

Containerised nursery systems: An efficient

technique for the production of quality planting material in Khasi Mandarin

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Khasi Mandarin is a valuable commercial fruit of the NEH region, India. The fruits are of premium quality and have a distinctive taste and flavour due to the ideal agro-ecological conditions in the area. The demand for citrus fruits is rising, offering great potential for future expansion. However, the productivity of this crop is only half the national average (10.36 t/ha). The lack of certified citrus quality planting materials (CQPM) can be a major constraint hindering the expansion of area and the commercial production of Khasi Mandarin. Furthermore, poor-quality propagules are vulnerable to many pathogens, which can be transmitted both through grafting and vectors. It is crucial to adopt containerised nursery systems (CNS), efficient methods of propagation and nursery management, pathogen detection and elimination, and an effective regulatory framework through the accreditation and certification of stocks. Furthermore, the shortage of CQPM can be addressed by doubling the windows of operations, i.e. during January-February through budding and July-August via wedge grafting. Therefore, strictly adopting a protocol and efficiency techniques may assist in producing the highest horticulturally superior and pathogen-free plants as the most strategic means for the success of the citriculture industry in the region.

Keywords: Certified citrus quality planting materials, Pathogen-free plants, Regulatory framework

KHASI mandarin (*Citrus reticulata* Blanco) is an ecotype of mandarin fruit and represents the most significant commercial crop in the NEH region, India. The fruits of this crop is of premium quality due to suitable agro-ecological conditions, and thus, a Geographical Indication (GI) tag was granted in 2014. The fruits of Khasi Mandarin have been exported from the region to the Middle East and neighbouring countries since 2022. However, the productivity of this fruit crop is relatively low (5.91 t/ha) vis-a-vis the average productivity in the country (10.36 t/ha). Unproductive citrus orchards can have an array of underlying causes, especially systemic viruses, virus-like diseases, and systemic bacteria. 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. Quality planting materials are a vital part of any citrus production system to minimise the spread of threatening pathogens, enhance adaptability, and ensure sustainability with better crop performance. Therefore, the success of the citrus sector in the region critically depends on implementing a citrus certification

programme for the production of certified citrus quality planting materials (CQPM). Certified CQPM is a healthy planting material with the highest yield and quality attributes for distribution to growers. Certified CQPM can only be obtained by establishing a strong certification system at the regional and/or national level through the adoption of the following main components of the certification programme.

Component 1: Identification and production of plus plants

The certification of CQPM begins with the identification and production of plus plants, which involve the following two steps:

Step I-Selection of a plus plant: The first step is selecting a candidate plant (plus plant), which can be achieved through the following three practices: domestic selection, new varieties (or clones), and exotic elite materials. Khasi mandarins are native to the region and available locally. Therefore, candidate plants of Khasi mandarin can be selected by a survey of the elite genotypes, followed by intensive testing using robust diagnostic criteria in the certification programme. In the event of introduction of any exotic citrus species, whether as scions or rootstocks, the material must

undergo a quarantine programme. The detection of any potential infection with an identified or unidentified virus or virus-like pathogens can be performed using high-throughput sequencing and then confirmed through standard molecular and serological tests.

There are two main criteria for selecting candidate plants. The primary criterion is selection based on horticultural excellence (A+), such as high yield and good quality. The second essential step is the pathogenicity test or viral indexing to obtain pathogen-free (B+) plants.

Step II-Testing of a plus plant: The selected candidate plants may adopt one of the two approaches, either Plan A or Plan B, depending on the presence or absence of viruses. In plan A (candidate plants A+ and B+), the candidate plants with horticultural superiority traits (A+) and freedom from diseases (B+) must be assigned a unique identification number. Such plants shall be relocated and maintained in the foundation block. Plan B (candidate plants A+ and B-) is implemented when the candidate plants have all the horticultural superiority traits (A+) but are found (+ve) with virus pathogens (B-). Such candidate plants (A+ and B-) must be reconstituted in a pot culture under controlled laboratory conditions. The representative plus plants (potted plants) should be carefully subjected to any of the following virus (pathogen) elimination techniques to produce candidate plants (A+ and B+). The following are the techniques for pathogen elimination:

- **Heat treatment (thermotherapy):** When using heat chambers for potted plants or tissues, thermotherapy protocols should be carefully followed to partly or entirely inactivate many viruses with minimal or no damage to the host tissues, for obtaining virus-free shoot tips.
- **Chemical treatment:** The use of therapeutic chemicals like virazole and vidarabine (antimetabolites) to eliminate viruses from infected plants in culture media.
- **Meristem tip culture:** The culture of a small, incision, pathogen-free apical dome under aseptic conditions can produce 'clean nucleus stock.'
- **Micrografting:** Grafting meristematic tissues onto a virus-free rootstock (seedling) *in vitro* can eradicate the virus.

Other *in vitro* methods for regenerating plant resistance to disease include somatic cell hybridisation, gene transformation, and somaclonal variation. The five key graft-transmissible infections that the candidate plants must be screened for are: i) Citrus greening disease (Huanglongbin), ii) Citrus tristeza virus (CTV), iii) Indian Citrus ringspot virus (ICRSV), iv) Citrus mosaic badnavirus (CMBV), and v) citrus exocortis viroid (CEVd). The candidate plants (A+ and B+) will be maintained in the foundation block following standard procedures after the elimination of pathogens.

Component 2: Development and maintenance of elite plants

Block 1-Provisional nucleus foundation plant stock: Planting materials identified as pathogen-free are regarded as clean, pathogen-tested, certified nucleus

material and must be maintained in the provisional nucleus foundation plant stock (Block 1). In this section, stocks will be verified for genetic purity and identification of genotype or cultivar using molecular tools. Non-true-to-type planting materials will be discarded, while genetically true-to-type ones will be sent to the Certified Clean Foundation Plant Stock (Block 2).

Block 2-Certified clean foundation plant stock:

The elite stocks of true-to-type obtained from Block 1 are classified as certified clean foundation plant stock (Block 2). This block maintains a small number of plants, usually 5 to 10 per variety. The nucleus planting materials under this block are maintained at a 2 m x 2 m spacing in an insect-free environment to prevent contamination by pathogens and insects, adhering to strict biosecurity protocols. Key diseases transmitted by vectors and clonally transmissible should be periodically tested for and tracked in the block. The stock of plants in this block should be maintained at the institutional level.

Block 3-Certified clean budwood increase stock:

A scion or budwood obtained from Block 2 shall be cultivated and maintained in the certified clean budwood increase block, also referred to as the certified clean registered nursery plant stock. This block serves as a source of bud wood and scion sticks for further propagation. The establishment of this block in the field facilitates the scaling up of the multiplication and distribution of CQPM to farmers. In such cases, the fields should be located in an isolated area where the risk of infection is minimal or nonexistent. If facilities are available, growing in a container under fully controlled environmental conditions is the ideal choice. The number of plants maintained within this block can be about 50 or more, depending on requirements and facility availability.

Component 3: Multiplication and distribution of elite planting materials

Block 4-Rootstock nursery block: In this block, seedlings of the recommended rootstocks are grown in a Containerised Nursery System (CNS), preferably under controlled conditions to avoid any vector-transmissible diseases. The concept of CNS is very significant, especially in regions with a high occurrence of soil-borne pathogens. Typically, once soil-borne pathogens like *Phytophthora* are present in field nurseries, eradicating them proves to be very challenging. To address this issue, the concept of CNS was introduced. Facilities required for CNS include a 50% shade net house for congenial growing conditions, UV-stabilised transparent polythene for solarisation, sterilised plastic trays for sowing seeds, UV-stabilised black polybags (100 µ) for transplanting seedlings, fumigation facilities for items such as potting materials, and a separate set of nursery equipment.

- **Media preparation:** The media mixture of virgin fertile soil, FYM, and sand (1:1:1 v/v) can be sterilised by steam. In the primary nursery, the sterile mixture is utilised to fill the plastic trays for

sowing seeds. The same sterile mixtures are utilised in the secondary nursery.

- **Primary Nursery:** Rootstock seedlings are grown in a sterilised plastic tray (60 cm x 40 cm x 12 cm). Sterilised mixtures are filled in a tray and set on a solid platform at least 1.5–2.0 feet above the ground to reduce soil-borne contamination. The nursery floor is layered with 2.0–4.0 inches of stone dust and boulders to prevent soil-borne pathogens from splashing, and it is regularly treated with a mixture of copper and lime through spraying or dusting. Fully ripened fruits of the recommended rootstocks (Rough lemon and Rangpur lime) are harvested from the rootstock mother block. The extracted seeds are carefully washed and surface sterilised. A prophylactic treatment of seeds was applied with Ridomil MZ 72 and Bavistin before sowing in plastic trays.

The treated seeds are sown at a depth of 1.0–1.5 cm, spaced 2.5–3.0 cm apart in a row and 5.0–7.0 cm between rows, under a 50% shade net house. Seed sown during November–December takes about 35–60 days for germination after sowing, with 85–90% seed germination in Rough lemon and Rangpur lime.

- **Secondary nursery:** Seedlings at 4–6 leaf stage and attaining the desired height are ready for transplanting to a secondary nursery. Only nucellar seedlings (based on visual observations), i.e. true-to-type, are identified as rootstock seedlings. The identified seedlings are carefully uprooted to avoid root damage during transplanting into polybags, followed by gentle irrigation. The stunted, thin, hook-necked, bent or twisted seedlings can be discarded. Monitoring is done regularly to avoid contamination from *Phytophthora* and other soil-borne diseases.

Block 5-Multiplication of elite planting materials:

In this block, budding and grafting are performed on a recommended rootstock.

- **Collection of scions/budwood:** The scion sticks and/or budwood can be collected from block 3. The buds originate from a shoot of an adequately mature current-year and non-bearing growth. The selected shoots contained a longitudinal white streak on the bark with swollen buds and were non-sprouting but ready to burst following grafting.
- **Multiplication techniques:** Khasi mandarin is generally propagated once a year, during January–February, by T-budding. Considering the high demand for quality planting materials, there is a need for a second window of propagation operations. The ICAR RC NEH Region, Umiam, has developed a wedge grafting for propagation during July–August. Furthermore, this technique can be carried out in six month old rootstocks, vis-a-vis one year old rootstocks in T-budding.

Table 1. Protocol for vegetative propagation of Khasimandarin

Particulars	Wedge grafting	T-budding
Type of rootstock	Vigorous	Vigorous
Sowing of rootstock seeds in the primary nursery	Plastic tray: 60 cm x 40 cm x 12 cm)	Plastic tray: 60 cm x 40 cm x 12 cm)
Timing of seed sowing	November to December	November to December
Transplantation procedures in the secondary nursery.	Polythene bag (LDPE)	Polythene bag (LDPE)
Size of a polyethene bag	30 cm x 15 cm	30 cm x 15 cm
Rootstock age	6–7 months	11–12 months
Rootstock diameter	4–6 mm	6.0 – 10 mm
Age of the scion shoot	3–4 months	5 – 6 months
Length of the scion	8–10 cm	---
Length of the vertical cut on the rootstock.	1.5–2.0 cm	---
Length of the slanting cut on both sides at the lower end of the scion.	1.5–2.0 cm	---
Length of the bud wood	---	2.0–2.5 cm
Length of the cut on the rootstock for inserting the bud at the T-point.	---	2.0–2.5 cm
Grafting or budding height	12–15 cm above the soil surface	20–22 cm above the soil surface
Timing of grafting/budding	July–August	January–February

- **Care and maintenance of budded/grafted plants:** Frequent light irrigation with automatic sprinklers and misters can be applied to propagated plants. In addition to the regular recommended fertiliser mixture, spraying of urea twice a month can improve the growth of nursery plants. Regularly remove any side suckers below the bud union. The multiplication of CQPM can be carried out at a government farm or through intermediaries such as registered private nurseries and other propagation and distribution agencies.

Block 6-Distribution block: The certified CQPM, whether multiplied by the government or recognised private nurseries, can be distributed through an authorised agency. The label "Certified Planting Materials" must be applied to all certified cleaned plant batches produced through CQPM. Each label, as "Certified Planting Materials", should include a unique barcode or QR code that provides details about the mother stock, genetic purity, and quality testing attributes. This certification can be carried out by a certified or recognised nursery following guidelines issued by a certified agency.

Traceability records

The accredited nursery should record and maintain the pedigree of the chain of custody for certified CQPM at every stage. This includes the certified mother stocks, maintenance, multiplication, and continuing until distribution or sale to growers.

Monitoring and registration

The "Certified Clean Foundation Plant Stocks" must be subjected to periodic testing for serious vector- and clonally-transmitted infections. Registration and certification of the Clean Foundation Block and Multiplication Stock should be compulsory to guarantee pathogen-free planting materials. An intensive approach is necessary to prevent viral infections via vectors and to maintain a clean, infection-free environment for the foundation blocks and budwood increase/multiplication stock. Any plants that test positive should be eliminated to stop the spread of infections.

Plant protection measures

The most prevalent disease in citrus nurseries is damping off. The pathogens, especially *Phytophthora*, pose a threat and can occur at any stage of plant growth in nurseries through contaminated water, soil, nursery staff, and tools. Regular monitoring and control

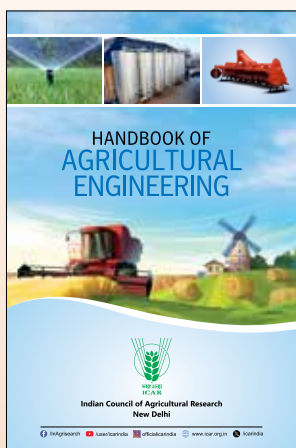
strategies include excluding, removing the source of infection, avoiding conducive conditions for disease development, and preventing entry. The most common insect pests in citrus nurseries are leaf miner (regular spray of Dimethoate @1 ml/L), leaf-eating caterpillar (Imidacloprid @0.3 ml/L), aphids (Quinalphos @1 ml/L), and mites (3 g/L wettable sulphur or 1.5 ml Dicofol). The alternate spray of neem oil (1%) with any insecticides demonstrated greater effectiveness.

SUMMARY

It is essential to establish standard procedures for producing quality planting materials with the highest horticultural quality and free from pathogens. Furthermore, propagation and nursery efficiency techniques, including two windows of operation each year, i.e. January-February by budding and July-August by wedge grafting using containerised nursery systems, will help to increase the production of CQPM. The traceability records and monitoring of the accredited nursery need to be updated to maintain the reliability and genuineness of the nursery.

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