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Innovative approaches for the production of certified quality planting materials in Khasi Mandarin (*Citrus reticulata* Blanco)

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ABSTRACT

Citrus reticulata is one of the most important commercial fruit crops in north-eastern India. The fruits are of premium quality with a unique taste due to the favorable agro-ecological conditions of the region. The demand for citrus fruits is increasing, providing enormous potential for future growth. The availability of certified citrus quality planting materials (CQPM) is the most strategic means to meet the desired growth and demand. Presently, there is a shortage of certified CQPM. Moreover, the citrus propagules are vulnerable to several pathogens, particularly viruses, viroid-like, and systemic bacteria, which are both graft- and vector-transmissible. Mechanisms are needed for an effective regulatory framework through accreditation and certification of mother stocks in the production of a certified CQPM. In this paper, a standard of procedures (SOPs) for the production of certified CQPM is discussed thoroughly. The major steps in the production of certified CPQM are: i) selection and identification of plus plants on the basis of two major criteria, i.e., horticultural traits and virus-free source plants; ii) maintenance of certified clean foundation stocks (Block 1); iii) multiplication of stock (Block 1); iv) labelling of certified CQPM with a unique bar or QR code for distribution. Periodic monitoring and maintaining the traceability records of a chain-of-custody of certified CQPM. Therefore, the strict adoption of a protocol will help in the production of the highest horticulturally superior plants and pathogen-free for the sustainable performance of citrus orchards in terms of tree longevity, fruit yield, and quality.

1. Introduction

The horticultural production in India has made remarkable progress, reaching 342.3 million metric tons (Agricultural Statistics at a Glance, 2022). It ranked second in area and production of horticultural crops in the world. Fruit crops share about 30% of the total horticultural production. Fruits can contribute significantly to India's agricultural economy through exports worth Rs. 6,219.46 crores (APEDA, 2023). Citrus is grown in large areas (1.09 million hectares) throughout the country with a production of 14.8 million metric tons, ranked as the second-largest commodity among fruit crops in the country, with a significant potential for exports. Exports of citrus fruits from India in 2019–20 were Rs 329.32 crore and reached Rs 590.4 crore in 2020–21 (Ministry of Commerce & Industry, 2022). Citrus is one of the most important fruit crops in northeast India. This crop supplements nutrition and is also an indispensable cash crop for both domestic consumption and export. The citrus fruit is consumed worldwide as both fresh and processed products. Northeast India is considered the center of origin of citrus species due to its unique position in terms of citrus biodiversity reserves, including 23 species and more than 68 varieties, or landraces, known to exist in the region. A variety of citrus species are cultivated in a wide range of climatic zones, from temperate to subtropical and further to the tropics. Khasi mandarin (Citrus reticulata Blanco) is an ecotype of mandarin fruit in Meghalaya and is the most important commercial fruit crop in the region (Rymbai et al., 2024). The fruits of Khasi Mandarin produced in northeast India are of premium quality due to the suitable agro-ecological conditions (Rymbai et al., 2022), therefore, a Geographical Identification (GI) tag was obtained for this

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crop in 2014. The Govt. of Meghalaya exported 2 tonnes of Khasi mandarin fruits to Middle Eastern countries in 2022.

In the region, the productivity of this crop is very low (4.90 t ha−1) as compared to the national average (10.36 t ha−1; NHB 2017). Unproductive citrus orchards are a common concern in citrus belts around the world, and there can be an array of underlying causes (Rymbai et al., 2024). Citrus orchards have been suffering from a number of systemic viruses, virus-like diseases, and systemic bacteria (Whiteside et al., 1988; Singh et al., 2017; Ganesh et al., 2018). These diseases affect longevity, productivity, and quality (taste, appearance, and nutrients), causing enormous financial losses to the producers. In such circumstances, volatility in prices and subpar fruit quality will have an indirect impact on customers as well. The disease has appeared to be one of the serious constraints for the citrus industry in the region. It is a fact that, in addition to good orchard management practices, the availability of reliable and good-quality propagules is among the various technical factors responsible for higher crop performance, both in yield and quality. Certified CQPM is an essential input in the horticultural production system to reduce the spread of threatening pathogens, improve adaptability, and ensure sustainability with higher crop performance. Therefore, the success of the citrus sector in the region thus depends critically on the implementation of a citrus certification program for the production of certified citrus quality planting materials.

2. Demand and supply of certified citrus quality planting materials

There is a constant increase in demand for citrus commodities, providing enormous potential for future growth in their production. The only way to meet the demand is through increased production, which can be achieved through area expansion, productivity increases, or both. The possibility of growth from area expansion is limited in the future, considering the rising population and increasing demand for land. Therefore, an improvement in productivity is the most strategic means to meet the desired growth and demand. However, the present productivity of clonally propagated fruits, including citrus, in India is lower in comparison to the correspondingly highest productivity in the world (Figure 1). The productivity of citrus in India is about 13.57 t/ha, which is 2.1 times less than that of productivity in Brazil (27.93 t/ha). It is a well-known fact that one major challenge to increasing productivity in citrus is the shortage of certified quality planting materials. The Seed Act in India was passed in 1966, which facilitated the setting up of state-level seed-certifying agencies. This Act allows for the certification of the clonally propagated potato among horticultural crops, but not other clonally propagated crops.

The requirement for certified quality planting materials of propagated crops in India is about 200 million; 20 million of those are for citrus crops alone (NAAS, 2022). In spite of the presence of a large number of nurseries, both public and private, only 35–45% of the demand for certified quality planting materials is met. Establishment of a pathogen-free nursery is of primary importance to prevent the prevalence of diseases (Su, 2008). The certified-quality planting material under the National Certification System for Tissue Culture Raised Plants (NCSTCP) so far has supported only bananas among fruit crops. Therefore, the development of an effective regulatory framework for the production of certified quality planting materials in citrus fruits is imperative. An effort is needed to develop a robust system for the production of certified CQPM from elite clones of citrus fruits on indexed desirable rootstocks in order to harness their full potential.

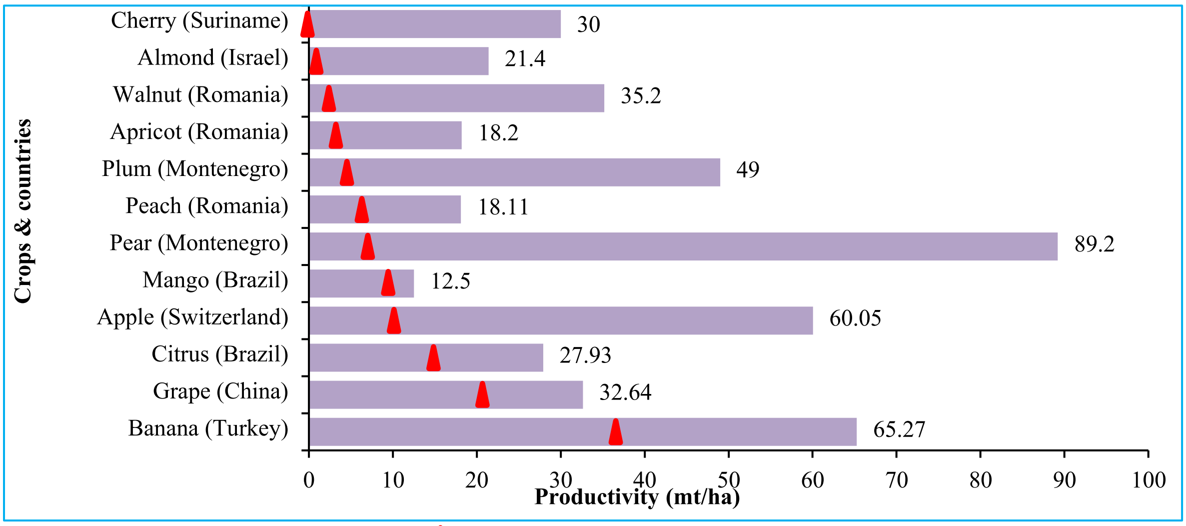

Figure 1. Productivity of fruit crops in India (▲) as compared to the countries with the highest productivity in the world given in parenthesis during 2019-20.

3. Viruses and virus-like pathogens

The primary challenge to the citriculture industry is viruses, virus-like diseases, and systemic bacteria, which are the biggest threats and constraints to improving the productivity of citrus. Although several pathogens infected citrus that limit yield and quality, the majority of these, such as bacterial and fungal pathogens, can be managed through various control measures, and the budwood does not transmit these diseases. On the other hand, the presence of graft-transmissible diseases in the propagules persists even during propagation and transmits to all the progenies. These pathogens that are graft-transmissible include viruses, viroids, phytoplasmas, spiroplasmas, systemic prokaryotes like *Xylella fastidiosa,* and systemic bacteria like *Candidatus Liberibacter asiaticus*. These pathogens often occur as latent infections as long as they are propagated on tolerant rootstocks or remain in tolerant scions, but if propagated on a susceptible rootstock, they will become destructive instantly (Lee 2007). Graft-transmissible, particularly viruses and virus-like pathogens, are often hard to detect in propagules like rootstocks and budwood and are unintentionally introduced even to places that were previously devoid of such infections.

Citrus is a perennial plant that is infested by a wide variety of viruses, viroids, and phytoplasmas (Table 1). Recent reports showed that numerous viruses, hitherto unreported in India, were found during investigations on the virome profiling of indigenous and exotic varieties of grapevine (Sidharthan et al., 2020). Similarly, two viruses and one viroid that had not been reported earlier in India were found during the virome investigation of three apple cultivars (Nabi et al., 2022). The most effective approach to preventing such inadvertent virus introductions is through a certification program for clonally propagated crops. This is possibly the most critical aspect of boosting yield and diversifying the citrus portfolio. In India, the citrus tristeza virus (CTV) and the huanglongbing bacterium *Candidatus Liberibacter asiaticus* (CLas) are the major threats responsible for the decline in citrus orchards. In the northeastern states of India, about 42–69% incidence of CTV in Khasi mandarin orchards has been reported (Singh et al., 2017). Surveyed in declined Khasi mandarin in Meghalaya showed the incidence of viral (30–70%) and fastidious bacterial (50–80%) based on symptomatology (Ganesh et al., 2018). Consequently, the viral and systemic bacteria seem to be among the most serious challenges plaguing the citrus sector in the region.

4. Citrus certification programme

The availability of quality planting materials is very vital for the success of horticultural development initiatives and the overall diversification of agriculture. It is particularly significant in fruit crops, which are perennial and have long gestations. In most fruit crops, the existing varieties are often poor in yield and quality; thus, producers rely on the introduction and import of superior exotic species. However, there is no regulation or monitoring mechanism available for the introduction of such exotic materials, leading to the simultaneous introduction of new pathogens, particularly viruses. Furthermore, the majority of crops, such as citrus, are vegetatively propagated and are vulnerable to several pathogens, particularly viruses. In citrus nurseries, vegetative parts and vectors such as the Asian citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*) are the most prevalent transmissible pathogens. Therefore, quality planting materials free of viruses can be produced only from accredited and certified bud wood mother stocks. The citrus certification programme may be defined as "the production of uniform, healthy, pathogen-free CQPM through vegetative propagation for assurance of the physiological, phytosanitary, and highest horticultural quality of the plants".

Table 1. Viruses and virus-like pathogens infecting Citrus

Viruses	Viroids	Phytoplasma & Fastidious prokaryotes
Indian citrus ringspot virus (ICRSV)*, citrus tristeza virus (CTV)*, citrus mosaic virus (CiMV)*, citrus yellow vein clearing virus (CYVCV)*, citrus leprosis virus (CiLV-C), citrus chlorotic dwarf-associated virus (CCDaV), citrus virus A (CiVA), citrus psorosis ophiovirus (CPV), citrus concave gum-associated virus (CCGaV), Citrus psorosis virus (CPsV), citrus vein enation virus (CVEV), citrus leaf blotch virus (CLBV), citrus tatter leaf virus (CTLV), satsuma dwarf virus (SDV), citrus variegation virus (CVV), citrus endogenous pararetrovirus (CitPRV), gummy bark (GB), Citrus leaf rugose virus, Bud-union crease of citrus trees, Leaf variegation with ring spots.	hop stunt viroid (HSVd)*, citrus dwarfing viroid (CDVd), citrus bark cracking viroid (CBCVd), citrus bent leaf viroid (CBLVd), Citrus gummy bark viroid (CGBVd) or Citrus viroid II, Citrus exocortis viroid (CEVd), Citrus viroid V (CBLVd)	*Candidatus* Phytoplasma cynodontis, *Candidatus* Phytoplasma Aurantifolia, *Candidatus* Liberibacter asiaticus and *Candidatus* Phytoplasma asteris

5. Scenario of citrus certification programme

The first citrus bud wood certification program was launched by Brazil in 1921 for sweet oranges. In 1939, it was further strengthened by a state regulation that established standards for the sale of citrus trees. Whereas for the citrus nurserymen, it was mandatory to register and undergo regular inspections. Subsequently, other citrus-producing nations, such as Israel, France, South Africa, and the United States, have enacted such regulations. In India, the need for certified CQPM production in citrus crops was realized long ago (Raychaudhury and Verma, 1992). Although efforts are underway and are gradually picking up towards achieving the goals of citrus virus-free QPM. In India, the ICAR-Central Citrus Research Institute (ICAR-CCRI) plays a very critical role in the certification of CQPM. The ICAR-CCRI is maintaining a mother block of different species and varieties of citrus. It has also supplied a large number, approximately 0.3 million, of index QPM of citrus varieties such as Nagpur mandarin, Mosambi, and acid lime to orchardists and registered nurseries in the country (NAAS, 2022). Similarly, the Citrus Research Station at Tirupati supplied about 1–4 lakhs of virus-free planting materials of sweet orange cv. Sathgudi to orchardists during 1996–1999. In Punjab, about 7-8 lakhs of Kinnow mandarin planting material are provided every year to citrus growers. About 1/4th of these planting materials are supplied by SAUs and line departments, and a large quantity of Kinnow mandarin is provided by private nurseries. The production and supply of planting materials are mostly done with little or no regulation of defined SOPs for supplying certified CQPM at any of these centers. Modern infrastructure, such as an insect-proof greenhouse, rooting chamber, efficient tools, machinery, and technical know-how, is lacking in many of the unorganized private sector nurseries for the production of certified CQPM that are free of pathogens and genetically true-to-type.

In view of the large annual requirement for certified QPM in citrus (about 20 million), the National Horticulture Board (NHB) has taken several initiatives by accrediting several nurseries for different fruit crops in various parts of the country. The NHB has made it mandatory for all accredited nurseries to meet all standards of certified CQPM, including the virus testing requirement (http://nhb.gov.in). The NHB has also established evaluation criteria for infrastructure, quality of seed and planting material, and the adoption of scientific nursery management techniques to guarantee true-to-type QPM. Locations, mother block features, propagation procedures, infrastructure, irrigation systems, operating manuals, personnel and quality, trade relations, biosecurity, and disease-free status were other parameters for consideration as criteria. Although biosecurity and disease-free conditions are specified in the set standards for nursery accreditation, regular screening and indexing of clonally propagated viruses and virus-like pathogens have not been taken into consideration.

A total of 688 nurseries nationwide have been accredited and given star ratings ranging from 1 to 3, according to a set of guidelines for the accreditation and rating of nurseries. The nursery accredited had a low rating (below 3 on a scale of 5 according to the accreditation standards); 1.31% are three-star rated, 10.61% (2 stars), and the majority are one-star rated (88.08%). This indicated that the majority of these accredited nurseries fail to satisfy the requirements of certified QPM, including virus testing (NAAS 2022). The examination of mother stocks (budwood source plants) in 11 nurseries of Kinnow mandarin (ranked 2 to 4 stars by NHB) in Punjab revealed the existence of the Indian citrus ringspot virus, the citrus yellow vein clearing virus, and Huanglongbing (Pant et al., 2018). The incidence of CTV in Khasi mandarin orchards in Northeastern states has been recorded at 42–69% (Singh et al., 2017).

This implies that farmers lack access to good-quality, certified disease-free, and true-to-type planting material, and as a result, orchard yield and quality suffer. There is an urgent need to develop an efficient regulatory system to produce certified QPM citrus fruits. There is also a need for an organized mechanism to undertake certification of CQPM supplied by accredited nurseries.

6. Protocol of Citrus Certification Programme

The availability of certified CQPM is a critical step towards increasing sustainable production and meeting exports. However, the non-availability of such quality planting materials has significantly hindered the productivity and export potential of citrus fruits. The certified CQPM can only be achieved through the establishment of a strong and robust national certification system on ‘Certification of Citrus Quality Planting Materials’. The key components in citrus certification programme are: i) identification and production of plus plants, *i.e.*, horticultural traits and virus-free source plants; ii) maintenance of plus plants; and ii) further propagation of plus plants for the production of healthy planting material with the highest yield and quality attributes for distribution to growers. The model of standard operating procedures (SOPs) for the production of certified CQPM is given below (Figure 2):

6.1. Step I: Selection of candidate plant or plus plant

The first step in the certification of CQPM is the selection of a candidate plant or plus plant. The initial candidate plants can generally be obtained in three ways: domestic selection, new varieties or clones, and exotic elite materials.

- ***i) Domestic selection***: In the case of Khasi mandarin and other citrus indigenous to the region, which are available locally, no introduction is required for the production of CQPM. A survey may be carried out among the elite genotypes of Khasi mandarin for the selection of the candidate plant. An elite domestic planting material needs to be extensively tested using robust diagnostic standards.
- ***ii) Exotic elite materials***: In cases of the introduction of a new genetic material or exotic material of high horticultural superiority and other desirable traits in the CQPM production system, such imported materials are required to be subjected to a robust quarantine system. Maintaining the imported exotic plant materials in a post-entry quarantine facility and conducting routine testing using reliable techniques periodically is critical.
- ***iii) New variety / clones***: A new variety of desirable traits and pathogen-free plants may be used as candidate plants.

The major selection criteria of a candidate plants

There are two major criteria for the selection of a candidate plant, i.e., horticultural superiority (A+) and pathogen-free (B+), as given below:

i) *Horticultural traits*: The most important horticultural traits are high yield and good quality. Color, texture, size, and flavor are the primary quality attributes that are crucial for the global marketing of horticulture products.

ii) *Pathogenicity test*: Following the identification of a plus plant consisting of horticultural superiority, the next step for the production of certified CQPM will be further determined by the detection of pathogens. Any exotic or new materials introduced in the certified CQPM production system will be subjected to a robust post entry quarantine system and tested periodically. The purpose of testing these materials is to detect any probable infection with an identified or unidentified virus or virus-like pathogens. The testing can be done by employing high-throughput sequencing (HTS for mRNAome or sRNAome). The HTS will identify the likely infection of all viruses and virus-like diseases in a single limited sample without the need for multiple detection assays. In this case, the standard crop-wise tests on samples, template preparation, the HTS pipeline, and the *in-silico* analysis pipeline should be developed and used for HTS-based virus detection. The tissues to be used for HTS analysis (targeting both RNA and DNA pathogens) are crucial here. For this, a gold standard procedure should be developed and followed. For instance, to detect all viruses and virus-like pathogens in citrus, a mixed sample (leaf lamina and phloem tissues) could be ideal. After the HTS-based testing, testing will be followed by standard molecular and serological tests. If the plant material is tested positive, the pathogens need to be eliminated by an appropriate method (thermotherapy, tissue culture, and shoot-tip grafting). After sanitization, the materials are to be retested for freedom from pathogens.

6.2 Step II: Testing of a plus plant plants

After the selection of candidate plants through horticultural traits and pathogenicity test, the following two approaches (Plan A or Plan B) may be applied depending on the absence or presence of viruses:

Plan A - The candidate plants (A+ and B+): In plan A, the candidate plants with horticultural superiority traits (A+) and freedom from diseases (B+) need to be registered with a unique identification number. The availability of such plants will be transferred to Block 1 following standard operation for the production of certified CQPM as given in step III:

Plan B - The candidate plants (A+ and B-): Plan B is followed when the candidate plants possess all the horticultural superiority traits (A+) but are detected with virus pathogens (B-). In such cases, the following standard operation may be adopted for the production of quality planting materials:

i) Reconstitute the plus plant (A+ and B-): The selected candidate plant containing A+ and B- may be reconstituted by collecting the bud wood or scion stick for growing in pot culture under controlled laboratory conditions to avoid any contamination with insects and other citrus species.

ii) Virus elimination of representative plus plants: The representative plants (potted plants) of plus plants loaded with pathogens may be eliminated using the shoot tip grafting technique, tissue culture, or thermotherapy. Following the elimination of pathogens, the genuine planting materials of true-to-type will be maintained in Block 1 as given in step III.

6.3 Step III: Maintenance and multiplication of elite plants

The selected candidate plants consisting of both horticultural traits and pathogen free will be following the following SOPs in different blocks.

Block 1: Development and maintenance of Foundation Block

Provisional nucleus foundation plant stock**:** If the planting materials are tested free of pathogens, such materials are considered a clean pathogen tested certified nucleus material and must be maintained in the provisional nucleus foundation plant stock. The planting materials in this stock will be confirmed for genetic purity and genotype or cultivar identification through molecular tools. The planting materials will be discarded if they are not true to type. However, if found genetically true-to-type, the materials will be sent to Block 1 (Certified Clean Foundation Plant Stock).

Certified Clean Foundation Block: The elite stocks of true-to-type obtained from the provisional nucleus foundation plant stock are considered Certified Clean Foundation Plant Stock. The first stage of nucleus stock materials is the Certified Clean Foundation Plant (Block 1). A small number of plants, often 5–10 plants per variety, may be maintained in this block. The scion wood or bud stick of the identified or selected candidate plants (A^+ & B^+) may be collected during July for wedge grafting and February for T-budding on any of the recommended rootstocks. A scion stick or budwood must contain plump, unsprouted buds. A defoliation must be carried out 7 days prior to collection and propagation. If the budwood is to be transported, it may be kept in a moist medium to avoid dehydration. The recommended rootstocks in Khasi mandarin are roughlemon, rangpur lime, and Volkameriana. The ideal time for budding is February, and grafting is June–July.

All the nucleus planting materials under this block should be maintained in an insect-free environment to avoid any contamination of pathogens and insects under high biosecurity. Plants in Block 1 may be maintained at 2 m x 2 m on an open field (only if the viruses and virus-like pathogens are not prevalent in that zone) or contained in controlled setups (greenhouses, screen houses, or tissue culture) with high biosecurity. However, it is always better to be maintained under fully protected conditions. All the nucleus planting materials under the 'Certified Clean Foundation Plant Stocks' should be monitored and tested for important clonally transmissible and vector-transmitted pathogens at regular intervals. The plant stocks in this block need to be maintained at the institution level, such as ICARs, SAUs, and the State Department of Horticulture and Agriculture.

Block 2: Development and maintenance of Certified Clean Budwood Increase Block

A scion or budwood collected from the Certified Clean Foundation Plant Stock will be grown and maintained in the Certified Clean Budwood Increase Block. This block is also called the Certified Clean Registered Nursery Plant Stock, and it will be a source of bud wood and scion sticks for further multiplication of planting materials. This block can be established in the fields to scale up the propagating materials and distribution to the farmers. However, if facilities are available, growing in a container under fully controlled environmental conditions is the best option. The number of plants maintained under this block may vary from 50 onwards, depending on the requirements and availability of the facility.

Block 3: Rootstock nursery block

In this block, rootstocks are raised through the seeds of recommended rootstocks. The size and number of seedlings raised depend on the requirements. The recommended practices for seed extraction, sowing, and seedling management must be carefully followed. The raising of seedlings is preferably done under controlled conditions to avoid any vector-transmissible diseases.

Block 4: Multiplication block

In this block, all the propagation operations are carried out, including grafting or budding of a budwood on a recommended rootstock, *i.e.,* rough lemon, rangpur lime, and Volkameriana. A propagation technique such as T-budding and wedge grafting can be adopted during February and July, respectively. The care and maintenance of the grafted plants must be carefully observed. The multiplication of plant materials can be carried out at a government farm or by stakeholders such as accredited private nurseries and other agencies involved in the propagation and distribution of planting material The registration and certification of these planting materials can be done by the State Department of Horticulture and Agriculture in collaboration with institutions like ICARs.

Block 5: Distribution Block

The certified CQPM multiplied either by the government or recognized private nurseries may be distributed through a recognized agency. All the certified clean plant lots produced through certified CQPM should be labeled "Certified Planting Materials" with a unique bar code or QR code containing information about the mother stock, quality testing attributes, and genetic purity. This certification can be done by a certified or recognized nursery following guidelines issued by a certified agency.

6.4. Traceability records

The accredited nursery should record and maintain the pedigree of the chain-of-custody of certified CQPM at every stage, including the certified mother stocks, maintenance, multiplication, and up to the point of distribution or sale, growers.

7. Monitoring and registration

- The healthy and clean materials in foundation stocks are very critical to the success of the production of certified CQPM. Hence, the Certified Clean Foundation Plant Stocks and Budwood Increase and Multiplication Stock are less likely to be vulnerable to regulated pathogens.
- Under the "Certified Clean Foundation Plant Stocks," all nucleus planting materials must be maintained free of insects and subjected to periodic testing for serious vector- and clonally-transmitted infections.
- Registration and certification of the Clean Foundation Block (Block 1) and Multiplication Stock (Block 2) should be mandatory to ensure pathogen-free planting materials.

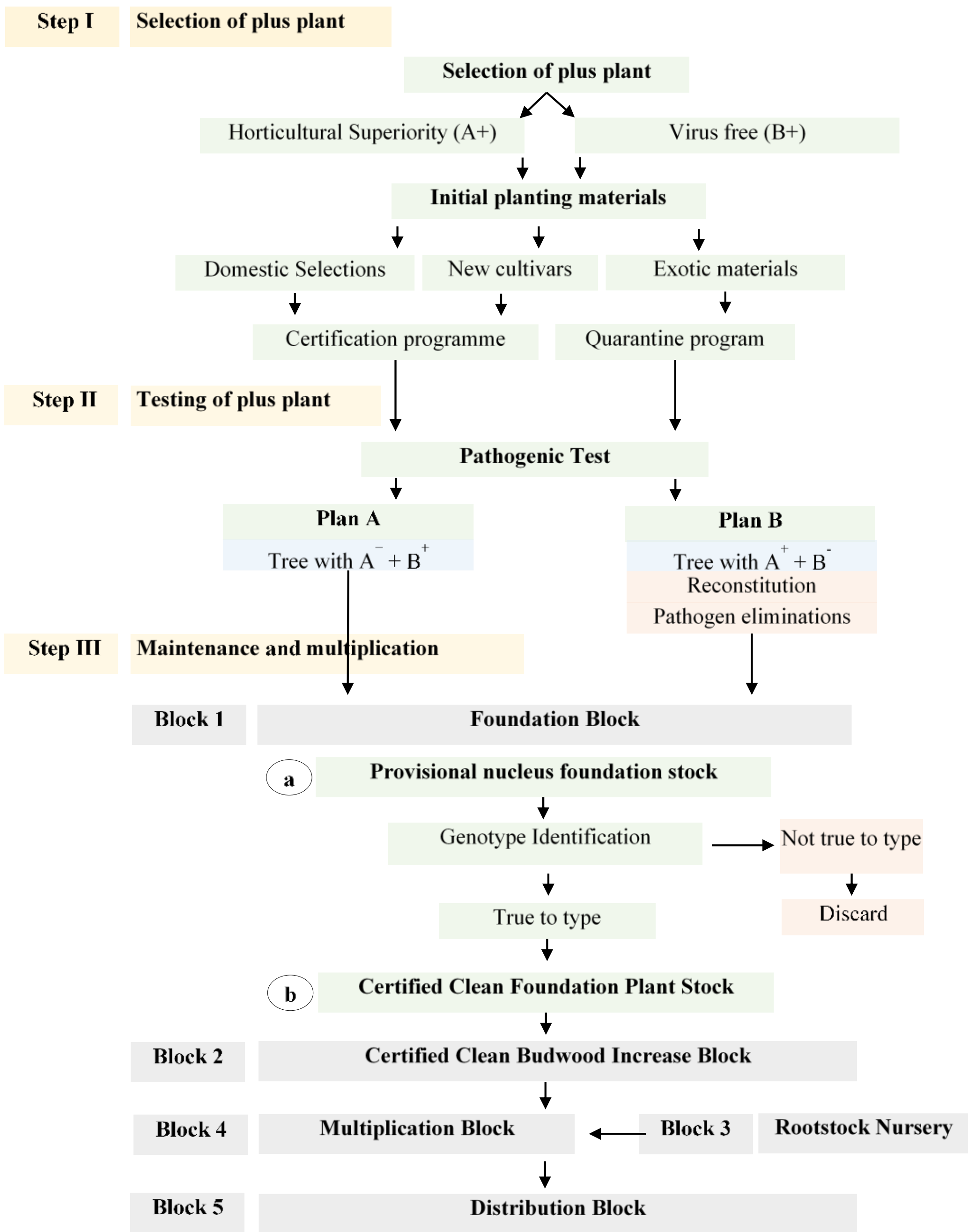

Figure 2. Standard operations procedures for Citrus Certification Programme

- An intensive strategy must be employed to prevent the entrance of viral infections through vectors and maintain a clean, infection-free environment for the foundation blocks and Budwood increase/multiplication stock.
- Although the initial rigorous testing of all the plants in the Clean Foundation Block was done, annual indexing is required in cases where the area has a high prevalence of vector-transmitted viruses and biennial in cases where there are no vector-transmitted viruses in the area.
- In cases of plants maintained in multiplication blocks
- (Block 2), indexing through a random stratified sampling of a sample size (0.01%) in a population size of 100 to 10000 plants (NAAS, 2022)
- The time of indexing for viruses is very critical, which may vary with season. For instance, there was a drastic decline in the copy number of viruses during December under tropical conditions (Sidharthan, 2022).
- If tested positive at any time, the diseased plants should be removed immediately to avoid the spread of pathogens.

- All certified clean plant lots generated have to be tagged with a distinct barcode or QR code that comprises details regarding the mother stock, quality testing characteristics, and genetic authenticity.

8. Potential challenges and limitations

There can be several challenges and limitations involved in the production of CQPM for citrus fruit trees.

- **Disease and Pest Management**: Citrus trees are susceptible to various diseases (e.g., citrus canker, greening disease) and pests (e.g., citrus leafminer, aphids). Nurseries must implement strict protocols to prevent the spread of diseases and pests, which can significantly impact the quality and health of planting materials.
- **Genetic purity and quality assurance**: Citrus trees exhibit genetic variability, leading to variations in traits such as fruit quality, yield, and disease resistance. Maintaining genetic purity and selecting desirable traits for propagation can be challenging due to factors such as variability in mother plants, rootstock block, environmental fluctuations, and handling during propagation and transportation.
- **Propagation Methods**: This process requires skilled labour and specific environmental conditions to ensure successful grafting and establishment.
- **Environmental Sensitivity**: Citrus young seedlings/ grafted plants are sensitive to environmental conditions such as temperature, humidity, and soil quality. Ensuring optimal conditions during propagation and growth is crucial to producing healthy and vigorous planting materials.
- **Seasonal Constraints**: The production of citrus planting materials may be limited by seasonal factors, particularly in regions with distinct growing seasons or climates that are less favorable for citrus cultivation.
- **Market Demand and Economics**: Meeting market demand for specific citrus varieties and rootstocks requires careful planning. Economic factors such as production costs, market prices, etc. also influence nursery operations.

Despite these limitations, advancements in research and technology continue to improve the efficiency and sustainability of citrus planting material production.

9. Future research directions

The future of certifying quality planting materials involves several promising research directions aimed at improving consistency, reliability, and sustainability in agriculture:

- **Genetic Characterization and Traceability**: Advances in genetic markers and sequencing technologies can enable more precise identification and characterization of plant varieties. This facilitates accurate tracing of planting materials from origin to destination, ensuring genetic purity and authenticity.
- **Disease Resistance and Tolerance**: Research focusing on breeding or selecting planting materials with enhanced resistance or tolerance to pests, diseases, and environmental stresses (e.g., drought, salinity) is crucial. This helps reduce reliance on chemical inputs and enhances long-term sustainability.
- **Quality Standards and Metrics**: Developing standardized metrics and criteria for assessing planting material quality can improve consistency and facilitate comparisons across different suppliers and regions. This includes parameters such as vigor, uniformity, root development, and absence of pathogens.
- **Technological Innovations**: Integration of advanced technologies such as remote sensing, drones, and artificial intelligence (AI) for monitoring and managing plant health can optimize production processes and early detection of issues affecting planting materials.
- **Climate Adaptation**: With changing climate conditions, there is a need to develop planting materials that are resilient and adaptable to varying climatic zones and extreme weather events. Research in this area can lead to the development of climate-smart varieties.
- **Biosecurity and Quarantine Measures**: Enhancing biosecurity measures and quarantine protocols to prevent the introduction and spread of pests and diseases through planting materials is critical. Research can focus on improving detection methods and developing resistant varieties.
- **Sustainable Production Practices**: Investigating sustainable production practices for nursery operations, such as low-cost media, efficient water and nutrient management, use of renewable resources, and reduction of greenhouse gas emissions, contributes to environmental stewardship.
- **Consumer Preferences and Market Trends**: Understanding consumer preferences and market demands for specific traits (e.g., flavor, shelf-life, organic certification) in planting materials can guide breeding and selection efforts to meet market needs effectively.
- **Collaborative Research and Knowledge Sharing**: Promoting collaboration among researchers, breeders, growers, and regulatory agencies facilitates knowledge exchange and accelerates innovation in certifying and producing quality planting materials.

- **Economic Viability**: Research focusing on cost-effective production methods, and economic analyses helps ensure that certified planting materials are competitive and accessible to a wide range of growers.

The focus in these research directions, stakeholders can contribute to the development of robust certification systems and sustainable practices that support the citriculture industry.

10. Conclusion

Citrus fruits are the most commercially important fruit crops, and the demand for citrus fruits continues to rise. The most effective approach to meeting demand and growth is through easy access to certified citrus quality planting material (CQPM). However, presently, the availability of certified CQPM is limited. Furthermore, the citrus propagules are prone to several pathogens and are both graft- and vector-transmissible. Therefore, there is a requirement for an effective regulatory framework through accreditation and certification of mother stocks in the production of a certified CQPM. This will contribute to the sustainable performance of citrus orchards through the availability of pathogen-free and horticulturally superior plants of the highest quality. There are several challenges and limitations involved in the production of CQPM for citrus fruit trees, such as disease and pest management, genetic purity and quality assurance, skilled labour, specific environmental conditions, and market constraints. However, technological advancements, collaborations among researchers, etc. can be effective strategies for sustainable and economic production of certified-quality planting materials.

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