

# Standard Operating Procedures

*for*

## Citrus Nursery in Bhutan

2021



**Department of Agriculture**  
**Ministry of Agriculture and Forests**  
**Royal Government of Bhutan**  
**Bhutan**



## EXECUTIVE ORDER

The Standard Operating Procedures (SOP) for Citrus Nursery in Bhutan 2021 is being issued to help citrus nursery operators ensure compliance with the requirements prescribed under the “**Seed Rules and Regulations of Bhutan 2018**” and more specifically with the “**Minimum Seed Standards of Bhutan 2019**” with particular reference to citrus planting materials production.

The “**Seed Rules and Regulations of Bhutan 2018**” empowers the Bhutan Agriculture Food Regulatory Authority (BAFRA) with the responsibility to open and maintain a central registry of all the seed growers in the country as prescribed under section 16, chapter II and authority to certify seeds under section 34 and 40 of Chapter IV. However, seed certification will require any seeds, either produced within country or imported, to be able to meet the minimum standards.

Towards meeting this minimum standard for seeds, the ministry has further issued “**Minimum Seed Standards of Bhutan, 2019**”, wherein the standard for citrus planting material has prescribed the Specific Minimum Standards requirement (site, structure, planting material). Citrus nurseries failing to meet these prescribed requirements shall not be eligible for registration as certified citrus nursery, and the planting materials produced shall be not be allowed to sell.

Therefore, all the citrus nurseries must adhere to this SOP to ensure that all requirements for certifications are fulfilled. Further, this will enable the overall development and sustainability of the citrus industry in Bhutan by putting in place the proper procedures and practices right from the production of the planting materials to crop management in the field.

Issued for strict compliance on 8 July 2021.



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## Section I: General Overview

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### 1. Introduction

Citrus is one of the main sources of economy and livelihood for the farmers in Bhutan. More than 22,158 households grow citrus in an area about 17,040 acres with annual average production of 31,020MT. Citrus production potential is projected to increase with climate change in the following years and will remain as one of the key export crops. Introduction of new public accessions both through imports and in-country selections has increased scope to expand citrus into diverse agro-ecological zones.

Of late, area under citrus cultivation is declining due to the climate change impacts, inadequate management practices and limited supply of high-health status planting materials to replace old, diseased and declining orchards. Current growing areas are facing rapid spread and invasion from pests and diseases, drought and water stresses, that heavily affect the overall production.

Most of the citrus orchards in the past were planted using planting materials from non-certified nurseries including private nurseries, backyards, and across the border areas. Besides limited awareness among the growers, availability of certified seedlings itself was limited, while many at the same time face the affordability and accessibility issues.

### 2. Rationale

Movement of planting materials from non-certified nurseries and free exchange among growers have been indisputably the main cause of devastating diseases like Huanglongbing (ex-citrus greening disease) and Tristeza Virus; that are transmitted mainly through the planting materials. These diseases along with other factors have a huge potential to rapidly wipe out the citrus industry. Huanglongbing (HLB) disease is easily spread in areas where the psyllid vector-*Diaphorina citri* is present. Free movement of planting materials without following any standard procedures pose high risk of carrying vectors from infested areas to others.

Most importantly, the planting materials supplied from the nurseries need to be completely free from pests and diseases followed by an appropriate field management practice. It must be of high-health status to completely avoid spread of disease to new areas and also to minimize the risk of infections from the surrounding environment once planted in the field.

The foundation of citrus industry development lies in providing and making the healthy planting materials available for new plantation and rehabilitation of declining orchards in the country.





Unless the orchards are planted with disease-free nursery stock, none of the potentially improved practices can be fully realized. The production of planting materials in nurseries with the minimum standards will ensure that the growers are planting disease free and healthy planting materials with assured horticultural characteristics.

In order to maintain minimum standards, it is essential to have in place a Standard Operating Procedure (SOP). Critical areas for SOP include: i) Nursery Location, ii) Nursery Structures, iii) Production Management, and iv) Transportation Systems. Further, Citrus Nursery SOP is required to meet and maintain the “Minimum Standards of Planting Materials” prescribed under the **“Seed Rules and Regulations of Bhutan 2018”**.

### 3. Objective

The overall objective of this SOP is to improve and maintain the standard and technical competency of the citrus nurseries for production of reliable and high-health status planting materials. It is also intended to facilitate overall compliance with requirements and restrictions prescribed under **“Seed Rules and Regulations of Bhutan 2018”**, and in particular to meet the minimum standards prescribed for the production and supply of citrus planting materials under **“Minimum Seed Standards of Bhutan 2019”**. Additional requirements are also prescribed herein to protect citrus industry in Bhutan from devastating diseases like HLB, and other economically important pests and diseases.

### 4. Legality of this Standard Operating Procedure (SOP)

**The section 16 of Seed Rules and Regulations of Bhutan 2018** mandates Bhutan Agriculture Food Regulatory Authority (BAFRA) to open and maintain a central registry of all the seed growers in the country. The section 23(2), (9) and section 29(a) of the Seed Rules also empowers BAFRA to regulate the import and export of seeds and authorizes BAFRA to certify and grant the seed certificate as per section 34 and 40 of the Rules. The certification shall be carried out in compliance with the **Minimum Seed Standards of Bhutan 2019** as required under section 32 and 33 of the Seed Rules and Regulations, 2018. Further, the Minimum Seed Standards of Bhutan, 2019 prescribes the standards for Citrus planting materials with the Specific Minimum Standards requirement for the site, structure and planting material. Therefore, this SoP is being formulated to guide the citrus nurseries to implement and comply to the requirements prescribed in the minimum seed standards of Bhutan, 2019.

### 5. Authority of this document

The SOP is formulated to guide implementation of **Seed Rules and Regulations of Bhutan 2018**, with particular reference to maintain ‘*minimum standards*’ for the production of citrus planting materials prescribed under **“Minimum Seed Standards of Bhutan 2019”**. This SOP may be



revised from time to time and will be available on the department's website: [www.doa.gov.bt](http://www.doa.gov.bt). The Department of Agriculture is the only competent authority to make any revision required based on general and expert's feedback and changing circumstances that demand alteration or improvements, if any.





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## Section II: Site Selection and Facilities

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### 1. Site requirements

The citrus nursery should be located in the areas free of psyllid to avoid the risk of HLB disease in the nursery. The site should have adequate area to establish all required facilities and have access to road. The specific requirements are:

- i. The nursery site should be above 1200 m above sea level.
- ii. The nursery site should be at least 1 km away from the citrus orchard (aerial distance).
- iii. The nursery site must be devoid of field with standing citrus trees and any other hosts of HLB and its vector.
- iv. The site should have an access to a clean source of water supply.
- v. The nursery site should be well drained and with no runoff from surrounding areas.
- vi. The site should have adequate sunshine hours.

### 2. Nursery Structures

The facility for propagation and growing of citrus nursery stock must be totally enclosed, with all doors, temperature regulation systems, blowers, vent closure mechanisms, sides, tops, joints and other parts maintained and operated to exclude insects. The specific structural requirements include the following:

#### **2.1. Exterior walls and top**

Any combination of solid surfaces and screening may be used, as long as the structure is completely insect proof.

#### **2.2. Doors and doorways**

- i. All structures should have double door entry system (1<sup>st</sup> door followed by the 2<sup>nd</sup> door before the nursery interior).
- ii. Any combination of solid surfaces and screening may be used for double door entry system, provided that structure is insect proof.
- iii. The double door should be developed adjoining the main structure on the outer side of the main structures.
- iv. The second door entry should not be straight through the first door entry.
- v. All doorways shall have a positive pressure air curtain, positive pressure double doors, or other mechanisms sufficient to prevent the entry of any insects; both during opening and closing of the doors.
- vi. All doors shall fit against the floor and door frame without gaps so that no insect can enter.





- vii. A footbath/dip structure has to be developed in between the first and second door entry system.

### **2.3. Screen mesh size**

- i. The mesh size for any screening materials used in walls, doors, vent covers, or other parts of a structure should be less than 266 x 818 $\mu$ m or 530x530 $\mu$ m designed to exclude psyllids, melon aphid and other aphids, leaf miners or other similar pests.
- ii. The metal wire mesh if used in place of nylon should be completely stainless and durable.
- iii. Non-permeable covering could be used to cover the entire structure in place of screen mesh.

### **2.4. Other openings**

Except for doors, all exterior openings for cooling pads, fans, vents or other parts of a structure to be covered with screen mesh to exclude all insects indicated in 2.3 (i).

## **3. Sanitation Requirements**

A precautionary notice must be placed in front of every individual propagation house to alert the worker(s) and visitor(s) for adherence to sanitation requirements and for strict compliance to bio-security measures. Sanitation procedures must be clearly written on the precautionary notice.

All equipment, plant material, supplies and personnel that enter or remain in a propagation facility shall subject to sanitation requirements.

### **3.1. Personnel**

- i. Nursery employees who work with citrus produced outside of the approved structure shall not return to work within the approved structure until the following day.
- ii. Prior to entering the nursery everyone must decontaminate with an approved decontamination product and wear a clean garment that should be provided by the nursery. If gloves are used, they must be a disposable glove or decontaminated each day and kept on the site itself.
- iii. All persons entering an approved structure or soil/media storage area shall walk through a sanitizing footbath containing a decontaminant that is approved by the National Plant Protection Centre (NPPC), such as copper sulfate products or chlorine bleach.
- iv. Designate nurseryman with skills and experiences to operate and function the specific block.
- v. The personnel working in the nursery must adhere to Occupational Health, Safety and Welfare rules and regulations.

### **3.2. Equipment**

- i. All equipment entering or leaving the nursery must be clean of all plant materials and soil, and must be disinfected.



- ii. Propagation tools and implements should be disinfected between different groups/lots of propagations during nursery plant propagation and between different plants while collecting budwood(s) from mother trees. Following procedures must be strictly followed;
  - a. A solution of 20% household bleach (sodium hypochlorite) or 75% Ethanol shall be used to disinfect tools.
  - b. Disinfection solution shall be prepared fresh each day.
  - c. If bleach solution is used, dip clippers, knives or pruning tools in the solution not more than few seconds.
  - d. Clean and dry the tools and equipment after completing the propagation works.
  - e. All equipment are recommended to be kept on site.

### **3.3. Materials**

All planting materials shall be raised in container on raised benches to avoid direct contact with soil by using the recommended media mentioned below;

#### **i. Potting media**

- a. All soil, peat, sawdust, mulch, manure or other plant-growing or potting media entering the approved site for the production of citrus nursery stock must be free of pests/diseases of citrus.
- b. All types of media must be completely sterilized before use.
- c. Media must be stored in an appropriate structure to avoid cross contamination.
- d. Media if recycled should be completely sterilized before use.

#### **ii. Container**

- a. All pots, cans, or other containers used to produce commercial citrus nursery stock must be stored in such a manner to prevent contact with the ground or contamination by flooding, rain-soil-splatter or ground water runoff.
- b. Growing containers including benches shall be cleaned between crops of citrus nursery stock.
- c. The size of the container should be minimum dimension of 25cm x9 cm (height x dia)

#### **iii. Propagules**

- a. Mother block for budwood collection shall be established strictly with the certified planting material supplied by National Citrus Repository Centre (NCR), Bhutan.
- b. Seeds must be collected from clean mother trees with known pedigree.
- c. Seeds must be treated as recommended by NPPC and must be stored between 4-5<sup>0</sup>C
- d. Seed must be germinated on a seed tray/seed bed using sterilized media.
- e. Non-certified citrus nursery stock shall not be grown in or introduced into the same greenhouse or structure with certified citrus nursery stock.





- f. Any citrus nursery stock or budwood source tree found infected or exposed to plant pest or disease infestation shall be immediately treated or removed from the certified citrus greenhouse if tested positive for HLB, CTV, and other diseases of quarantine concern.
- g. All planting materials must be raised in containers on raised benches to avoid contamination from soil and running water to prevent exposure to soil borne pathogens.

### **3.4. Site Quarantine Measures**

- i. Any visitors to the nursery must adhere to quarantine rules and must strictly follow site sanitation procedures.
- ii. Prevent encroachment at the nursery location from Rutaceous subfamilies- *Aurantioideae*, *Rutoideae*, and *Toddalioideae*, plants and noncertified citrus plant materials, to avoid the nursery site becoming infested with injurious pests or diseases of citrus.
- iii. Completely avoid bringing in any Rutaceous subfamilies, unless authorized and certified by BAFRA or other competent authority.
- iv. Follow established sanitation procedures to prevent nematode, psyllid, aphid or other common plant pest infestation of the nursery site.
- v. Nursery areas shall remain weed free.
- vi. Any citrus nursery stock or budwood source tree found infected or exposed to plant pest infestation shall be subject to immediate quarantine action and will not be eligible for certification until treated as prescribed by the NPPC and released from quarantine.
- vii. Nursery compound must be well fenced and protected.

### **3.5. Budwood Mother Block (BMB)**

- i. All the budwood multiplication tree must be grown exclusively under protected conditions as described under Section 2.3 (i)
- ii. Budwood trees shall be sourced only from NCR and raised as described under Section 3.3
- iii. The collection of budwoods shall be done by the designated professional nurseryman only.
- iv. All individual budwood trees must be properly tagged to identify their pedigree for traceability.
- v. At a minimum, the following measures shall be taken to prevent disease contamination from internal or external sources.
  - a. If any BMB trees tested positive for HLB, CTV, ECV, or a vector of such disease they must be removed and destroyed.
  - b. The BMB trees must undergo PCR testing after every two years.
  - c. Tools and equipment used to cut or prune BMB trees shall be used only in the BMB and shall be disinfected before use on any other trees.
  - d. Irrigation of the BMB shall be performed in such a manner as to minimize the risk of transmission of diseases through the irrigation system.





- e. The BMB shall follow the scheduled spray program as prescribed by NPPC.

## **4. Rootstock**

### **4.1. Rootstock Mother Block**

- i. A clean Rootstock Mother Block of released rootstock varieties must be established and maintained to source seeds for rootstocks multiplication.
- ii. All rootstock seeds must undergo a thermal (hot water) or other treatment recommended by the NPPC to reduce the risk of citrus pathogens, including *Phytophthora*.
- iii. The plant protection measures must be carried out on a regular basis as recommended by NPPC.

### **4.2. Rootstock Multiplication Block**

- i. A Rootstock Multiplication Block of released rootstock varieties must be established and maintained to source rootstock seeds.
- ii. All rootstock seeds must undergo a thermal (hot water) or other treatment recommended by the NPPC to reduce the risk of citrus pathogens, including *Phytophthora*.
- iii. The plant protection measures must be carried out on a regular basis as recommended by NPPC.

### **4.3. Rootstock Seedling**

- i. Rootstock(s) should be a released or notified rootstock variety collected from Rootstock Multiplication Block.
- ii. An appropriate seed treatment should be strictly followed as recommended by NPPC.
- iii. The seeds should be sown in raised seedbed, seed trays, or containers.
- iv. The seedlings should be transplanted into container/poly pots when the seedlings attain 3-4 leaves.
- v. The seedlings with deformed main stem and bent/crooked /J-root should be completely discarded.
- vi. The diameter of the rootstock should be minimum of 10mm (pencil dia.) at the time of grafting.

## **5. Propagation procedure**

- i. Whole citrus nursery stock must be produced and maintained under enclosed structures as described Section 2.3(i)
- ii. Citrus nursery stock must be propagated only by using budwoods/scions collected from Budwood Multiplication Block trees or being directly supplied by NCR.
- iii. The requirement of rootstocks seedlings and grafting standards shall be as described under Minimum Standards in Part III of this document.





- iv. Citrus nursery stock propagated for supply;
  - a. Shall be inspected prior to 12 months post-budding for citrus tristeza and HLB or any other pest of regulatory concern.
  - b. The nursery stock showing symptoms of citrus tristeza and HLB must further undergo a PCR tests for confirmation.
- v. All citrus nursery stock and propagative plants shall remain within the approved structure at all times, until delivered for sale or use.
- vi. Citrus nursery stock may be moved directly from one approved structure into another, provided the plants are prevented from exposure to psyllids/other pests of concern at all times during transit.
- vii. Certified citrus nursery stock should not be brought along with non-certified citrus nursery stock or any other citrus plant materials.
- viii. The nursery stock must be sprayed/treated with recommended pesticides at least 14 days prior to delivery.

## 6. Stock-taking and Labeling

- i. Proper documentation of all the nursery stocks must be maintained by the nursery and produced when asked by the competent authority.
- ii. All nursery stock must be properly labeled at all stages of propagation, indicating the details of rootstock and budwood sources.
- iii. Citrus plants for sale or distribution shall be labeled in order to maintain the traceability and identification/verification of the supply. The label must clearly indicate the rootstock and scion variety, and the source of supply.

## 7. Certification of Nursery and Planting Materials

- i. Any person or agency who propagates citrus nursery stock for the purpose of commercial must hold a valid trade license and must be registered with BAFRA to operate and run the citrus nursery as required under section 15 and 16 of the **Seed Rules and Regulation of Bhutan 2018**.
- ii. Any person who propagates citrus nursery stock for the purpose of sale must have undertaken the technical training on plant propagation and nursery management practices.
- iii. Any person who propagates citrus nursery stock for the purpose of commercial use must obtain Certificate for Sale from BAFRA as required under section 40 of the **Seed Rules and Regulation of Bhutan 2018**.
- iv. The valid nursery registration certificate shall be kept on display at the citrus nursery site, where it is readily visible to the public, and shall be produced when asked by any competent authority.





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## Section III: Minimum Standards

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### 1. Minimum Standards for Planting Materials

The 'Seedling' means-planting materials propagated directly from seeds and 'Grafts' means planting materials propagated through grafting and budding.

#### 1.1. Seedlings

- i. The seeds should be collected from released and notified mother trees free from diseases.
- ii. Seedling shall be genetically pure in absolute terms.
- iii. The planting material should be at least one-year old at the time of sale.
- iv. The planting material should be 0.8-1m tall and 6-10mm girth at the base.
- v. The seedling must be of single stem and have strong and well-developed root system.
- vi. The material should be certified free from viruses, canker, HLB and other known pests and diseases.
- vii. The seedlings shall be grown in container/poly pots.
- viii. The seedlings lot shall be free of deformed main stem and bend/crooked /J-roots.
- ix. The lot, seedling not conforming to specified size shall not exceed 5.0% (by number).

#### 1.2. Grafts

- i. The rootstock and scion wood should be sourced from released and notified varieties.
- ii. The BMB trees should be registered and tested at least once in two years and certified free from graft transmissible diseases.
- iii. Both rootstock and scion wood should be genetically pure in absolute term and free from graft transmissible diseases.
- iv. The grafts shall be produced from budding and grafting; and shall not be produced through cuttings or air-layering.
- v. The overall height of the plant should range from 40-60 cm at the time of sale.
- vi. The diameter of grafts at 10cm above the graft union should range from 1.0-1.25cm.
- vii. The grafts should be of single stem and have strong and well-established root system.
- viii. The graft should be free from rootstock sprouts/suckers.
- ix. The graft should be maintained completely free of flowers and fruits.
- x. The graft should be properly tagged and labeled with the name of variety (scion) and rootstock.
- xi. The material should be free from viruses, canker, HLB and other known pests and diseases at the time of sale.
- xii. In the planting material lot, materials not conforming to specified size shall not exceed 5.0% (by number).





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## Section IV: Preparation and Delivery of Planting Materials

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- i. The demand for planting materials should clearly specify the rootstock, commercial varieties, intended agro-ecological zone for plantation.
- ii. The planting materials intended for supply should be hardened for 15-30 days prior to supply.
- iii. The planting materials should be delivered with the poly pots/container intact.
- iv. The planting materials intended for supply should be treated with recommended plant protection products at least 15 days prior to supply.
- v. The planting materials lot must be properly tagged and labeled for identification and verification at the time of delivery.
- vi. The planting materials must be handled with care at the time of loading, transportation, unloading and transported with proper protective covering.
- vii. The consignment should be accompanied by invoices attached with movement permit issued by BAFRA.



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## Section V: Acknowledgement

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In the absence of comprehensive minimum citrus nursery standards for citrus nurseries in Bhutan, the Texas Citrus Nursery Stock Certification Manual and the rules prescribed by the Department of Plant Industries, Florida Department of Agriculture and Consumer Services are used with modification to suit our context. Further, reference was made from other relevant documents including Nursery Manual for Citrus and Mango, 2016, Published by Australian Centre for International Agriculture Research, Australia and Technical Guide for Citrus Nursery Management, 2015, Department of Agriculture, Bhutan.

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