular based efforts are going on globally. Extraction of high amounts and purity of DNA and RNAincluding viruses is fundamentalto rapid diagnosis of diseases. Spin-column based extraction protocols have been preferred for effective extraction. However, it is limited to process a small initial sample volumeper column that leads to low nucleic acid yield. As a result, the spin column method is unable to meet the sample pooling method's requirements. Thus, testing or screening of big citrus orchards for theses pathogens is a challenging task to the researchers. We have developed a novel magnetic bead-based platform (Machine andkits) for single sample in single cartridge, processing 100mg to 3gm of citrus leaf sample with excellent DNA(260/280 ratio = 1.8-1.9) purity and much higher yield (upto 200ng/ul).We have designed and developedHiPurA Pre- filled Cartridges for Plant DNA (MB571PC16- HiPurA®Super Plant Pre-filled Cartridges), RNA(MB603PC16- HiPurA® Pre- filled Cartridges) and total nucleic acidextraction (MB571PC24- HiPurA® SuperPlant Pre-filled Cartridges). These developed single sample processing cartridges tobe used with HiMedia's innovative magnetic automated DNA and RNA extraction platforms, InstaNX<sup>®</sup> Mag16 series (Sample capacity: 1-16), InstaNX<sup>®</sup> Mag24 series (Sample capacity: 1-24) and InstaNX<sup>®</sup> Mag32 (Sample capacity: 1-32) only within "90" minutes. Overall, the developed innovative and advanced magnetic bead-based technology with a capacity to process 3gm of citrus leaf including midrib section, the citrus industry can benefit usingInstaNX<sup>®</sup> Magplatform for surveillance (pooling method model) and diagnosis of Citrus Tristeza Virus and Candidatus Liberibacter spp.

**Keywords:** HiPurA<sup>®</sup> Pre- filled Cartridges, InstaNX<sup>®</sup> Mag16,InstaNX<sup>®</sup> Mag24, InstaNX<sup>®</sup> Mag32,Magnetic bead-based technology, *Candidatus* Liberibacter asiaticus, *Citrus tristeza* virus



# Virulence of entomopathogenic nematode, *Heterorhabditis indica* against developmental stages of fruit piercing moth, *Eudocima materna* L.

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Fruit piercing moths, *Eudocima* spp. (Lepidoptera: Erebidae), are considered as serious pests of fruit crops grown throughout the tropical and sub-tropical belt from Africa, Asia, Australia and to the Pacifc Islands. The use of entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* offers an eco-friendly alternative to chemicals and as biological control agents against different insect pests. Virulence of the EPN, *Heterorhabditis indica* against larvae (3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> larval instars) and pupae of citrus fruit piercing moth, *Eudocima materna* were evaluated under laboratory conditions. Results revealed that application of increasing concentrations of infective juveniles (IJs) (10, 25, 50, 100 and 200 IJs larva<sup>-1</sup> and pupa<sup>-1</sup>) induced higher mortality on larval instars and pupae with mean mortality ranging from 26.6 to 100% ( $LC_{50^-}$  3<sup>rd</sup> (14.43 IJs larva<sup>-1</sup>), 4<sup>th</sup> (17.08 IJs larva<sup>-1</sup>), 5<sup>th</sup> (23.63 IJs larva<sup>-1</sup>)) and 10–70% ( $LC_{50^-}$  85.91 IJs pupa<sup>-1</sup>) after 48 h post-exposure, respectively. *H. indica* successfully reproduced in the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> larval instars of *E. materna* and their offspring emerged from the cadavers. The highest reproduction was recorded in 5<sup>th</sup> instar larvae (1,082,855 IJs larva<sup>-1</sup>) at 600 IJs larva<sup>-1</sup> in *E. materna*. The present work is the first investigation on using EPNs against fruit piercing moth larvae and pupae and provides some preliminary evidence for potential use of *H. indica* for biological control of *E. materna*.

**Keywords:** Biological control, Citrus fruit piercing moth, *Eudocima materna*, Entomopathogenic nematode, *Heterorhabditis indica*, Mortality



Advancing Citriculture for Agro-economic Prosperity

#### TS-3-P-45

# Diversity of plant parasitic nematodes in citrus species: A comparative study between raised bed and flat planting systems in plains of north eastern India

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A systemic investigation was undertaken at Regional Research Centre for Citrus (RRCC), ICAR-CCRI, Biswanath Chariali, Assam to assess the diversity and prevalence of plant parasitic nematodes in the soil rhizosphere of nine different citrus species *viz.*, Khasi mandarin, Nagpur mandarin (budded), Nagpur mandarin (STG), Cutter Valencia, Sweet orange Mosambi, Hamlin, Flame Grapefruit, Assam lemon and NRCC Acid lime-7 on raised bed and flat planting systems during the year 2022. Based on the morphological characters, four PPN genera *viz.*, *Tylenchulus semipenetrans*, *Helicotylenchus*, *Pratylenchus*, *Hemicycliophora*, *Tylenchorhynchus* and *Criconematid* were identified. In addition to this, several dorylaimid, rhabditid and predatory nematodes were also encountered. Among the PPNs, *T. semipenetrans* was highly abundant in Cutter Valencia on raised bed system followed by Nagpur mandarin (budded) and Assam lemon on raised bed system. While, other PPN genera were moderately abundant in other citrus species grown on both raised bed and flat systems.

Keywords: Diversity, Plant parasitic nematode, Tylenchulus semipenetrans, North Eastern India



# Spatial and temporal prevalence of plant parasitic nematodes of citrus in vidarbha region

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The present investigation was conducted at Plant Protection Division, ICAR-Central Institute for Cotton Research, Nagpur during the year 2014-2018 respectively. During the present studies 11 genera were identified from 57 samples collected from 19 talukas of 05 districts in Vidarbha region of Maharashtra state. The absolute and relative frequencies along with densitieswas recorded highest invarious genera viz. *Helicotylenchus, pratylenchus, Tylenchulus* and *Tylenchus* genera. However, the highest absolute and relative frequency was recorded in *Helicotylenchus*(93.99 & 18.53%) followed by *Pratylenchus* (84.51 & 16.80%) in citrus ecosystem. Whereas, the *Helicotylenchus* and *Pratylenchus* genera noticed the highest absolute and relative density of (2.10 & 17.46%) and (1.89 & 15.76%) respectively. The prominent value indicates the prominent presence of the genera *Helicotylenchus* (7.62), *Tylenchulus* (6.97), *Pratylenchus* (5.77) and *Tylenchus* (2.59) in citrus ecosystem. The high density spots recorded at 19 spots in 16 talukas of 05 districts.

**Keywords:***Helicotylenchus, pratylenchus, Tylenchulus, Tylenchus Rotylenchulus, Hoplolaimus, Tylenchorhynchus, Hirschmanniella, Meloidogyne, Aphelenchoides,* Absolute fequency, Relative frequency, Absolute density, Relative density, Prominence value



# Premature fruit drops of Nagpur mandarin (*Citrus reticulata*): Associated fungal species and its climate impact in central India

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The aim of this research was to determine the incidence and possible causal pathogen(s) of premature post bloom fruit drop (PFD) disease of citrus in present climate situation. Mandarins occupy largest area and provide more than 43 per cent production among all citrus fruits grown in India. The commercial cultivars being grown in different regions are 'Nagpur' mandarin (Santra) in state Maharashtra of Central India. Citrus decline is associated with a combined effects of biotic and abiotic factors. Hence, the loss comes in a series of waves varying in the different citrus fruits in length of time between them. In citrus, the shedding of flowers and fruits comes more or less in three distinct waves known which are also known as post-bloom drop, June drop and pre-harvest drop. Disease related fruit drop usually starts during August and continues till harvest with its peak in mid-September to mid-October. However sometimes depends on natural climates in beginning fruit settings. However heavy small fruit drops initially due to attack of C.gloeosporioides ranging between 21-64 percent were observed in off time rainfall where rainfall 16.4mm and humidity (75-91percent) in the months 2<sup>nd</sup> week of March up to April last week in 2023. The fruit drop in the months of September-October is the most detrimental, as the fruits are near maturity and have drawn nourishment from the tree. Several disease causing phytopathogenic fungi, including terrible pathogens Alternariacitri (8.5%), Colletotrichum gloeosporioides 22.8%, Diplodia natalensis 2.5% were associated with citrus fruit drop. C. gloeosporioides was the most frequently isolated during the investigation. The confirmation of emerging new pathogen showing phytotoxic symptoms initially on Nagpur mandarin circular small spots of 5 to 20mm diameter sometimes very large area covered on peels mostly depressed on fruits peels before maturity and ripe citrus fruits were found from all accessions of citrus cultivar and farmers field in the last last week of August pick period was October till harvest in the year 2022 heavy damaged orchard were found due to high rainfall and humidity wide- spread in citrus belts of central India. Even same occurred on sweet orange at market in the months August 2023 and Koch's postulates was fulfilled. Due to new pathogen observed not a single fruit drops but very serious quality damaged all over experimental orchards severity 40-100 percent and totally fruit physiology destroyed.

Keywords: Mandarin orange, Fruit drop, Climate, Pathogens, Climate, Emerging new pathogen



# Studies of LysR family transcription regulator from *Candidatus* liberibacter asiaticus

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Citrus greening or Huanglongbing (HLB) is an extremely destructive, fast-spreading disease that causes huge economic losses to citrus industries. Candidatus liberibacter asiaticus (CLas) is a phloem-limited, unculturable, fast-spreading, and gram-negative bacteria that is the causative agent of HLB. The CLas is transmitted by Asian citrus psyllid. The HLB symptoms contain blotchy leave, irregularly-shaped fruit, premature defoliation, undersized growth, and demise of plants finally. Till date, there is no control strategy to completely eradicate the disease. Effective strategies to control CLas could include the development of inhibitor molecules antimicrobials against the CLas proteins critical in bacterial survival through proteome and in-silico analysis. Proteins in the LTTR family are among the most abundant types of transcriptional regulators in prokaryotes and members are involved in regulating diverse sets of genes that influence a variety of biological processes including virulence of CLa. In the present study, we are targeting one of such LysR transcription regulator proteins. We have cloned, over-expressed, and purified recombinant LysR protein from CLas. In bioinformatics studies, the three-dimensional structure of LysR was predicted by the alpha fold server and further validated by Ramachandran plots. The validated model was used to screen the potent inhibitor molecules against the LysR protein from CLas. The docking and molecular dynamics simulations were performed to characterize the protein. We are optimizing the condition for crystallization of LysR protein to analyze of three-dimensional structure and its key residues on the active site.

Keywords: Citrus, Huanglongbing, LTTR, LysR, Molecular docking

# Thematic Area -4

Innovations in Post-harvest Management, Valorization and Bioprospecting of Citrus



### **Oral Presentation**

### TS-4-0-01

### Essential oils amended coatings in citrus post-harvest management

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Synthetic fungicides have been used as a major tool for reducing postharvest decay and improving the shelf life of citrus fruits. The growing concerns about consumers health, environmental pollution and high handling cost of chemical fungicides have forced to move the modern citrus industry into safer alternatives. The edible coatings enriched with essential oils have been developed as an alternative and eco-friendly approach to control post-harvest decay and maintain the quality and shelf life of citrus fruits. This paper intended to review the effcacy of essential oils amended coating on citrus fruits with respect to disease control (blue mold and green mold), physical quality attributes [physiological weight loss, firmness, total soluble solid (TSS), titratable acidity (TA) and vitamin C] and physiological processes (respiration rate and ethylene production) and tried to correlate their applicability in the Nepalese context. The integration of antifungal edible coatings along with the existing management practices (sanitation, physical methods and cold storage) could provide effective results in postharvest quality and disease management of citrus. The applicability and efficacy of essential oils amended coatings should be tested on a commercial scale to variable environmental conditions of Nepal.

Keywords: Citrus, Essential oils, Postharvest, Quality attributes, Shelf life



# Performance of Rangpur lime (*Citrus limonia* Osbeck) fruits treated with organic and in-organic coatings under ambient conditions

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The influence of several coatings, including guava leaf extract (15%, 30%, 45%), lemon extract (5%, 10%, 15%), potassium permanganate (4 gm, 8 gm/ kg fruit), calcium chloride (1% and 2%), silver nitrate (2 % and 4%) and bamboo leaves, on post-harvest guality and shelf life of 'Rangpur lime' were studied. Present investigation was carried out at Department of Fruit Science, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh during the year 2021-2022. The physio-chemical traits of stored fruits were analysed four days interval up to sixteen days. Over the course of sixteen-day storage period, physiochemical traits underwent modifications. Our findings depicted that the fruits treated with guava leaf extract, lemon extracts, and bamboo leaves exhibited the greatest results in managing percent weight loss, percent juice content, acidity, TSS, vitamin C, and total sugars compared to treated fruits of potassium permanganate, calcium chloride, and silver nitrate. The 45% guava leaf extract in combination with 15% lemon extract were discovered to be the most effective coatings for preserving acidity, TSS, and vitamin C throughout the storage. The fruit surface appearance (colour) was found better in fruits treated with bamboo leaves and KMnO<sub>4</sub> @ 8g + CaCl<sub>2</sub> @ 2%. Although covering lime fruits with bamboo leaves and KMnO<sub>4</sub>@ 8g + CaCl<sub>2</sub> @2% produced noticeably superior outcomes for the fruit's surface colour compared to control, the reducing and non-reducing sugars continued to rise up to the 12<sup>th</sup> day of storage. This study unequivocally shows that guava leaf extract @ 45 % + Lemon extract @ 15 % and bamboo leaves treatments reported as best organic coating material for rangpur lime fruits to preserve nearly all the post-harvest quality parameters as well as extending their shelf life.

Keywords: Post-harvest quality, Shelf life, Organic and inorganic coatings, Rangpur lime



# Effect of drying methods on bioactive compounds and functional properties of Nagpur mandarin (*Citrus reticulata* Blanco) peel powder

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Nagpur mandarin (*Citrus reticulata* Blanco) fruit after processing leaves large proportion of peels, which is burden to the environment as solid waste. The peel is rich source of bioactive compounds so to utilize this peel for industrial purpose it need to recover in stable form. In present investigation Nagpur mandarin peel was dried by four drying methods (Freeze, hot air, microwave and refracto-window drying) followed by grinding in hammer mill. Ground mandarin peel powder was examined for bioactive compounds (total phenol content, total flavonoid content, DPPH, FRAP, hesperidin, water activity and vitamin C) and functional properties (bulk density, true density, water absorption capacity, swelling capacity, solubility index and oil absorption capacity). The drying methods (freeze, hot air, microwave and refracto window) were found to have a significant effect on the bioactive compounds and functional properties of mandarin peel powder of all studied samples. The freeze drying method was found to be a highest, total phenol content (163.70 mg GAE/100g), total flavonoid (184.69mg QE/100g), DPPH (34.91%), FRAP (2.56µgAA/ml), vitamin C (3.28 mg/100g), water activity (0.26), hesperidin (2.82%) and functional properties bulk density (0.23g/cm<sup>3</sup>), true density (0.24g/cm<sup>3</sup>), water absorption capacity (4.86g/g), swelling capacity (5.93g/g), solubility index (0.41g/g), oil absorption capacity (3.07g/g). Freeze drying method is the best method for drying that gave the quality of Nagpur mandarin peel powder good.

Keywords: Nagpur mandarin peel, Drying methods, Functional properties, Technological properties



### TS-4-0-04

# Bio-prospecting of fungal endophytes inhabiting *Citrus macroptera* Montr: an endangered ethnomedicinal plant used in folk medicines in north-east India

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A total of 13 endophytic fungal isolates, inhabiting Citrus macroptera growing in Assam, India, were studied for their ability to synthesize bioactive metabolites for bioprospecting. The isolates were tested for antimicrobial as well as antioxidant activity. Ethyl acetate extracted secondary metabolites of endophytic Talaromyces assiutensis, exhibited the highest antimicrobial activity (zone of inhibition 18.17±0.76 mm) against Staphylococcus epidermidis followed by Acremonium falciformae(zone of inhibition 17.69±0.63 mm) which inhibited Sclerotinia sclerotiorum. Talaromyces assiutensis showed highest antioxidant activity. However, the difference between antioxidant activities of Talaromyces assiutensis and Acremonium falciformae were statistically insignificant (P>0.05). Based on these activities, Talaromyces assiutensis was considered for further analysis. Internal transcribed spacer r-DNA sequence analysis was done for confirmation of taxonomic identity of *T. assiutensis*. The secondary metabolites produced by T. assiutensis revealed the presence of flavonoid at the highest quantity followed by alkaloids, saponin and terpenoids as phytochemicals. FT-IR spectrophotometry of the extracted metabolite of T. assiutensis showed the presence of alcohol, alkane, acid anhydride, alkene, aromatic, alkyl halide and amine as functional groups. Nine major cmpounds, viz., 3-undecene-5methyl-, 2,5-dihydroxy-3-methyl-2,5-cyclohexadiene-1,4-dione, 3-n-hexylthiolane, S,S-dioxide, Methyl 8-Methylnonanoate, (4H)4a,5,6,7,8,8a- Hexahydrobenzopyran-5-one-3-carboxamide,2, Cyclopentane undecanoic acid, methyl ester, Trans-2,4-Dimethylthiane, S,S-dioxide, 5-Hydroxy-4-hydroxymethyl-1-(1-hydroxy-1-isopropyl)-Cyclohex-3-ene and 3-Nonyn-1-ol were identified from the bioactive metabolites of *T. assiutensis* using GC-MS. Some of these compounds are antimicrobial whereas some are antioxidants. These results indicate that the metabolite containing different bioactive compounds produced by T. assiutensis isolated from C. macropteramay be used in pharmaceutical as well as agricultural industries.

**Keywords:** Citrus macroptera, Endophytic fungi, Bioprospecting, Bioactive metabolites, Phytochemicals, FT-IR spectrophotometry, GC-MS



# Application of composite edible coating-based on guar gum and chitosan to maintain the bioactive compounds and extends the shelf life of Kinnow mandarin fruits during storage

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The present investigation aimed to elucidate the effects of chitosan (CH) and guar gum (GG) based composite edible coating on physicochemical quality and storability of Kinnow fruit under ambient conditions. Composite coating materials were prepared by using tamarind (TAM) seed starch and jackfruit (JACK) seed starch with CH and GG separately. The results indicated that GG+TAM coated fruits maintained significantly (P $\leq$ 0.01) lower physiological losses in weight (PLW) (3.12%), decay incidence (0.83%), and respiration rate (13.57 ml CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) compared to control. The reduced activity of pectin methylesterase (PME) (0.97 µmol min<sup>-1</sup> g<sup>-1</sup> FW) and lipoxygenase (LOX) (1.97 µmoles min<sup>-1</sup> g<sup>-1</sup> FW) were noted in GG+TAM coated fruits. Also, the GG+TAM coated fruits retained higher fruit firmness (6.77 N), titratable acidity (TA) (0.94%), ascorbic acid (AA) (27.83mg 100g<sup>-1</sup>), total phenols content (TPC) (213.05 µg GAE g<sup>-1</sup> FW) and total antioxidants activity(TAA) (23.57 µmol TE g<sup>-1</sup>) over the control. However, total soluble solids (TSS) were reported lower in GG+TAM coated fruits. Based on findings, it can be concluded that GG+TAM coating could be gainfully utilized for prolonging the shelf life of Kinnow fruits up to 25 days without losing desirable quality traits at ambient storage.

Keywords: Antioxidants, Decay incidence, Hydrocolloids, Enzymes



### Post harvest management of citrus mould by plant extracts and food preservatives

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In over 100 countries across six continents, citrus is one of the world's most widely cultivated fruit crops. Citrus production is not only affected in the field, but also in the post-harvest phase. One of the most destructive post-harvest diseases of citrus is citrus green mould (caused by Penicillium sp.) which causes 50 - 60% post-harvest losses. The research studied the effect of post-harvest dipping of citrus fruits with plant extracts and food preservatives on reducing mould infection. Of 25 plant extracts tested, Eucalyptus, Neem, and Zimmu were found to be effective in controlling pathogen at 2.5, 5 and 10% concentration under laboratory conditions. In case of fruits dipping in plant extracts prior to inoculation, maximum control was achieved at 10% concentration. Similarly, out of 12 food preservatives tested, Bronidiol, Potassium metabisulphite, soda ash and baking soda showed 100% inhibition at 1,3 and 5% concentrations under *in-vitro* condition. Also, the fruits dipped in these food preservatives prior to inoculation showed maximum control. In addition, the effect of pre-cooling was also tested on mould growth on fruits. Precooling the fruits by dipping them in cold water to 10 ± 20°C for 15 min helps to maintain fruits healthy without any symptom of infection up to 28 days from harvest at 4°C. Hence, it is inferred from this study that, green mould disease of citrus caused by Penicillium sp.can be managed by either treating the fruits with 10% plant extract like Eucalyptus, Neem, Zimmu, or by treating the fruits with 1, 3 or 5% food preservatives like Bronidiol, Potassium metabisulphite, soda ash and baking soda. Storage of pre-cooled fruits at 4°C helps in maintaining the guality of the fruits up to 28 days.

Keywords: Citrus green mould, Plant extracts, Eucalytus, Food preservative



### TS-4-0-07

# Understanding the volatile composition of peel extract of grapefruit (*Citrus paradisi* Macf.) varieties cultivated in India

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One of the alternatives to effectively utilize the large quantities of citrus peels (40-50%) is to produce oil, as they possess oil-producing potential. Thus, in the present study, the peel oil and juice extract of nine different grapefruit varieties were analyzed to determine their respective volatile composition. A total of 107 volatile components were found in peel oil from nine grapefruit varieties using Gas chromatography-mass spectrometry analysis. The varieties differed with respect to their relative composition of volatile components in peel oil. Major volatile constituents from peel oil were Monoterpenes (45%) followed by alcohol (26%), sesquiterpene hydrocarbons (21%), aldehyde/ketones (6%) and esters (2%) of volatile. Further the most abundant monoterpenes and their cumulative per cent composition in grapefruit peel oil were, coumarin (13.46%) and D-limonene (10.43%). The D-limonene content varied from 3.23% (Star Ruby) to 16.06% (Marsh Seedless). Varieties like Star Ruby and Ruby Red contained a relatively lower amount of D-limonene (3.23% and 3.99%, respectively) compared to Marsh Seedless (16.06%), Ray Ruby (15.15%), Flame (12.84%), Foster (12.57%), Rio Red (11.51%), Oroblanco (10.98%), and Red Blush (7.55%). Overall, the monoterpene content was higher in varieties namely Ray Ruby, Foster, Flame, Marsh Seedless, and Rio Red. Alcohols were relatively higher in Ruby Red, Star Ruby, Ray Ruby, Oroblanco and Flame. Sesquiterpenes were more in Marsh Seedless, Ruby Red, Star Ruby, Red Blush, and Rio Red. Aldehyde/ketones were predominant in Red Blush, Ray Ruby, Marsh Seedless and Rio Red. While, the content of esters was comparable among the grapefruit varieties, Red Blush contained a relatively higher amount of esters in its peel oil. Also, polymethoxylated flavones namely tangeretin and nobiletin were detected in the peel oil of few grapefruit varieties such as Foster, Marsh Seedless, and Star Ruby. The study concluded that grapefruit varieties exhibited variation in volatile component irrespective of similar qualitative traits.

Keywords: Grapefruit, GC-MS analysis, Juice extract, Peel oil, Volatile compounds



# Fruit quality of Nagpur mandarin orchards of Katol tahsil, Nagpur district

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The present investigation was undertaken to study "fruit quality of Nagpur Mandarin orchards of Katol tahsil, Nagpur District". Seven locations of Katol tahsil were selected to study fruit quality of Nagpur mandarin. In all fifteen orchards were selected from seven selected locations. The fruit samples were collected from all fifteen mandarin orchards to study nutritional status of mandarin. The initial analysis of soil revealed that, pH of soil was slightly acidic to slightly alkaline in nature. No much variation in electrical conductivity of soil was observed at all locations. Soils were medium to high for organic carbon content and slightly calcareous to calcareous for calcium carbonate content. The soils are low to medium in category for both available nitrogen and phosphorous. Available potassium was found high to very high in availability of potassium in soil. Available Sulphur in surface indicates its availability low to moderate in soil. The fruit weight of Nagpur mandarin was ranged from 143.9 to169.6 g. The lowest and highest fruit weight observed was 143.9 g and 169.6 g in location 14 Parsodi and location 2 Ladgaon of Nagpur mandarin orchards. The juice content ranged from 34.86 to 57.90% of Nagpur mandarin orchards fruits. The lowest juice content 34.86% were observed in the fruits of location 5 Dhiwarwadi and maximum fruit juice 57.90% were observed in the fruits of location 9 Lamdhan. The TSS ranged between 6.8 to 9.0%. The lowest TSS 6.8 % were observed in the location 9 Lamdhan fruits and highest TSS 9.0% were observed in the location 1 Ladgaon, location 7 Fetri and also in location 12 Kalkuhi. The ascorbic acid content in Nagpur mandarin fruits ranged from 30.65 to 38.7 mg 100 ml<sup>-1</sup>. The lowest ascorbic acid 30.65 mg 100 ml<sup>-1</sup> content observed in location location 1 Ladgaon and higesht 38.70 mg100 ml<sup>-1</sup> in location 15 Amnergondhi (rithi). From the above study, it can be concluded that, quality of Nagpur mandarin is very good in Nagpur mandarin orchards from Katol tahasil of Nagpur district.

Key words: Mandarin orchards, Katol Tahsil, Fruit quality, TSS, Ascorbic acid, Juice content



### Solar powered portable citrus fruits sorting machine at farm levels

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Mahatma Gandhi Institute for Rural Industrialization is an autonomous institute under the Ministry of MSME, Govt. of India with the main objective of providing science and technology inputs in the rural sector. The Rural Energy and Infrastructure Division of MGIRI has developed a Solar Powered Portable Citrus Fruits Sorting Machine at Farm levels as oranges being one of major Citrus fruit and they are very sensitive and needs careful handling. Further, these oranges need to be transported to pack houses where they are graded as per sizes, washed, waxed and packed for dispatch to markets. The ungraded guantity of these oranges is normally transported back or sold on spot at negligible cost. Manual grading is time consuming and incurs fatigue and cost however, the accessibility of compact sorting machinery poses a challenge, while larger sorting machines often entail prohibitive costs and dependency on erratic grid power availability at farm level. Various orange grower federations and organizations along with prominent leaders have raised this issue at various forums as oranges among the citrus fruit is one of the major horticulture activities in suicide prone Vidarbha region. Hence, there was a need for the development of a viable techno-economical model of a solar powered portable automated orange grading machine at farm levels. This machine can process about 5 to 6 quintals per hour and the system is powered by DC motor and control system connected to a 120 Wp Solar PV module. The grading sizes are adjustable for variable grading size standards so that this machine can be used for variety of Citrus fruits.

Keywords: Citrus fruits, Farm level, Machine, Oranges, Portable, Solar, Sorting



### Orange processing and product development at any growth stage of orange

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Harsh weather conditions are making Indian orange farmers to think on processing of oranges at any stage of orange growing. This might help orange farmers to protect their invested money and returns on it. But farmers don't have the proper economic estimate to sell out their orange for processing at any stage of orange growing. This might be possible with proper technological intervention to find out the sum of returns at different stage of growing orange and subsequent processing. The present research is a part of work to accesses the products which can be developed from premature stage to final grown up stage along with its economic analysis. One month old premature oranges of Ambiya Bahar were divided in three parts, first one peels, second juice and third pulp. The products recovered from peels were pectin and orange oil found to be 15.4% and 4.5% by weight respectively. The Juice was converted into bioliquid fertilizer and is in process of characterization. The pulp, third remaining part of oranges was converted into edible fibre and hesperidins. The total pulp recovered from the oranges gives 26.31% edible fibres and hesperidins are under the process of characterization. Also, we are in process of converting of total orange into powder and its characterization. The objective of this research study is to support the orange farmers in Vidharbha Region to avoid loss in the orange farming and to stop wastage of oranges due to drastic climatic conditions. Along with this, the study will help in verification of valuable components recovery from premature to fully grown-up stage of orange fruits.

Keywords: Premature Oranges, Orange Oil, Pectin, Hesperidins, Edible Fibers



### TS-4-0-11

# Effect of different levels of wine yeast inoculum and pH of must on wines prepared from *mrig bahar* fruits of Nagpur mandarin

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The investigation on "Effect of different levels of wine yeast inoculum and pH of must on wines prepared from *mrig bahar* fruits of Nagpur mandarin" was conducted at Post harvest Technology Laboratory, Department of Horticulture, College of Agriculture Nagpur during the year 2016-17. The experiment consisted of two different factors viz., levels of wine yeast (Saccharomycescerevisiae var. ellipsoideus) inoculum and levels of pH of must with three replications using Factorial Completely Randomised Design. The biochemical analyses of the composition of wines prepared indicated that the different levels of wine yeast inoculum as 3, 6 and 9 per cent and levels of pH of must as 3.0, 3.5, 4.0, 4.5 and 5.0 significantly influenced the quality of mandarin wines prepared from Mrug bahar fruits. On the basis of findings of the investigation and specification suggested for different chemical constituents of Indian standard wineit can be concluded that, the wine prepared from Mrig bahar fruits of Nagpur mandarin using 6 per cent wine yeast inoculum and 4.0 pH of must yielded highest alcohol percentage, ascorbic acid content, excellent flavour, taste and sensory qualities at the end of storage.

Key words: Mrig bahar, Mandarin, Must, Yeast



#### TS-4-0-12

# Optimizing clarification of sweet orange juice by integrating ultrafiltration and centrifugation with pectinase enzyme

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The objective of this study was to enhance the efficiency of the juice clarification process for sweet orange (Citrus sinensis Osbeck) cv. Mosambi juice by employing commercially available pectinase enzyme and other clarifying processes. A total of eight different treatments were employed by combining pectinase enzymes at concentrations of 0.5% and 1.0% (with an incubation temperature of 40°C for 60 minutes), both individually and in combination with centrifugation (7,500 rpm and 30 mins time) and ultra-filtration (tubular membrane of polyvinylidene fluoride polymer material, molecular weight 5 kD, surface area 0.2 m<sup>2</sup>, operating pH 2 to 11, temperature 0-55°C and pressure 0.8-5.5 bars) process. The control group consisted of non-clarified juice. The results obtained from the measurements of absorbance and viscosity indicate that the highest level of clarity was found in the juice that underwent ultra-filtration + 0.5% pectinase (with an absorbance of 0.080 and a viscosity of 3.747 cP). This was followed by the juice that was clarified through ultra-filtration + 1.0% pectinase (with an absorbance of 0.087 and a viscosity of 3.777 cP). The maximum recorded concentration of ascorbic acid was achieved with the combination of centrifugation + 0.5% pectinase, resulting in a content of 40.333 mg/100 mL. The juice samples that underwent centrifugation + 0.5% pectinase treatment exhibited the maximum flavonoid content, with hesperidin at 124.857 ppm, naringin at 9.987 ppm, and narirutin at 130.32 ppm. This was followed by the ultra-filtration + 0.5% pectinase treatment, which yielded 109.067 ppm of hesperidin, 8.337 ppm of naringin, and 115.087 ppm of narirutin. These results were found to be superior to those obtained from the other treatments. The non-clarified juice displayed the highest total phenol content at 15.208 mg GAE L<sup>-1</sup>, whereas the juice treated with 0.5% pectinase alone and centrifugation alone had a somewhat lower concentration at 13.374 and 13.097 mg GAE L<sup>-1</sup>, respectively. The non-clarified juice samples exhibited the maximum *in-vitro* antioxidant activity, as evidenced by the ABTS, DPPH, and FRAP values of 5.151, 9.537, and 2.231 mM L<sup>-1</sup> Trolox, respectively. Similarly, the antioxidant activities was preserved in juice treated with ultra-filtration + 0.5% pectinase, yielding ABTS, DPPH, and FRAP values of 3.938, 8.741, and 1.653 mM L<sup>-1</sup> Trolox, respectively. Ultra-filtration as a standalone method or in conjunction with a 0.5% pectinase treatment can be recommended when the sole objective is to obtain a clear juice. On the other hand, the combination of a 0.5% pectinase enzyme with either centrifugation or ultra-filtration can be employed to achieve juice clarification while preserving the biochemical and nutritional characteristics to some degree.

Keywords: Sweet orange, Juice clarification, Pectinase, Centrifugation, Antioxidants



Advancing Citriculture for Agro-economic Prosperity

#### **Poster Presentation**

#### TS-4-P-01

# Studies on post-harvest application of different chemical on shelf life and quality of sweet orange cv. Nucellar

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The present investigation entitled studies on post-harvest application of different chemical on shelf life and quality of sweet orange Cv. Nucellar was carried out at Department of Horticulture, V.N.M.K.V, Parbhani, Maharashtra. The experiment was laid out in Completely Randomized Block Design (CRD) with thirteen treatments and three replications. The observation on physiochemical changes in fruits were recorded at 10 days interval up to 50 days at room temperature. Initial observation was recorded before keeping the fruits in boxes. The fruit treated with BA-100 ppm + 6 % wax recorded shelf life of 49.93 days as against 12.67 days in control. The fruit had high ascorbic acid content (47 mg/100 g of juice) and less TSS (13.39 %) during 50<sup>th</sup> day of storage of sweet orange at ambient temperature. The maximum acidity (0.52 %), low pH (3.50) and minimum TSS: Acid ratio (24.06 %) was observed in the treatment  $T_7$  (GA<sub>3</sub> 200 ppm + 6 % wax) and the maximum TSS (12.67 %), high TSS: Acid ratio (29.47 %) and low ascorbic acid (43.10 mg/100 g of juice) was recorded in the treatment T<sub>2</sub> (CCC 250 ppm + 12 % wax) during the 50<sup>th</sup> day of storage of sweet orange at ambient temperature. The maximum reducing sugar (3.77) was found in the treatment  $T_{12}$  (CCC 250 ppm + 12 % wax). However, the treatment  $T_{10}$  (CCC 250 ppm + 12 % wax) recorded high pH (3.75) and minimum acidity (0.41%). Low reducing sugar (3.37%) was noticed in the treatment  $T_1$  (CCC 250 ppm + 6 % wax) and higher non-reducing content was found in the treatment  $T_4$ (CCC 500 ppm + 12 % wax) during 50<sup>th</sup> day of storage of sweet orange at ambient temperature. Wax plays important role in increasing the shelf-life sweet orange Cv. Nucellar. It helps to reduce the gaseous exchange (CO<sub>2</sub> and O<sub>2</sub>) between fruit skin and environment due to which less respiration and transpiration losses occurs. However less moisture loss, least changes occur in reducing sugar, nonreducing sugar, TSS, acidity, pH and ascorbic acid.

Keywords: Sweet orange, Shelf life, Physiochemical, Wax, Post harvest



# TS-4-P-02

# Standardization of lime blended mandarin marmalade

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Marmalade is a product that is prepared from citrus fruits, where the peel of the fruit is suspended. Marmalade is a good source of vitamin C, dietary fibres and minerals which offers numerous health benefits. Blending is one of the best methods to improve the nutritional quality of value-added product by providing the nutrients of various fruits in single product, hence giving better quality and nutrition. Thus, keeping this objective in mind, the present investigation was done for finding the best suitable combinations of blended marmalade where we have prepared blended marmalade using mandarin and lime in different proportions and stored it for 150 days, and analyzed with respect to physicochemical and sensory parameters. The experiment was conducted at the PHT Laboratory, Department of Fruit Science, Dr. PDKV, Akola during the year 2019-20. The variety Nagpur mandarin and PDKV lime were used. It was observed that the treatment having 55% mandarin and 5% lime juicy vesicles was found to be best for the parameters TSS, total sugars, reducing sugars, acidity, ascorbic acid, pectin and sodium content and sensory attributes.

Keywords: Blended, Citrus, Juicy vesicles, Lime, Mandarin, Marmalade



# Sweet orange peel candy : A breakthrough for value addition

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The present investigation was carried at the Department of Horticulture, V.N.M.K.V. Parbhani, Maharashtra. The sweet orange peel candy was made up by using different treatment i.e., dipping time and concentration of sugar solution. There are three dipping times i.e., 12 hours, 24 hours and 36 hours and three levels of sugar concentration i.e., 50%, 60% and 70%. Candy was prepared by combining the dipping times and sugar concentration. Nine treatment combinations i.e., T<sub>1</sub>(T<sub>1</sub>S<sub>1</sub>-12hours in 50% sugar concentration), T<sub>2</sub> (T<sub>1</sub>S<sub>2</sub> -12hours in 60% sugar concentration), T<sub>3</sub> (T<sub>1</sub>S<sub>3</sub>-12hours in 70% sugar concentration), T<sub>4</sub> (T<sub>2</sub>S<sub>1</sub>-24hours in 50% sugar concentration), T<sub>5</sub> (T<sub>2</sub>S<sub>2</sub> -24hours in 60% sugar concentration), T<sub>6</sub> (T<sub>2</sub>S<sub>3</sub> -24hours in 70% sugar concentration), T<sub>7</sub> (T<sub>3</sub>S<sub>1</sub>-36hours in 50% sugar concentration), T<sub>8</sub> (T<sub>3</sub>S<sub>2</sub> -36 hours in 60% sugar concentration), T<sub>9</sub> (T<sub>3</sub>S<sub>3</sub> -36 hours in 70% sugar concentration) were taken in factorial randomized block design with three replications. The fresh sweet orange peels were evaluated for various physico-chemical characteristics. On fresh weight basis, moisture content and total solids of fresh peel was found to be 74.8% and 25.6. Total soluble solids, pH, titratable acidity and ascorbic acid were analyzed to be 11.5, 3.88, 0.48% and 35.6 g/100 g, whereas, reducing sugars, total sugars, pectin and tannins and were found to be 4.5%, 6.75%, 13.34 (% cal pectate) and 170 mg/100 g respectively. The result regarding storage (120 days) of candy revealed that the maximum moisture content and titrable acidity reported from treatment T<sub>1</sub>(T<sub>1</sub>S<sub>1</sub>i.e., 12.91% and 0.61%) while minimum value reported from treatment T<sub>9</sub> (T<sub>3</sub>S<sub>3</sub>i.e., 8.77% and 0.48%). Maximum total solids, pH, Total Soluble Solids, reducing sugars and total sugars were reported from treatmentT<sub>a</sub>(T<sub>3</sub>S<sub>3</sub>i.e., 91.13%, 3.15 84.66%, 22.40%, 68.54%) respectively while minimum values were reported from treatment  $T_1(T_1S_1)$ i.e., 87.00%, 2.22, 60.33%, 18.08% 44.55%) respectively. Maximum ascorbic acid content reported from treatment T<sub>3</sub> (T<sub>1</sub>S<sub>3</sub>i.e.,2.05 mg/100g) while minimum from treatment T<sub>7</sub> (T<sub>3</sub>S<sub>1</sub>i.e.,0.21 mg/100g) and maximum pectin and tannins were reported from treatment T<sub>3</sub>(T<sub>1</sub>S<sub>3</sub> i.e., 1.54% cal pectate and 74.99 mg/100g) while minimum values reported from treatment  $T_7$  ( $T_3S_1$ i.e., 0.90% cal pectate and 53.06 mg/100g). Organoleptic quality evolution was done in 9-point hedonic scale and it reveal that the treatment T<sub>a</sub> (T<sub>3</sub>S<sub>3</sub>)showed maximum score regarding colour, texture, flavour and overall acceptability during storage period and remained more acceptable in comparison to other treatments.

Keywords: Peel candy, Sugar concentrations, Sweet orange



# Biochemical and antioxidant dynamics during fruit development in sweet orange cultivars

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Kinnow in the citrus industry has been dominating since the last few decades, especially in North-West India which has led to monopolisation and increase in chances of collapsing industry. This research work has been carried out on three pigmented sweet orange varieties to increase their utilisation by highlighting the health benefits obtained from antioxidants present in its pulp and peel which would help mitigate the dominance of the single crop. Escalating health issues like cardiac arrests and strokes, declining immune system and ageing related issues, also blood pressure and sugar levels illnesses necessitates the incorporation of natural and healthy diet that is rich in bioactive compounds exhibiting a variety of health benefits. The fruit samples of three cultivars evaluated were collected from Punjab Agricultural University - Fruit Research Station, Jallowal. The peel and pulp of the fruit samples were analysed for their antioxidant activities throughout different maturity stages. The three cultivars were analysed for their total phenol content (TPC), total flavonoid content (TFC), beta carotene, DPPH activity, FRAP, hydroxyl ion activity and total reducing power (TRP). Sweet orange cv. Vaniglia Sanguigno amongst other two cultivars surpassed the levels of TPC, TFC, DPPH, FRAP in fruit peel of the sample. Whereas the beta carotene, TRP and hydroxyl ion activity were hiked in fruit peels of cv. Moro and Tarocco respectively.

Keywords: Antioxidant activity, Bioactive compounds, Carotene, Phenols, Pigmented varieties



# Bioflavonoids from citrus biomass: From wastes to functional hydrogels

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Citrus fruits when processed by industries produce tons of peels, pulps, and seeds as horticultural byproducts. These inexpensive residual sources deserve attention due to bioactive compounds present in them. 'Kinnow' mandarin (Citrus nobilis X Citrus deliciosa) is a potential source of bioflavonoids with antioxidant properties, but their potential is hindered due to pre-systemic metabolism. Colloidal delivery systems can overcome this barrier in question. This study involved the extraction and identification of key flavonoids from mandarin biomass, the peels and seeds, after processing kinnow into juice. Supercritical fluid extraction at 330 bar, temperature 40 C, and co-solvent 10% ethanol were employed for extraction. In this study, two monolayered systems, that is, polylactic-co-glycolic acid (PLGA) nanoparticles, and alginate hydrogels entrapping bioflavonoids were fabricated and compared for in vitro cell free gastrointestinal (GI) release of flavonoids from them. The release data was fitted into kinetic models to predict the mechanism of release of bioflavonoids from the carriers. From the results it was observed that total flavonoids extracted from the mandarin biomass were 47.3 ±1.06 mg rutin equivalents/ ml SFE, with presence of polymethoxyflavones (PMFs), tangeretin and nobiletin in abundance. The flavonoid-PLGA nanoparticles exhibited a particle size between 200-250nm with an entrapment efficiency of nearly 80.0%. in vitro GI studies revealed the role of primary carriers in providing gastric protection to the bioflavonoids, which in the free system degraded 50% in the gastric phase, whereas 80.0% of flavonoids were protected by using PLGA as a matrix, and 90.0% of the flavonoids were protected when alginate hydrogel was used as a carrier. Therefore, both the systems provided adequate protection to the bioflavonoids, they also offered sustained release in the intestinal environment. Kinetic modeling results suggested Korsmeyer-Peppas model as the best fit both cases, the difference being in the mechanism of release. This work underpins the role of carriers in efficient delivery of bioflavonoids, in addition to the importance of extracting their valuable bioactive from wastes, and their conversion into products.

Keywords: Flavonoids, Alginate, Hydrogels, Nanoparticles, Gastrointestinal, Kinetics



# Increasing shelf life of Nagpur mandarin by coating bio-synthetically formed silver nano-particles

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The present investigation was carried out in the Department of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The objectives of this study was to investigate the effect of green silver nanoparticles coating in prolonging the shelf life of Nagpur mandarin, to find out best nano-particle treatment for better shelf life of Nagpur mandarin and to study the physico-chemical changes and microbial status during the storage of Nagpur mandarin. The experiment conducted with eleven treatments and four replications under RBD experimental design. Nagpur mandarin fruits coated with different GSNp treatment and stored in cold storage conditions remained in good condition up to 60 days of storage in mrig bahar and 45 days of storage in ambia bahar without disturbing the guality of Nagpur mandarin fruits. The green silver nano particles synthesized by using different leaf extract with silver nitrite solution. The fruits coated with these biologically synthesized nano particle with gaur gum as sticking agent and stored in cold storage conditions. Maximum retention of quality parameters such as TSS, PLW, Titratable acidity, Sugars, Ascorbic acid and Phenols found in Nagpur mandarin coated with GSNp Tulsi followed by GSNp Neem coating. Microbial contamination and fruit decay rate was negligible in the fruits coated with GSNp Tulsi, the end of storage minimum chilling injury was recorded in the fruits coated with GSNp Tulsi and according to sensory evaluation report, the fruits coated with GSNp Tulsi gave highest score of overall acceptability according to Hedonic scale followed by GSNp Neem coated fruits.

Key words: Mandarin, Mrig bahar, Hedonic Scale, Shelf-life



# Utilization of citrus fruit peels by extraction of essential oil

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Citrus is an important genus belong to the family Rutaceae, which includes crops like lemon (*Citrus limon*), limes (*Citrus aurantifolia*), orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), bergamot (*Citrus bergamia*) and kumquat (*Citrus japonica*) etc. The crops have growing for their valuable nutritive fruits under various climatic conditions in different parts of the world. After removing the peels from the fruits, the pulp is consumed for several purposes, and the remaining peels are thrown out as waste materials. But the peels of citrus fruit are the richest source of valuable essential oil, and the percentage of available essential oil was observed to range from 0.5-2.5% in citrus sp. The essential oil of citrus and its constituent has been used in aromatherapy, pharmaceutical, cosmetic, food and flavour industries because it has a range of properties like antioxidant, anti-inflammatory, anticancer etc. Therefore, the objective of this review is to attract attention to the utilization of waste citrus peels through the extraction of essential oil, which can provide extra income to citrus growers as well as related industries.

Keywords: Fruit peels, Utilization of wastes, Essential oil, Extra income



# Comparative Profiling of *Citrus macroptera* and *Citrus reticulata* essential oil of North East India & assay for its antimicrobial and antioxidant activity

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The chemical composition essential oil was extracted from the Citrus macroptera from lunglei, Mizorum Citrus reticulata from Arunachal Pradesh and from Manipur were analysed by GC-MS. The results of the GC-MS analysis indicated the presence of the of the D-Limonene as a major compound which is predominant in Citrus reticulata Arunachal Pradesh (84.14 %) followed by Citrus reticulata Manipur (64.00%) and the least in Citrus macroptera lunglei, Mizorum (25.80%). In addition to D-Limonene the other minor compounds include beta-Myrcene,  $\alpha$ -Terpineol,  $\gamma$ -Terpinene,  $\beta$ -Pinene, Linalool, etc. The extracted essential oil was evaluated for their anti-fungal activity against the phytopathogens Fusarium oxysporum, Macrophomina phaseolina and Colletotrichum gloeosporioides. The antifungal results Citrus macroptera oil exhibited remarkable inhibition percentages against Fusarium oxysporum (77.31%), Macrophomina phaseolina (92.96%), and Colletotrichum gloeosporioides (98.89%) at the highest concentration tested (1000ppm). Similarly, Citrus Arunachal Pradesh oil displayed substantial antimicrobial effects, with inhibition percentages of 85.64%, 83.89%, and 87.04% against Fusarium oxysporum, Macrophomina phaseolina, and Colletotrichum gloeosporioides, respectively, Remarkably, Citrus Manipur oil demonstrated exceptional antimicrobial efficacy, achieving complete inhibition (100%) against Fusarium oxysporum, and substantial inhibition percentages of 96.85% and 96.34% against Macrophomina phaseolina and Colletotrichum gloeosporioides. The results of antioxidant scavenging activity showed that Citrus macroptera from lunglei exhibited the strongest antioxidant activity (IC50: 33.63 µg/mL), while Citrus reticulata from Arunachal Pradesh had the weakest (IC50: 48.81µg/mL). In conclusion, the GC-MS analysis indicated the presence of the D–Limonene as major compounds. The antifungal assays showed that these essential oils have significant inhibitory effects against tested fungal pathogens. These findings suggests their potential for use in various industries and prompting further exploration into their bioactive components and their use as botanicals in agriculture.

Keywords: Citrus Macroptera, Citrus reticulata, Essential oil, GC-MS



# Storage studies of wines prepared from Nagpur mandarin

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In this research abstract, we describe a study aimed at the interaction effect of different levels of yeast inoculum and pH of the must on physico-chemical parameters of Nagpur mandarin wine. The ultimate objective of the study was to find out appropriate levels of yeast inoculum and pH of the must to prepare 'Superior' quality wines from ambia and mrigbahar fruits of Nagpur mandarin. The experiment was laid out with treatment combinations of three different inoculum levels of Saccharomyces cerevisiae var. ellipsoideus(viz., 3, 6 and 9 per cent), with five different levels of pH (viz., 3.0, 3.5, 4.0, 4.5 and 5.0). Different treatment combinations of the levels of yeast inoculum with the pH of the must exerted variable effects on different physico-chemical characteristics viz., alcohol content, residual sugars, acidity, pH and non-enzymatic browning of wines during storage upto 9 months. Based on the sensory evaluation of wines at 9 months storage, wines prepared by using 6 per cent inoculum with 3.5 pH of the must adjudged first rank during both the trials, with a total sensory score of 17.58 in Trial 1, in which ambia bahar fruits were used, and with a total sensory score of 17.88 in Trial 2, in which mrig bahar fruits were used. Though some variations were observed in physico-chemical characteristics of wines during storage upto 9 months, the overall conclusion of the present investigation is that 'Superior' quality wines can be prepared from both ambia and mrig bahar fruits of Nagpur mandarin using two different treatment combinations viz., (i) 6 per cent inoculum of Saccharomycescerevisiae var. ellipsoideus with 3.5 pH of the must, and (ii) 6 per cent inoculum of Saccharomycescerevisiae var. ellipsoideus with 4.0 pH of the must.

Keywords: Citrus reticulata Blanco, Nagpur mandarin, pH, Saccharomycescerevisiae, wine



### TS-4-P-10

# A comparative analysis of drying processes on selected phytochemicals and antioxidant action of sweet orange peel

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Sweet orange (Citrus sinensis Osbeck) peels represent a valuable and abundant source of bioactive compounds within the citrus processing sector. Given their rich bioactive composition, it is essential to convert these peels into a stable form for various industrial applications. Drying serves as an effective method to reduce the high moisture content inherent in these peels, rendering them suitable for incorporation into food products, thereby enhancing their nutritional value. The purpose of this study was to investigate the effects of the hot air oven drying (OD), microwave oven drying (MD), and freeze drying (FD) process on the peel of sweet oranges cv. Mosambi. Sweet orange peels were dried in a hot air oven at four different temperatures viz. 40°C, 50°C, 60°C and 70°C. In a microwave oven, three power levels viz. 360, 540, and 720 watts were utilized to dry the peels. The dried and ground sweet orange peels were subjected to analysis of phytochemical and *in-vitro* antioxidant activity. In terms of ascorbic acids (38.73mg/100g), tartaric acid (4.67 mg/g), malic acid (5.36 mg/g), DPPH radical scavenging activity (8.83mmolL<sup>-1</sup>Trolox), FRAP value (3.77 mmolL<sup>-1</sup>Trolox), among eight drying treatments, FD was shown to be the optimum drying technique; whereas, maximum hespiridin and malic acid content (32.87 and 6.72 mg/g, respectively) was recorded with MD at 540 Watt. We did not observe any significant variation in citric acid (7.15 mg/g with MD at 360 Watt to 9.91mg/g with FD) and total phenolic contents (22.96 mg GAE L<sup>-1</sup> with OD at 50°Cto 24.96 mg GAE L<sup>-1</sup> with MD at 540 watt) among the drying processes. The lowest limonin content (2.8 mg/g) was observed in FD, while the highest concentration (5.54 mg/g) was associated with MD at 360 Watt. Thus, taking phytochemicals into account, freeze drying method can be recommended for dehydrating sweet orange peel followed by microwave drying at 540 or 720 watt.

Keywords: Sweet orange peel, Microwave drying, Oven drying, Freeze drying, Phytochemicals, Antioxidant



# Green extraction of nutraceuticals from waste orange pomace via-molecular imprinting technique

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Citrus fruit produces around 7.8 million tons of citrus waste per year in India. The sustainable valorization of these inexpensive agro-industrial citrus wastes has abundant polyphenolic antioxidants whichcan be used for plant-derived nutraceutical applications, not only to benefit environmental but to also comply with the sustainable practices. In this regard, molecularly imprinting technique was explored for the selective recovery of pharmacologically important bioactive named Hesperidin from the citrus waste pomace extract using Deep Eutectic Solvents (DES) for the development of nutraceutical resource.

Molecularly Imprinted Polymers (MIP) were used for hesperidin recovery preceded by the DES extraction method. Three DES i.e. Choline chloride: Acetic acid: water (1:1:10), Lactic acid: Glucose (5:1), L-proline: Malic acid (1:1), and one Benchmark solvent (Ethanol: water-1:1) were selected. The extraction efficiency of DES was optimized for time, temperature, solid/liquid ratio, and water content. The polyphenolic antioxidant extraction efficiency was compared in terms of total phenolic content and antioxidant capacity such as total flavonoid content, ABTS, DPPH, and FRAP and antimicrobial analysis. MIPwere synthesized using dummy template strategy using hesperetin as the dummy template, chitosan as the biomaterial, calcium chloride as the crosslinker, and L-proline: Malic acid (1:1, w/w) DES as the porogen solvent.

Among all the tested DES the L-proline: Malic acid DES was found best, proven by the total phenolic content concentration and antioxidant capacity studies. The variable conditions such as temperature: 60 °C, time: 120 min., solid liquid ratio: 1:30 g/mL, and water content: 30% were optimized best for optimal extraction of targeted bioactive polyphenols in L-proline: Malic acid DES. Moreover, the pre-polymerization and characterization results reveal the successful preparation of MIP and controlled non-molecularly imprinted polymer (NIP). The rebinding studies of MIP indicated that the adsorption capacity of the prepared polymer towards hesperetin was found to be much significant as compared to the control NIP.

Keywords: Citrus Orange pomace, Deep eutectic solvent, Polyphenol, Molecular imprinting

# Thematic Area -5

Latest Developments in Technology Outreach, Citripreneurship, Trade & Export, Value Chain, Group Dynamics and Policy Formulation in Citrus Sector



# **Oral Presentation**

# TS-5-O-01

# Exploring the six pronged model of institutional support to farmers for boosting citrus export of India

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India is a tropical country with a variety of perennial fruits that grow throughout the length and breadth of the country. Citrus fruits, the third most important fruit crop of India after mango and banana, is cultivated in 10.86 lakh hectares with an annual production of 142.62 lakh tons and contribute about Rs.28000 crores in GDP every year, ranging from fresh fruits to processed products. Maharashtra, Madhya Pradesh, Andhra Pradesh, Telangana, Punjab and North East India are the important states of citrus cultivation. India is the third highest producer of citrus in the world and successfully exports the fruit to different countries of the world. Bangladesh is the biggest market for Nagpur mandarins. Nagpur and Amravati districts of Maharashtra together produce 7 lakh MT of Nagpur mandarins on 1.26 lakh hectares. Of this, Bangladesh alone imports 25% of the produce. But to boost citrus export, research institutes of ICAR and other government research centres have also a major role to play. The authors explore the possibilities and propose a 6-pronged promising model of institutional support solely for citrus exporters which has the potential to benefit the citrus industry in long run. In this model research institutes and other State and Central Government regulatory bodies need to work in coordination. The research institutes can work as Knowledge Hubs for Nucleus Planting Material, Varietal Base, Identifying Package of Practices, Referral Lab (Viral and Bacterial Indexing), Disseminating Knowledge to Core HR and Supply Chain Consultant. Institutions related to agri-export, research and development have a major role to play in enhancing the agricultural export of the country. Once farmers, exporters and other related stakeholders utilize the services of the institutes efficiently, they can reap the benefit. As Nagpur mandarin has huge export potential; sustainable production of desired quality fruits, GI, favourable climate of the region and above all institutional support; it is high time for citrus growers to focus on export and increase their profitability by leveraging all favourable factors. Necessary polices to empower research organizations with more funds and delegation of multi-faceted export oriented services can prove as a new impetus to citrus export industry of India.

Keywords: Citrus, Export, Institutional Support, ICAR, Research Institutes, Model



# Demonstration of rejuvenation technology of Nagpur mandarin in farmer's field

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Citrus decline is a major problem in Central Indian conditions and ICAR- Central Citrus Research Institute, Nagpur had conducted demonstration of rejuvenation technologies of Nagpur mandarin in a farmer's orchard. The experiment was started in the year 2020. The orchard was selected from Nimji village, Kalmeshwar taluka, Nagpur district of Maharashtra state of India. All the treatments were given to the selected trees for rejuvenation. There were around 45 trees which were either in declined or near-decline stage with no to minimal fruiting of spring blossom noticed. To the selected 7 year old declined and 18 year old semi declined trees; FYM, macro and micronutrients were applied. All the plant protection measures were taken time to time for all the treatment plants except controlled ones. Observations taken in 2023 from 7 year old treated declined trees have shown higher canopy growth (22.90m3), tree height (4.28m), TSS (7.3%), fruit yield (8.22 t/ha) as compared to controlled trees (22.51m3, 4.26m, 7.2% 6.08 t/ha respectively). Similarly, 18 year old treated semi-decline trees have shown slightly higher canopy growth (89.56m3), maximum average fruit weight (163.9g), TSS (7.9%), fruit yield (9.92 t/ha) as compared to controlled trees (84.10m3, 152.1g, 7.8% 7.31 t/ha respectively). No visible symptoms of disease were seen except gummosis noted in 2-3 plants of 18 years old. Incidences of insect pests were recorded in 7 and 18 year old Nagpur mandarin orchard, the average incidence of citrus psylla, leaf miner and black fly were observed in both the orchards during the observation period. Plant parasitic nematodes Tylenchulus semipenetrans were less in no. in treated trees as compared to controlled trees. However, Pratylenchus sp. recorded higher count in 7 year old treated decline trees as compared to controlled trees, but in 18 year old trees showed low count in treated trees as compared to controlled trees.

Keywords: Decline, Fruit yield, Nagpur mandarin, Plant protection, Rejuvenation, TSS



# ICAR-CCRI technologies transforming the livelihood of citrus growers: a micro-level study

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While establishing a citrus orchard, the most crucial factor which should be taken into consideration is purchasing good quality, healthy, disease-free planting material. The technology of containerized disease-free planting materials of citrus of ICAR- CCRI, Nagpur; is a boon to citrus growers who suffer from lack of availability of good quality planting materials. The farmers who had purchased planting materials from ICAR-CCRI are satisfied of their orchard production and health. A glance into the success stories of 5 farmers; viz., Shri Prashant Vamanrao Gulande, Shri Rupesh Gupta and Shri Prashant Khursunge of Nagpur, Shri Laxmikant Khandelwal of Amravati and Shri Kundan Jadhav of Jalgaon district of Maharashtra; would be enough to depict the successful development, assessment, refinement and transfer of containerized disease-free planting material technology of ICAR-CCRI. Along with this, the farmers have also adopted the scientific package of practices recommended by ICAR-CCRI and also maintain steady contact with scientists of the institute for keeping themselves updated on latest technologies of citrus cultivation. Mr. Gulande, Mr. Khursunge and Mr. Khandelwal have established their nursery business also by setting up mother blocks of citrus and are earning around Rs. 5.5 lakhs/year, Rs. 15 lakhs/year and Rs 10 lakhs/year respectively. These progressive farmers also have citrus orchards established with disease-free planting materials of ICAR-CCRI and presently they earn Rs.7-8lakhs/year, Rs.10lakhs/year and Rs.8-10 lakhs/year respectively. Mr. Jadhav and Mr. Gupta, on the other hand, focusing only on cultivation aspect, have orchards of Nagpur mandarin and sweet orange and are able to generate about Rs. 40-45 lakhs/year and Rs. 6-7 lakhs/year respectively. Mr. Jadhav sells his produce in markets of metro cities like Mumbai at premium prices. The difference in average annual income of all these farmers is basically due to the differences in sizes of their nurseries and orchards and age of the bearing plants. Nonetheless, each one of them are getting premium prices for their good quality fruits and nursery plants which is the result of adoption of disease-free plants of ICAR-CCRI and related package of practices. The case studies serve as an inspiration to other citrus growers of the country.

**Keywords:** Adoption, Income, Case Studies, Disease-free planting materials, Progressive citrus growers, Technologies, Scientific package of practices



# A Study of common people's choices: citrus consumption habits and preferences

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People's choice and consumption behavior, including healthy perception and the pleasure aspect, given the heterogeneity and the multiplicity of the qualitative aspects that can characterize Citrus group of fruits, have been widely investigated. Citrus fruits have long been known for their nutritional and health benefits for humans. The poll was carried out as an online survey. Surveys were also distributed via social media connections. The questionnaire examined the frequency and amount of citrus consumption and included a variety of forms, including whole fruits, juices and processed products. The aim is to identify the motivating factors that drive these consumption habits, such as taste preferences, nutritional aspects, and lifestyle choices. In addition, the study explores preferred types of citrus fruits and whether individuals prefer popular choices such as oranges, lemons and limes, or whether they option for lesserknown varieties. Evaluate factors such as flavour profile, perceived health benefits, and culinary applications to reveal underlying preferences. Finally, by analysing the data collected from diverse sample of respondents, the poll reveals a varied range of citrus eating patterns and preferences among the general public. The findings highlight the complexities of citrus consumption, which is influenced by flavour, nutrition, and lifestyle. The data indicates noteworthy trends ranging from intake frequency to preferred citrus types. Notably, health consciousness and culinary applications have a considerable impact on preferences. Furthermore, shopping decisions are influenced by a combination of affordability, organic concerns, and promotional activities. These findings are useful for dietary advice, marketing initiatives, and public health campaigns. Understanding these consumption dynamics becomes critical in promoting healthier choices and building a better appreciation for this key food group as citrus continues to maintain a role in common diets.

Keywords: Citrus fruit, Flavor, Health, People, Questionnaire, Survey



# Problems and prospects of citrus production in Rajasthan

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Major cultivated fruit of Rajasthan is belongs to Citrus spp. It contributes 54 per cent area (42131 ha.) and 70 per cent of total fruit production (672940 MT) of Rajasthan as per record of department of horticulture, govt of Rajasthan in 2021-22. Mandarin grows around 63 per cent (38718 ha.) of total citrus cultivation. Sri Gaganagar and Hanumangarh are kinnow cluster, while Hadoti region is Santara cluster known for distinctive taste and color of citrus. The department of horticulture, govt of Rajasthan decided to market Rajasthan orange under the brand, "Raj Santara". Recently, citriculture industry of Rajasthan facing problems due to climate change (cause unfruitfulness of citrus orchard, biennial trend), citrus phytophthora, citrus decline, water scarcity at critical stages and early closing of canal in command area. These problems leads to downswing production of citrus in Rajasthan. Government has taken initiative by establishing Center of Excellence for citrus (Citrus group and Mandarin) at Kota and Jhalawar. Some more efforts can be taken to overcome these problems are to allot more budget for research and extension, law enforcement to ensure quality planting material, establish nursery to raise healthy disease and virus free plantlets, develop biotic and abiotic stress resistance root stocks, avoid canal closer during April to May in Kinnow growing areas, insure irrigation during hot summer, practical training through front line demonstration & exposer visits to motivate farmers, special training programmes to train field staff etc. will boost citrus industry in Rajasthan.

**Keywords:** Citrus, Raj Santara, Climate change, Unfruitfulness, Phytophthora, Decline, Critical stages, Command area



# Participatory mode: An effective way to disseminate the technologies for citrus in NE region of India

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Citrus fruits are present in various forms and have their centre of origins in the north-eastern parts of India. The region has a rich diversity of citrus, with Khasi mandarin (Citrus reticulata Blanco.) an ecotype among citrus, being the most cultivated citrus. Khasi mandarin has excellent taste and quality; hence, a GI tag was obtained, and its premium fruits are being exported. Despite the recent advancement in citriculture, the adoption rate of technologies is limited among the growers of these hilly states, and productivity is stagnant, which is a matter of concern for the stakeholders associated with its trade. To sustain the production with a quality harvest, the dissemination of need-based location-specific technology via participatory mode was the best alternative. On the basis of the PRA technique, the key areas, viz., irrigation water, canopy and nutrient management, disease and pest control, guality planting material, etc., were identified for technological intervention. A need-based technology developed by the Institute wasdemonstrated, viz., Julkund, half-moon terraces, and scientific package of practices (i.e., Bordeaux paste; mulching; removal of diseased/dry shoots; nutrient application schedule; weed management; insect pest and disease control, maturity indices, etc.).A model Khasi mandarin orchardat Mawryngkneng Village, East Khasi Hills and a citrus nursery were developed at Sonidan village, Ri-Bhoi District, Meghalaya. These two-pronged strategies for quality planting materials and orchards management model serve as field clinic orchards for the other growers in the region. To strengthen the performance and effectiveness of the disseminated technologies, a strong network of communication with the growers, SHGs and scientists was established through a mobile-based short message service (SMS) in the local language. The outcome indicated that the location-specific need-based approach through participatory mode is an effective way for demonstration and adoption of scientific interventions among the growers of hilly terrain. Furthermore, such approaches will help sustain production and improve the livelihoods of Khasi mandarin growers.

Keywords: Citrus, Participatory mode, Demonstration, NE India



# Planning of resources by Orange growers for production and marketing of Oranges in Vidarbha region of Maharashtra

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To be successful, today's orange growers must be able to plan their resources to meet the challenge of varying costs, prices and climatic condition. Planning of resources is helpful to the farmers to move to a higher level of production and income. Hence this study was carried out in order to assess the planning of resources by orange growers for production and marketing of oranges and to find characteristics of orange growers influencing their planning of resources. The present investigation was carried out in Amravati and Nagpur districts (Vidarbha region) of Maharashtra. A sample of 240 respondents constituted for the research study. The present study revealed that majority of orange growers (59.17%) planned their resources moderately. The study found that many orange growers in this region do not plan post harvest management, drainage management, soil and water testing, etc. The study revealed that innovativeness, economic motivation, knowledge and adoption about recommended orange growers. The present study indicates that orange growers with more innovativeness, more economic motivation, increased knowledge and adoption about recommended orange cultivation practices would help in better planning of resources that ultimately led to efficient resources management and increasing profitability.

**Keywords:** Improved Production Technology, Marketing, Orange Cultivation, Orange Growers, Planning of Resources, Production, Relational Analysis, Resource Management



# Value added products by orange processing and dissemination

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The most important commercial citrus species in India are the mandarin (Citrus reticulata), sweet orange (Citrus sinensis) and acid lime (Citrus aurantifolia) sharing 41, 23 and 23 % respectively of all citrus fruits produced in the country. Orange is found in large quantities in Vidarbha region. The orange fruits are an important source of energy and vitamins for human-beings but they are perishable in nature; hence, efforts have been made to convert into imperishable orange products by suitable processing technology. But the small orange farmers in this region do not have the suitable technology to convert their oranges into available market products. The objective of this work is to disseminate the orange processing technology which will support the orange farmers in Vidharbha Region to avoid loss in the orange farming. So MGIRI started its dissemination technology through Livelihood Business Incubation (LBI) for orange farmers & Farmers Producer Company (FPC). Under this technology, MGIRI developed some products like squash, ready survey, jelly, jam, orange crush, orange gummy Toffee, Orange Marmalade, Orange Peel Toffee, Face pack, Orange cookies, with the help of region farmers. Many orange farmers and Farmers Producer Company (FPC) participated into LBI orange processing training conducted at MGIRI. In these trainings the total orange fruits it converted into products, and no part is waste. It is calculated from over experimentation at LBI that orange farmers can get of 60% to 70% profit if they converted their orange fruit into above mentioned products.

Keywords: Orange Products, Processing technology, Technology Dissemination



# Insurance choice and revenue risk reduction in citrus crops: A machine learning approach

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Crop insurance has become a popular tool in developed and developing countries to protect farmers against downside revenue risk and stabilize farm income. However, insurance uptake has been challenged in the Indian context even with federal incentives in the form of premium sharing. Exposure to climate vulnerability conditions insurance uptake. In this study, I develop a Conditional Inference Tree (CIT) – a supervised machine-learning algorithm that operates with recursive partitioning to improve the understanding of insurance uptake in presence and absence of climate vulnerability. It identifies variables of relevance with permutation-based significance tests, removing inherent biases in Classification and Regression Tree (CART) and other algorithms. I study farm household profiles growing citrus crops and their decision characteristics reported in the Situation Assessment Survey of Agricultural Households, surveyed by the National Statistical Office of India during January-December 2019. The choice of risk management – whether or not a farmer insures his crops, either by availing credit at formal financial institutions or independently, is modeled using farm and household characteristics as features. Initial results show that citrus farmers vulnerable to climate risks prefer insurance. Among the less/nonvulnerable, irrigation appears to be a major driver of insurance choice, which is surprising given the belief that positive uptake is associated with weather risk. Econometric models validate these findings and offer additional insights. They show that off-farm income encourages crop insurance, but female farmers are less likely to insure their crops. Further CIT analysis underway, e.g. boosting, can provide strong learners, which avoids over fitting while enhancing prediction accuracy. Nonetheless, these results guide context-specific risk reduction policies and support farm-nonfarm linkages.

Keywords: Conditional inference trees, Citrus crops, Crop insurance, Machine learning, India



# Present status on commercial cultivation of citrus species in Tamil Nadu

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Tamil Nadu, with its diverse climatic conditions, cultivates various citrus species such as Acid lime (Citrus aurantifolia), Lemon (Citrus limon), Citron (Citrus medica), Mandarin orange (Citrus reticulata), Pomelo (Citrus maxima), Sweet orange (Citrus sinensis), Indian rough lemon, Narathai (Citrus taitensis), Sour orange and Indian wild orange (Citrus aurantium) and Kaffir lime, Kolumichai (Citrus hystrix). The total area and production of these citrus species in Tamil Nadu are 16,416.07 ha and 241840.3 MT, respectively. Tamil Nadu is home to several fruit crops, including acid lime, lemon, citron, mandarin orange, pomelo, and sweet orange. Acid lime, a key crop for the pickling industry, covers 11,889.38 hectares and has a production and productivity of 1,67,084.96 MT and 14.05 t/ha. Lemon is grown in 1803.11 hectares and has a production of 1,67,084.96 MT and 14.05 t/ha. Citron fruit is used for candy and liqueurs, with oil used in sweets and beverages. Mandarin orange, a popular fruit, is grown on 853.28 hectares and has a production of 13524.94 MT and 15.85 t/ha. Pomelo is cultivated for edible fruit and medicinal applications. Sweet orange, rich in potassium and citrate, can help prevent high blood pressure and stroke. Indian rough lemons are a rich source of fiber, vitamin C, potassium, copper, vitamin B6, calcium, magnesium, and iron. They stimulate the digestive tract, strengthen the immune system, boost skin collagen production, and reduce inflammation. Sour oranges are grown in Tamil Nadu's 129.48ha cultivation area, with production and productivity of 4998.55MT and 38.61t/ha, respectively. Kaffir lime, rich in compounds like limonene, nerol, and alkaloids, offers health benefits and is used as a flavoring agent in dishes, herbal medicines, beauty products, cosmetics, and perfumes. Predominant insect-pests are black fly, whitefly, psylla, thrips, leaf miner, scale insects, bark eating caterpillars, fruit fly, and mites. Citrus diseases include Phytophthora gummosis, citrus tristeza virus, citrus greening and canker.

Key words: Acid lime, Citrus, species, Cultivation, Tamil Nadu



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# Institutional finance in the establishment of containerized citrus nursery

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In India, most of the citrus nurseries are grown as field nurseries. In field nurseries the eradication of soilborne pathogens like Phytophthora once introduced becomes very difficult as also the dreaded viral and bacterial diseases and later acts as a premier cause of citrus decline. The total annual demand for planting material of Nagpur mandarin orange is about 50 lakhs. There is tremendous scope for private nursery entrepreneurs to produce disease-free planting materials by adopting containerised nursery systems. ICAR-CCRI, Nagpur a pioneer institute in the development of the state-of-the-art technology of containerised citrus nurseries has been instrumental in licensing the technology to private nursery owners. The investment cost for the establishment of containerised citrus nursery model is very high. The establishment of a mother orchard of mandarin orange, rootstock, and related infrastructure involves a substantial investment. The nursery growers need institutional finance to establish such a hi-tech nursery. The substantial income generation starts from the sixth year after repaying the principal amount and interest. Taking into consideration the recommendations of CCRI, Nagpur, and the views/experience of mandarin orange growers, a model bankable scheme for the production of disease-free planting material has been prepared. The cost is indicative in nature and can be modified to suit different areas taking into account the local condition, techno-economic parameters prevailing wage rates, etc.

Keywords: Containerised nurseries, Disease free planting material field nurseries, Institutional finance

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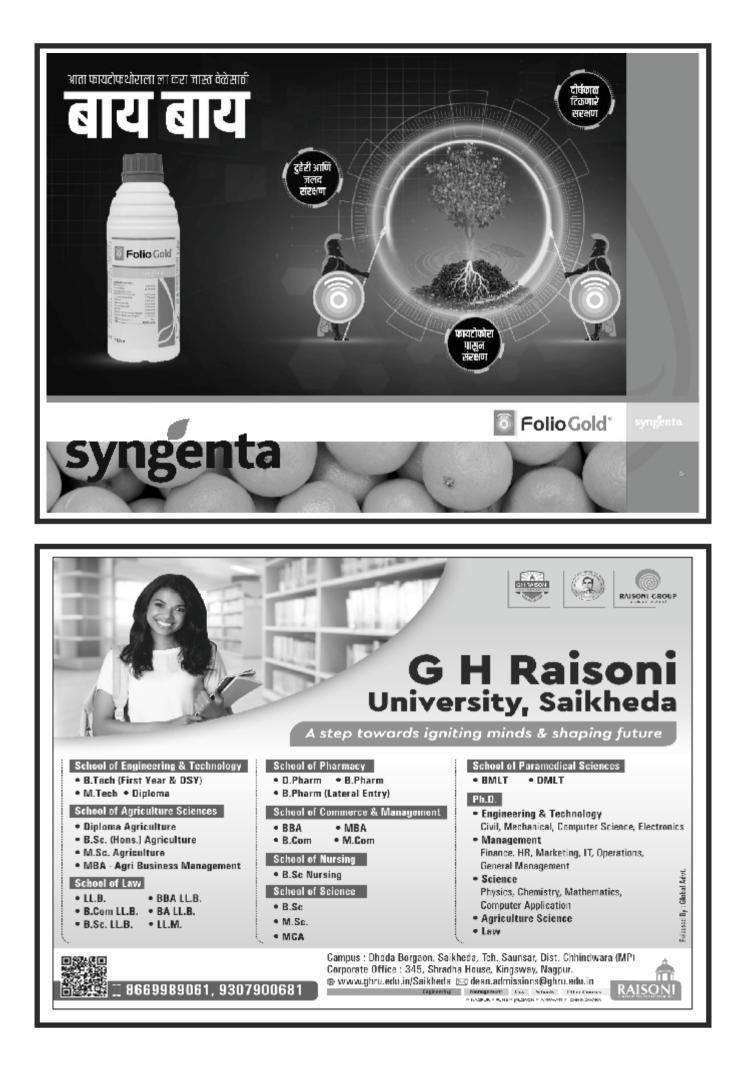
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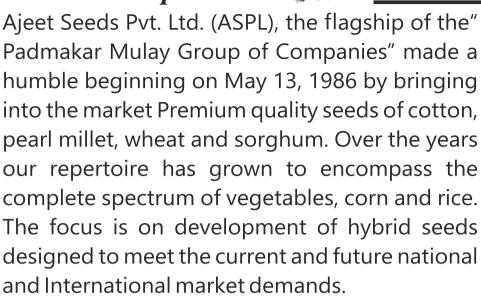
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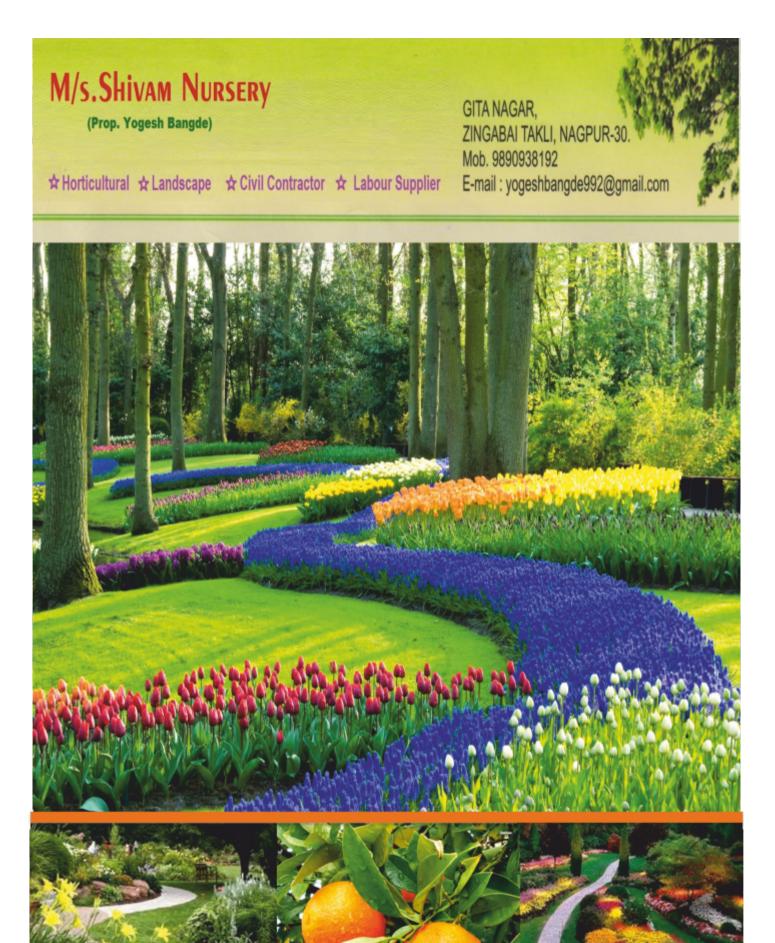


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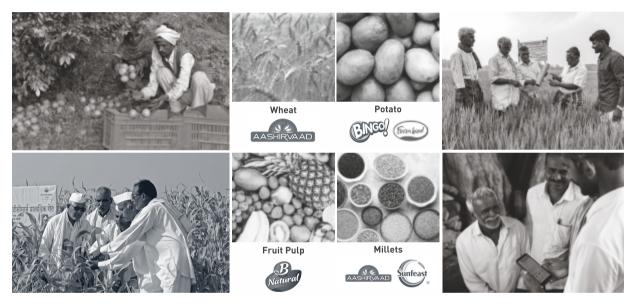
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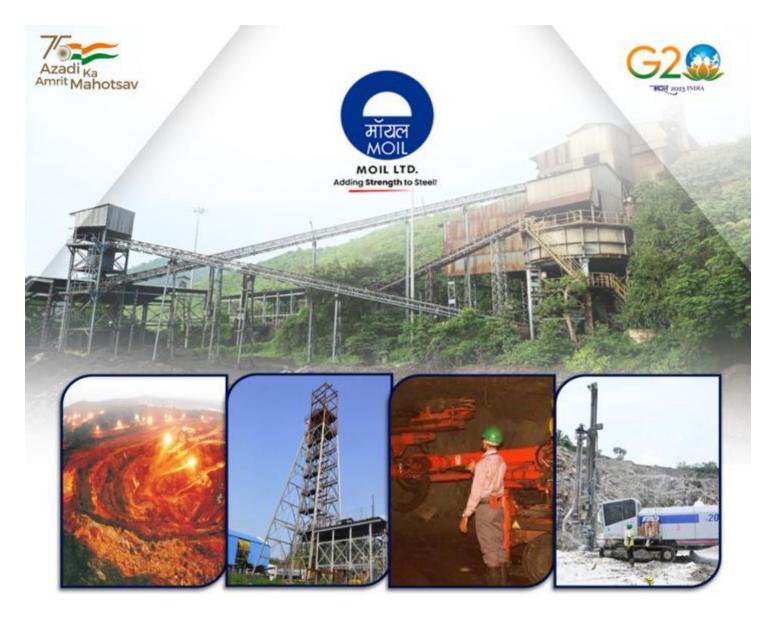
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