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CITRUS NURSERY MANAGEMENT
A Technical Guide

RENEWABLE NATURAL RESOURCE RESEARCH AND DEVELOPMENT CENTER BAJO WANGDUEPHODRANG BHUTAN (2015)

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Kinley Dorji and Lakey

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Preface

Citrus is a very important export commodity for Bhutan, but fruit production has declined due to poor orchard management and the impact of significant pests and diseases. Major diseases of citrus include Huanglongbing (HLB) (also known as greening disease), powdery mildew and viral diseases such as strains of Citrus tristeza virus (CTV) and Citrus exocortis viroids, etc.

One of the approaches widely used in the world to combat citrus HLB and other diseases is the production of high quality disease-free seedlings in enclosed nursery structures such as insect proof green or net houses. Therefore, the Department of Agriculture, Ministry of Agriculture & Forests, has accorded high priority to the development of citrus nursery. Nursery production capacity at the National Seed Centre (NSC) has been enhanced substantially, both in terms of infrastructure and production technology. However, the NSC depends on the RDCs for budsticks. The Department of Agriculture has set up the National Citrus Repository at the Research & Development Center (RDSC) Tsirang. One of the objectives of the repository is to make available high-health status budwood from registered mother trees in the country in the coming years.

Since NSC Bhur is the only commercial citrus nursery in the country, this manual would help nurserymen, researchers and upcoming nursery operators in better understanding the institutional mandates of different agencies, viz. Research Development Centers (RDC), National Citrus Repository, National Seed Center (NSC), Bhutan Agriculture Food & Regulatory Authority (BAFRA) and the National Citrus Program and their association vis-a-vis the process and protocol of a certification scheme.

The guide is prepared keeping in mind the status of Bhutanese citrus industry and the present status of citrus nurseries. We hope this guide book would provide basic information on citrus nursery principles with special reference to record keeping and maintaining the traceability of propagation process.

FOREWORD

The Bhutanese citrus industry today faces numerous challenges of which citrus Huanglongbing (previously known as 'citrus greening') disease is a primary concern. Across the globe today citrus Huanglongbing (HLB) management is limited to three strategies: (1) reduction of bacterial inoculum (by rouging of infected trees), (2) controlling vector population, and (3) use of disease free seedlings. While roughing out infected trees and control of vector population has been sporadically practiced in our country, production of disease free seedlings is still a major constraint. Seedling demand far exceeds supply forcing growers to produce substandard seedlings of their own. Thus, these seedlings not only spread diseases but also die prematurely before they attain productive stage.

There are many citrus nursery manuals published elsewhere. However, they are found to be not very relevant to the Bhutanese citrus nursery situation due to the obvious level of technological gap. This technical guide conceptualizes and incorporates ideas and experiences accumulated over the years under local (Bhutanese) conditions so that the nursery practices can be easily adapted. Therefore this guide includes in brief all the relevant components of citrus nursery management right from rootstock seed extraction to variety registration and certification while maintaining and assuring a clear path of plant material movement (quarantine measures) and their traceability. Special focus is also given on the need for further and stronger linkage amongst concerned collaborators, and on aspects of nursery record keeping.

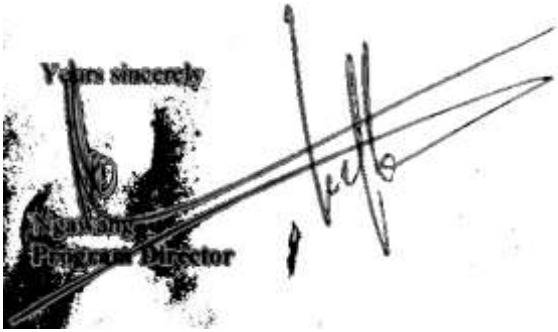
I hope this technical guide will be useful to all stakeholders involved in management of citrus nursery and help achieve production of high quality citrus seedlings which in turn is required to make the Bhutanese citrus industry not only economically viable but also a sustainable source of income for our rural communities.

I commend the two authors who initiated and put together the manual and worked on several drafts, and also acknowledge everyone in the Department

of Agriculture who provided valuable suggestions. As this is a first of its kind, we are committed to continuously improving the content of the manual. We look forward to your feedback. You can leave your comments at https://www.facebook.com/groups/Bhutancitrusgroup/. Hard copies of this publication are available in our center (RDC Bajo) and at the Horticulture Division, Department of Agriculture, Thimphu. Conveniently, you can access a soft copy from our following websites

www.moaf.gov.bt , www.rcbajo.gov.bt,
https://www.facebook.com/groups/Bhutancitrusgroup/

We thank the Australian Centre for International Agricultural Research (ACIAR) Citrus Project for financially supporting the publication of this booklet. This is also one of the outputs of the project activities.



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Acronyms

ACIAR: Australian Center for International Agricultural Research

BAFRA: Bhutan Agriulture Food and Regulatory Authority, Thimphu Bhutan

CB: Citrus board (Functioned in Bhutan by TRC)

CTV: Citrus Trestiza Virus

DoA: Department of Agriculture

DTI: Department of Trade and Industry

FYM: Farm yard manure

HLB: Huanglongbing

JICA: Japanese International Cooperation Agency

MoAF: Ministry of Agriculture and Forests

NCP: National Citrus Program, Horticulture Division, DoA, MoAF Thimphu.

NCR: National Citrus Repository, RDSC Mithun (under management of RDC Bajo), Wangduephodrang.

NPPC: National Plant Protection Center, Semtokha, Thimphu Bhutan.

RDC: Research and Development Center

RDSC: Research and Development Sub Center

RGoB: Royal Government of Bhutan

TRC: Technology Release Committee

Glossary

Air filled porosity is the percentage of air space in a media when the soil moisture is at field capacity.

Apomixis is a condition where embryo is developed without fertilization

Budwood or Budstick is a season old twig (with two to three buds) of a desired variety.

Certification is the accreditation process wherein seedlings produced have undergone through a set of prescribed conditions ensuring its high health status.

Citriculture is the art of cultivating citrus fruit.

Dormancy refers to ability of plant part to (seeds) to suspend metabolic process until congenial environmental conditions occur.

EC is a measure of amount of salts in soil to indicate the soil health status

Foundation block refers to a block of citrus trees from different habitat (wild, cultivated, backyard, landrace etc) basically maintained to conserve genetic resources and to improve crop performance through genetic improvement

Grafting is a process in which a part of a plant (two to three buds) is inserted onto rootstock.

Increased block is a block of registered trees (high health status) maintained with nursery centers to produce budwood. The increased block trees are obtained from mother block of authorized institute (NCR).

Introduced varieties are those varieties that are publicly accessible and brought in from other countries.

Local mandarin is a collective name referring to mandarin grown in Bhutan.

Media is any kind of material that provides anchorage and ideal conditions for plant development.

Mother block in citrus production refers to a block of high health status registered budwood trees maintained by an authorized agency. It is also known as germplasm block.

Multiplication block is synonymous to Increased Block or Daughter Tree Block meant for the supply of high health status budwoods in large quantity.

Nucellar seedlings is a seedlings grown from nucellar embryo of seeds without fertilization.

Pasteurization is a physical process where in the media is heated to a desired level of temperature (65 degree C) to render it safe from harmful pathogens.

pH is a measure of soil acidity or alkalinity

Propagation refers to multiplication of plants of specific cultivar or variety that posses one or more desirable characteristics (yield, fruit shape, fruit quality and resistance to biotic and abiotic stress).

Registered tree is a tree of a registered variety tested for high health status and allowed legally for multiplication.

Registered variety is a type of plant evaluated and registered for commercial cultivation by competent authority.

Rootstock is a lower part of plant used in a process of plant propagation in which a shoot is a scion wood is inserted onto it.

True-to-type is a condition of plant (type or seeds) that retain identical characteristics of parent plant in process of propagation. It is usually achieved through asexual or vegetative propagation.

Water holding capacity is the ability of the media to retain the available soil moisture.

Zygotic seedlings are sexually produced seedlings from fertilized seeds

1 Introduction

Bhutan is a mountainous country located in the Eastern Himalayas that falls within the center of origin of citrus as considered by many researchers, particularly due to its species diversity. Arable land constitutes about 2.93 % of the total country area (38394 km^2) and the Bhutan currently has a population of over 0.73 million.

Citrus is one of the important export crops of Bhutan, and is grown over 5086.42 hectares (ha) and ranks first among other agriculture commodities, both in terms of values and production. Therefore, citrus industry in Bhutan is a booming farm business.

Annually, on average (2008-2011), about 22300 metric tons of mandarin is being exported to neighboring countries of Bangladesh and India worth Nu. 325million. However, Bhutanese citrus industry suffers from poor management accrued to traditional system of cultivation. Moreover, due to Bhutan's porous border, lack of high health status propagation

materials and the absence of stringent quarantine measures, citrus Huanglongbing (HLB) remains widespread.

Since then, review of citrus program activities and germplasm collection began in earnest. Pertinent issues were identified by both native researchers and ex-country experts who defined specific approaches and formulated strategies. An ordinance titled "Citrus Ordinance" was passed initially in 2003 and later in 2009, and an "Executive Order" was issued by the then Ministry of Agriculture in 2003. Capacity development was enhanced and nursery infrastructure was strengthened. The national citrus repository was established. The Department of Agriculture hopes to sustain and develop citrus industry in the country through linkage and effective collaborations amongst these important agencies.

Citrus nursery requirement differs slightly from other fruit nurseries although most of the practices remain similar. The entire additional requirement – infrastructure and regulation - are due to Huanglongbing (HLB) and other diseases spread through insect vectors and propagative materials. This manual

defines the minimum standards and protocols required for Bhutanese citrus nurseries.

1.1 Rationale

HLB is a threat that could possibly lead to the decimation of the citrus industry in spite of all the interventions by the government. One of the main contributing causes is the lack of certification scheme although substantial efforts are being put into production of disease-free seedlings.

Currently, in the wake of confirmation of HLB disease in most citrus production areas in the country, the National Seed Center (NSC) is the only authorized producer of citrus seedlings. There is an acute shortage of seedlings. This booklet is also geared towards putting in guidelines to eventually help upcoming private nurseries, researchers, extension officials in the technical requirement as well as define minimum standard for compliance. The following basic requirement and minimum standards must be followed to establish and manage a citrus nursery.

2 Nursery standards

The following basic rules must be followed

1. Nursery must be located at least one kilometer away (in radius) from commercial orchards.
2. Double door entry into the nursery.
3. Upon the consent of DOA (National Citrus Program), register nursery with BAFRA and obtain license from Department of Trade and Industry.
4. Nursery stock must be propagated in approved/prescribed insect proof structures
5. Strictly adhere to existing citrus nursery standard and stock certification program
6. Testing program for HLB and other pathogen in alternate years through biological indexing
7. Record keeping of the nursery stocks and tested proofs and submission to Plant Quarantine unit (BAFRA) and TRC/Citrus Board whichever is appropriate. Alternately, the records must be made available at time of inspection by BAFRA.

2.1 Registration of varieties

Most of the citrus trees cultivated in Bhutan are mainly local mandarin although they constitute genetically and morphologically different types of mandarin leading to non-uniformity in fruit quality and maturity time. Therefore, to improve the fruit quality and enhance market demand, the variety must be evaluated for its performance prior to commercial release. Currently, research centers have evaluated and identified many promising varieties (local and introduced). The bud lines of these promising varieties can be obtained from the research and development centers and the NSC. However, such promising lines must be proposed for release and the high health status trees should be registered). Currently, the system of tree registration (disease free) is yet to be initiated

2.2 Certification

Many types of Bhutanese mandarin are found to be symptomless carrier of many strains of Citrus Trestiza Virus (CTV) and other graft transmissible diseases. Therefore,

certification process ensures that propagation material used (rootstock and budwood) in producing grafted seedlings are from registered stock, free from graft transmissible diseases. However, the quality parameters specific to varietal characters will be the responsibility of the farmers and nursery centers (NSC or private). The BAFRA will only monitor for compliance of certification system with respect to biosecurity front. The choice of the varieties will be determined by owner preference for variety and purpose.

The details on procedures of high health status mother trees and linkage of nurseries and NCR can be found in repository's working protocol (Annex-5).

2.3 Objectives

1. To institute citrus certification process to sustain Bhutanese citrus industry.
2. To make available high health status seedlings in sufficient quantity to meet the public demand

3 Tools and equipment

The efficiency of the nursery operation largely depends on the availability of important equipment and tools. Some of the equipment required are

1. Sprayer (liquid sprayer and duster)
2. Media mixer
3. Media sieve (3mm mesh)
4. Chain saw
5. Budding or grafting knife
6. Secateurs
7. Hand trowel
8. Hand rack
9. Plug trays
10. Pruning saw
11. Spade
12. Shovel
13. Hand hoe
14. Watering can
15. Bucket

4 Nursery structures

Ideally, a citrus nursery must have the following sructures;

1. Office and store
2. Soil disinfection shed
3. Media preparation and potting shed
4. Propagation room (for budding/grafting)
5. Mother block/increase block (double door insect proof screen house)
6. Double door propagation house (green house equipped with humidity and temperature regulation facilities)
7. Rootstock production house (double door insect proof screen house)
8. Irrigation facilities

However, considering the current capacity of Bhutanese nursery, the following structures would be adequate.

4.1 Soil or media disinfection shed

Foot rot disease caused by (*Phytophthora nicotianae*) is a serious fungal disease of citrus. To reduce the incidence of this

disease in nursery and to prevent its further spread into orchard, disinfection of soil or soil pasteurization is a must. Since chemicals are not readily available, we recommend steam pasteurization of soil media for half an hour at 65 degree Celsius. This physical method is advantageous over other chemical methods as it renders noxious weeds seeds non viable.

4.2 Double door screen or green house:

Insect proof screen house with double door is a primary requirement to keep away insect pests and vectors. Visits/entries to mother and propagation blocks must be strictly regulated.

4.3 Rootstock production house:

Plant production for rootstocks can be taken up in the propagation house. However, they must be arranged batch wise for proper recording. Due to high incidence of soil borne diseases in the nursery bed, nursery growers are recommended to initially germinate rootstock seeds in plug trays. If plug trays are not available, rootstock seeds can be raised on seed

beds prepared from sterile soil media in another shaded plastic house.

4.4 Propagation house

This is the main component in a citrus nursery. Budded seedlings are kept in this house as it has provision for regulating temperature and humidity. In general, double door plastic house can be used as a substitute for propagation house but the management of temperature and humidity is often difficult leading to increased seedling mortality.

4.5 Mother block/ Bud line

Registered and release varieties of citrus need to be maintained in the mother block depending on the capacity of the mother block screen house. The mother trees should be labeled and each tree per variety should be numbered serially. Initially, high health status seedlings can be obtained from the National Citrus Repository (RDSC-Mithun), Tsirang. It is the responsibility of the nursery centers to maintain daughter trees (multiplication block) which must be biologically indexed for

diseases. The indicator plants' budwood can be obtained from the NCR.

5 Media preparation

There are several commercial growing media and mixtures available in the market. However, they are not only expensive but also not readily available in Bhutan. Some of the important commercial media are:

1. Peat: It is made from marsh swamp residues generally used for newly rooted or germinated seeds. It is prepared from the remains of dehydrated acid hog plants. It is known for its water holding capacity and acidic nature (with a pH of about 3.5), and most of them are relatively free of pathogens.

2. Vermiculite: It is a micaceous mineral, chemically hydrated silicate of calcium and magnesium, and is neutral in reaction. It has a high cation exchange and water holding capacities, but has slightly low air-filled porosity.

3. Perlite: It is gray white material of volcanic origin without any mineral nutrients. It is highly porous and provides high moisture holding capacity and a good air-filled porosity.

Equally good growing media can be prepared from locally available materials such as saw dust, sand, biogas wastes, soil, top soil, leaf litter, rice husk, etc. At the same time, addition of animal wastes manure such as cow dung, poultry manure, goat manure, etc in correct proportion enhances the nutrient availability to plants. As such, ideal growing media should posses following characteristics

1. The growing media should be firm enough to provide anchorage to seedlings
2. It should hold water sufficiently for longer duration of time
3. It must have good air filled space for soil aeration and drainage of excess water
4. It must be free from weed seeds, nematodes, and soil pathogens

5. The media should have a pH range /of 5.5 to 6.5

No medium is perfect if not mixed in correct proportions. Leaf mould is found to have a very high percentage of air space initially and tends to get very compact at later stages. The water holding capacity is also found very low with pH of 4.5 – 5. Low pH reduces nutrient availability to plants while low water holding capacity needs frequent irrigation. Low air filled porosity can lead to increased foot rot or root rot disease incidence. Therefore, leaf mould (leaf litter) is not a good potting media.

Note: Do not use soils as growing media as they harbor pathogens

6 Media sterilization disinfection

Locally prepared growing media are the source of inoculums for many nursery diseases. Besides, media are also source of nursery weeds. Therefore, to prevent nursery diseases, two types of disinfections (chemical and physical) are commonly being followed by well established citrus nurseries. Since chemical treatment is risky and not environmental friendly, we

suggest media sterilization through physical disinfection methods (pasteurization).

Physical disinfection includes steaming of media for an hour at 65 degree Celsius ideally. However, based on the nursery capacity, different steam sterilization techniques can be devised. Steam pasteurization completely controls weeds in nursery.

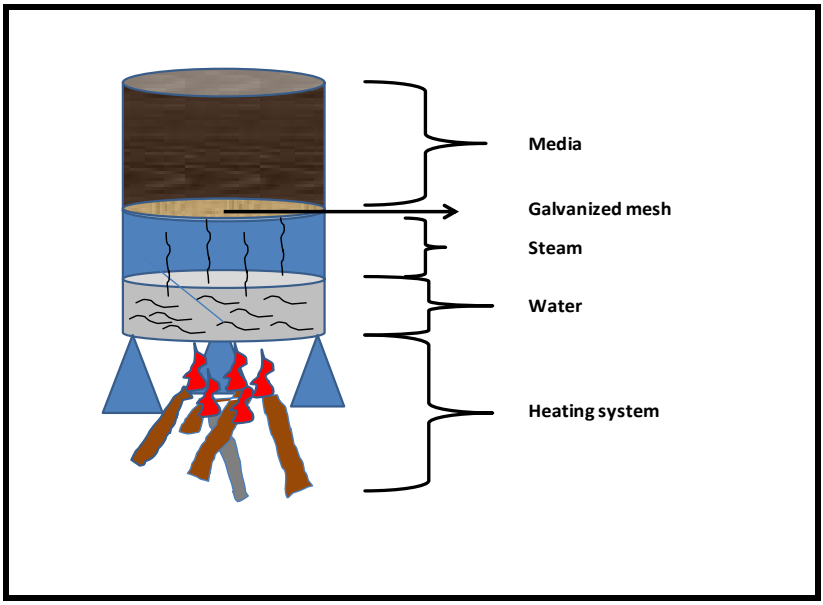

Figure 1 Pasteurization of media (physical method)

Soil/media solarization is another physical technique that can be used for disinfection. The media is spread on a raised platform (1 or 2 feet) using concrete construction and then wrapped with UV-stabilized transparent plastics. The media must be turned over at the 2 to 3 weeks interval depending on sunshine hours for about 2 to 3 months, again depending on weather conditions.

7 Raising rootstock

Rootstock is defined as the plant onto which the scion is inserted, and it produces the root system and lower trunk part of the tree. Rootstock part not only provides anchorage and nutrient supply to the budded or grafted scionwood but also possess ability to withstand salinity, water stress, nematode, phytophthora rot etc. Rootstocks must be grown from seeds only. Rooted cuttings or layers are not advised. Usually citrus rootstock is raised from cultivars with high percent of nucellar seedlings (unfertilized seedlings) to retain desirable parental characters.

7.1 Seed Selection

Presently, Bhutan has about nine different collections of rootstock varieties with desirable horticultural traits. However, only few roots stock varieties' seeds are currently being produced. Rootstock selection can be made on the following criteria:

1. Vigor (dwarfing or semi dwarfing)
2. Compatibility of rootstocks with known commercial variety
3. Resistance or tolerance to stress (abiotic and biotic)
4. Ease of propagation through vegetative means
5. Well developed root system

The most commonly used rootstock are:

1. Local mandarin (*Citrus reticulata*)
2. Troyer citrange
3. Carrizo citrange
4. Cleopatra mandarin
5. *Poncirus trifoliata*
6. Rangpur lime
7. Citrus volkameriana

These varieties differ in their traits and performance. Details are as indicated in the table below.

Table 1 Rootstocks and their characters

Rootstock	Germination	Desirable trait	Thorn	Vigor	Susceptible	Graft take	Hardiness
Local mandarin	Good	Tolerant to CTV	Medium	Good	HLB, foot rot	Good,	Very
Troyer citrange	Good	Highly resistance	Medium	Good	None	Good	Medium
Carrizo citrange	Good	Highly resistant	Medium	Good	Psorosis	Good	Medium
Cleopatra mandarin	Good	Tolerant to CTV	Medium	Good	HLB, for foot rot	Good	Good
Poncirus trifoliate	Good	Highly resistant	Very thorny	Medium	None	Good	medium
Rangpur lime	Good	Tolerant to nematode	Medium	Good	Exocortis, Trestiza, foot root	Good	Very hardy

Select appropriate rootstock varieties based on local requirements. The fruits need to be collected from trees of selected varieties as fallen fruits might contaminate seed beds and growing media. The harvested fruit must be packed and brought to the nursery within a short period of time.

Do not use seeds removed from mouth after eating the fruits as the seeds are contaminated.

Carefully choose the rootstock varieties of your choice and collect the fruits separately for seed extraction. Pack and label it for entry into Rootstock Register (Annex-1).

7.2 Seed extraction, treatment and storage

Extract seeds within a day or two after harvest as delay may cause the fruits to rot and increase the chances of infection. Cut the fruit (T.S) with a sterile knife to a depth of about one cm around the center of fruit (depending on fruit size) and separated the halves by twisting. Avoid complete cutting of fruits as this would result in cutting the seeds too. Remove the seeds manually or by using a hand seed extractor.

After removing the seeds from pulp, wash them thoroughly with water. Remove the shriveled, underdeveloped floating seeds to separate and collect the best viable seeds. Immerse the collected viable seed in hot water (52 degree Celsius) for about 10 minutes and air dry it in a dark room for 24 hours. Treat the seeds with fungicide (Captan or Thiram at 2 g kg^{-1} seeds), pack air tight in polyethene bag with rubber band and store in

cool temperature (7-10) degree Celsius. Seeds can be stored up to eight months. Take care not to dry seeds below 70% moisture content. Storing seeds above 10 degree Celsius reduces the seed viability and shelf life.

7.3 Sowing

The seeds can be directly sown in the polytubes or transplanted. The polytube sown seeds can be grown in propagation house at reduced light (shade). Otherwise, the seeds should be sown in seedbeds designed for sowing and germination. The seeds can be either broadcasted or line-sowed in furrows. Sowing can be done year round under tropical conditions. However, planting in dry seasons is preferred due to low disease incidences.

In broadcast method, the seeds are broadcast evenly over the medium and covered with the medium at around 2.5 cm, or in 2.5cm deep furrows spaced 10 cm apart. Care must be taken for a successful and high percent germination. Reduce the light reaching the seed bed by using shade net. Soil moisture and

temperature can be maintained by covering the plug trays or seed beds with transparent plastics.

7.4 Cultural practices

Proper fertilizer application, irrigation, and weed control are important cultural operations. Do not apply too much of FYM. Consult nearest research centers or extension office for advice. Ensure that nutrient is applied in correct quantities. Apply Suphala (15:15:15) initially at the rate of 5 kg per 3 m^2 area. Once germinated, spray micro nutrient solution at a regular interval. Also provide irrigation based on soil moisture content. If the medium is pasteurized, weeds are taken care off. The common diseases observed in seed beds are damping off (*Phytophthora*, *Rhizoctonia* and *Sclerotinia*).

7.5 Transplanting or potting

Seeds broadcast and/or sown in furrows should be transplanted. The seed bed must be watered thoroughly before digging to avoid damage to root system. It must be dug up on the same day of transplanting.

Seedlings should be transplanted/moved into ploypots when they attain heights of 30-40 cm (Local, Troyer, and Trifoliate) above the medium level. This duration varies with the species and growing conditions. In Tsirang region, under green shed net conditions, transplanting/potting stage is achieved in two months. However, seedlings older than 5 months require trimming of tap roots. The best economic size of polyethene tube (poly pot) is found to be one liter capacity (15 by 21 cm). Large sized poly pot requires large volume of media and occupies larger space in the growing room.

** sterilize poly pots before filling them with sterilized media*

Select healthy seedlings with well developed root system. Discard seedlings with off-shape (J-shape) roots. Grade them according to the size (small, medium, large). The large and small seedlings are generally said to be of sexual origin (zygotic) while the medium sized seedlings are asexual (apomictic. Maintain the seedlings of base on grades. Only medium size seedlings can be true to type due to its nucellar embryony. The other two may not exhibit parental characters.

Once seedlings are uprooted, protect their roots from direct sunlight before they are transplanted. It is important to maintain a between balance of aerial and root systems. Any tool used in transplanting must be sterilized using 10% sodium hypochlorite before and after use. A schematic representation of media mix preparation to direct seeding in poly pot is shown below in Figure 2.

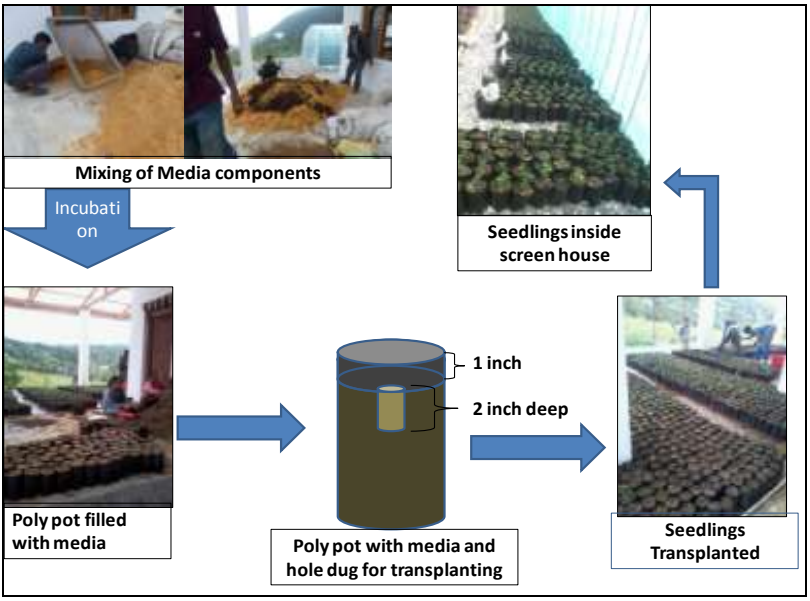

Figure 2 Media mix preparation and direct seeding in poly pot

8 Preparation of budwood or scionwood

The scion is the part which is inserted into the other plant (rootstock) and thereby produces the top of the plant, including branches, leaves and, ultimately, fruits. It is usually a bud or few buds (scion) with desired characters that can be used for vegetative propagation.

In citrus, budwoods must only be obtained from registered and certified institutions or nurseries. In many countries, sourcing budwood from other uncertified citrus area is considered illegal. Therefore, contact nearest Research Centers or Extension Offices for clarification.

8.1 Selection of bud lines

The mother block (inside screen house) or the increased block of the nurseries has many varieties either promising (identified superior through on station research evaluation but not released) and released varieties maintained exclusively for budwood. Bud line selection is very important as variety differ in their performance depending on location, altitude, soil type etc. Some of the varieties perform better under high altitude

condition while some do well in low altitudes. Similarly, some of the varieties are early maturing while some are late. It is very important to choose suitable varieties.

In Bhutan citrus is commonly propagated through grafting due to the very less cost involved in production of budwoods. When periodic test for diseases are initiated and certification and quarantine measures implemented, the cost may eventually rise.

Budding is therefore suggested. The high health status bud of released and promising varieties can be obtained from Citrus Repository, RDSC Tsirang. However, if nursery centers have capacity (human resource and infrastructure), they can maintain their own rapid multiplication block/daughter trees from registered trees.

8.2 Collections, packing and labeling

Scion woods or budwoods are usually collected in dormant season for deciduous species. However, budding in citrus can be done year round depending on the infrastructure available. One year old twig (current season's growth), cylindrical shape

budwood is preferred for budding while angular budwood can be conveniently used for grafting. Collect bud stick during early hours of the day when it is turgid and the temperatures are low.

Steps involved in preparation of budwood are:

All the tools used in collection process needs to be sterilized or disinfected with 10% sodium hypochlorite

Verify the status of the variety and locate the correct variety of your interests. The details (access map, tree map, certificates etc) can be obtained from budwood files.

1. Treat each scion tree as an individual tree.
2. Select suitable one year old twigs with prominent buds. Twigs without conspicuous buds need defoliation prior to its collection for about a month in advance.
3. Once the budwoods are cut, remove the leaves at the petiole base.
4. Bundle the bud sticks (25-30) tree wise, wrap in newsprint paper and tie with rubber band.

5. Tag the bundle with labels:
   a. Cultivar name
   b. Serial number of source tree
6. Bring them to nursery shed; dip the bud sticks in 10 % Sodium hypochlorite solution and air dry them.
7. Enter the details of the scionwood cut into the "Budwood Record Register" or "Nursery Plat."
   a. Date of collection
   b. Location/Institutions
   c. Variety
   d. Tree Number
   e. Tree location (Row: _____, Tree position: row____, Tree sequence number)
   f. Varietal status
   g. Number of bud sticks collected
   h. Number of buds per bud stick
   i. Name of collector
8. Treat them with 0.5 % captan, air dry and wrap with newspaper and store in plastic bags.

These budwood can be kept for about a week without losing viability if stored at 4-7 degree Celsius

8.3 Waxing or para-filming

It is very important to prevent desiccation of bud sticks. The bud sticks are either waxed or wrapped with thin layer of parafilm. Waxing is usually done for other fruit species although it is in practice for citrus usually if they have to be stored for longer duration or transported to a distant place. However, care must be taken not to injure bud sticks when immersing them into melted wax. As of now, waxing is not a common practice as budding is done within few days of obtaining bud sticks. Instead, para-filming of bud sticks was found more effective. The parafilmed bud sticks retain viability for almost about 3 months when stored at room temperature (18 to 23 degree Celsius) in opaque plastic bags.

9 Propagation

Citrus (mandarin) in Bhutan are usually grown from non grafted seedlings and hence graft transmitted diseases did not spread. Now, with the graft transmissible disease (HLB, CTV, viroids, citrus psorosis virus, citrus tatter leaf virus) already rampant in citrus growing area, use of non-grafted seedlings

proved uneconomic due to its long juvenility (7-8 years). The seedlings die before they attain bearing stage. Therefore, one of the strategies to rehabilitate citrus orchard is to promote high health status grafted seedlings produced from registered and certified budwood.

In Bhutan, citrus is either budded or grafted. Grafting is done only if there are enough bud sticks. Currently, budwoods are free of charge (provided by Research Centers). The scenario may change after citrus registration and certification process are initiated. Usually two eyed budwood are used in citrus grafting. Budding or bud grafting is quite recent to Bhutanese citriculture. Emphasis is now being put on budding as high quality bud sticks will soon be limited in supply if quarantine and certification scheme is adopted.

9.1 Grafting or budding

Grafting refers to the process of inserting a part of a desired plant into or onto another plant in such a way that they grow as a single plant. Depending on the number of buds in the scion, this process is of two types; budding and grafting.

Budding is a grafting where only single bud is used and inserted on rootstock while grafting involves two or three buds. In Bhutan, chip budding is being promoted because of its relative ease and convenience. Chip budding can be done year round while other budding techniques depend on the season when bark slips easily.

9.2 Chip budding

Chip budding is a common practice in citrus propagation. In Bhutan, modified chip budding is being promoted for citrus because of its convenience and higher success rate.

In this method, instead of removing a chip from bud stick, a "chip bud" is prepared using the bud with the entire stick behind (Figure 4). The technique is standardized by JICA Expert (Mr. Yuichi Tomiyasu), RDC, Wengkhar.

The steps involved in modified chip budding are

1. Ready grafting/budding tape, budding knife, secateurs by sterilizing it with 10% sodium hypochlorite after budding each batch of budwood.

2. Select pencil sized (1 to 1.5 cm diameter) roots stock for grafting (usually 8 to 12 months old depending on growing condition).
3. Prepare the rootstock by defoliating it and cutting it at a height of 1.5 feet.
4. At a height of 1 foot, make a slanting downward cut, slipping the bark of about 2 cm and remove the cut by giving another slight cut at the base (Figure 4, Step2)
5. Prepare budwood (Figure 3) by giving a complete slanting cut at the base of bud stick at 0.5 to 1cm below the bud. Then slice little deeper than the bark from behind the cut end so as to exactly fit the rootstock. (this slice from behind must match the notch made on the rootstock)
6. Cut the bud stick using secateurs at about half a cm (above the eye or bud) and insert the bud into rootstock notch.
7. Tie hard with budding tape, once around the bud and several times below and above the bud (Step 2, Figure 5). Ensure all the cut ends are made air tight with

budding tape. Be careful not to dislodge the bud while tying with tape.

8. Label the plant number, variety of budwood and rootstock used along with the date of budding, source of budwood, name of nursery (Figure 3).
9. Record the details of the batch budded/grafted in propagation register (similar to The Bud Record Register). The details of the rootstock must have been included.
10. Transfer it to the propagation room where temperature and humidity can be regulated. Arrange the budded seedlings in batches to enable easy management and record keeping. Label the batch (lot) with Nursery Plat. This arrangement of seedlings in batches aids in disease testing and diagnosis process.

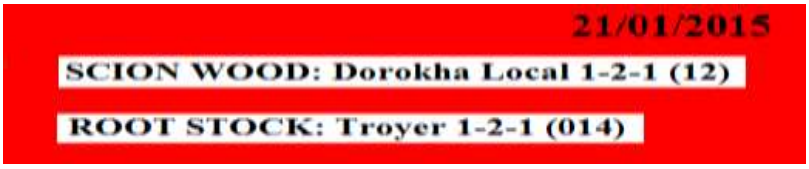

Figure 3 The label provided for a budded seedlings with Dorokha variety, plant number 1, on 2nd row, 1st Position from scion wood batch number 12 on Troyer rootstock seedling grown from tree no. 1, in 2nd row, 1st position, 14th batch seeds

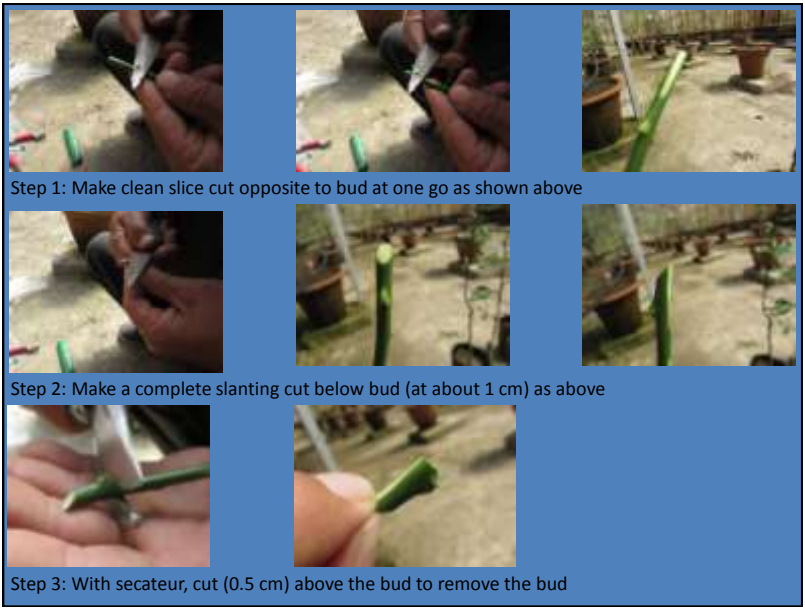

Figure 4 Preparation of bud for budding

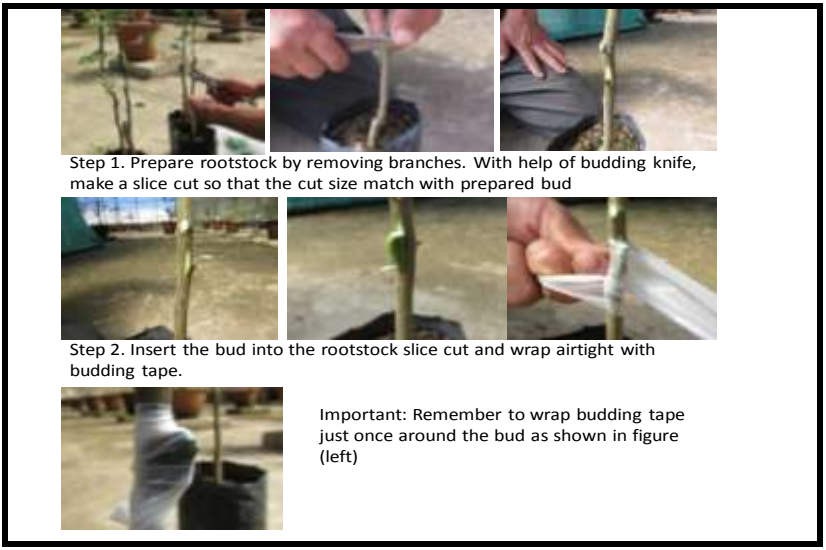

Figure 5 Preparing rootstock and inserting budwood (Budding)

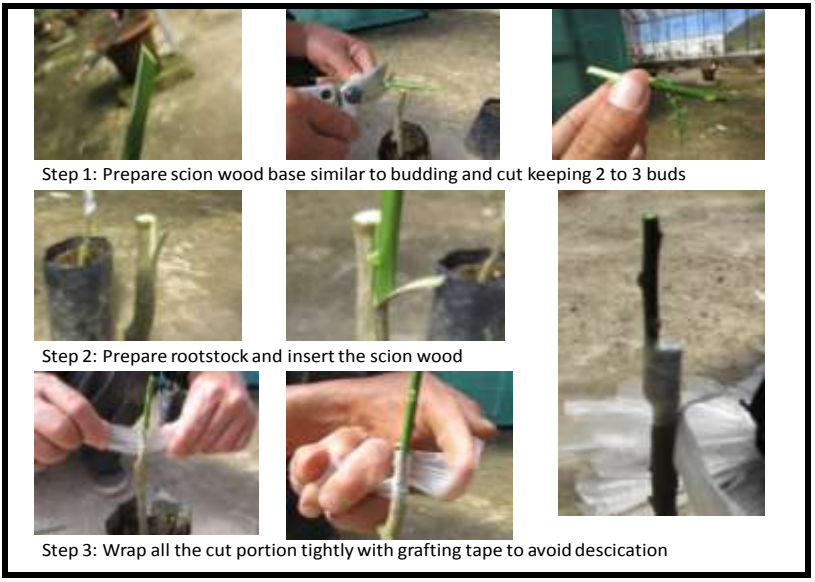

Figure 6 Steps in citrus side grafting

10 Aftercare

The success rate of budding or grafting depends on graft aftercare. Most of the graft failure is caused by negligence in carrying out after graft operations.

10.1 De-suckering of root stock

New shoots that grows below the graft union are the main cause of graft failure. These water shoots draw the nutrients

and make it unavailable to the graft union. Even after the union has formed and the grafted bud sprouted, removal of rootstock water shoot must continue. Delay in removal of suckers may result in stunted growth of budded trees or may dry up at later stages. Therefore, regular monitoring and de-suckering must be conducted.

10.2 Irrigation

Irrigation is a must for healthy citrus seedlings. There are several methods of irrigation. The most common are

1. Manual irrigation
2. Drip irrigation
3. Over head sprinkler

Manual irrigation is the most commonly followed practice in citrus nursery. The potted seedlings are provided irrigation with the water from pipe. Drip irrigation is usually provided to the mother block where the trees are space at distance. The flow is regulated by pressure compensating emitters or drippers place at the root zone. Over head sprinkler is used at later stage of seedling growth. Irrigation can be schedule by

directly observing the soil water content or through the use of tensiometer / irrometer.

10.3 Nutrition

Supply of optimum nutrient is a must for healthy seedlings. Seedling health determines the productivity of trees at later stages. Therefore proper nutrient in complete form must be applied to seedling nursery.

There are different ways of nutrient application in nursery based on separate objectives. Potting media must be sufficiently enriched with nutrient based on nutrient tests.

While preparing potting media ensure that about 20 % of the mix constituent should be well decomposed FYM or other similar manures (goat manure, chicken manure, etc). It is also important to incorporate micronutrient mix if available.

If possible, apply calculated amount of micronutrient (any commercial form) monthly or it can be done as and when deficiency symptoms develop. In Bhutanese nursery where

maximum of the media constitute leaf litter, deficiency in Manganese (Mn) and Magnesium (Mg) is very common.

10.4 Removing budding/grafting tape

Once the graft union is formed, it is important to remove the budding tape. The tapes can be removed ideally six weeks after grafting. However, before removal, check if the cut wound around graft union is healed. If the bud is not healed properly, wait for another few weeks. On the other hand, delay in removal can cause girdling or ringing, thereby interrupting translocation. Cut the plastics and remove it completely.

10.5 Cutting rootstock above graft union

Once the graft union is formed, part of the rootstock above the union should be cut off right after removing grafting tape. The cut must be sloping about a cm above the bud union. Or alternately, the rootstock can be bent slightly above the bud union (to force nutrient to bud union).

10.6 Record keeping

Each and every activity carried out must be recorded properly. The quantity and type of propagation materials (rootstock seeds, budwood, cultivars, etc) obtained from individuals or institutions should be entered in the Nursery Stock Register. BAFRA office should inspect for compliance and issue Nursery Stock Inspection Certificate.

10.7 Hardening off

Hardening is process of getting plants adapt to field conditions. This step is crucial for plants grown under controlled shaded conditions. Seedlings need exposure to direct sunlight for a minimum of two weeks prior to field transplanting.

11 Nursery pests and diseases

Although citrus seedlings are grown inside protective insect proof structures as required, they are never free from pests and diseases. Many pests and diseases affect the production of citrus seedlings. Most of these pests and disease can be controlled by agriculture chemicals. For detail

recommendation, contact the nearest Extension Office or Research and Development Centers.

11.1 Diseases

Many nursery diseases can cause potential economic loss. Most important diseases are:

1. Huanglongbing (Ex- Greening)

   Huanglongbing is one of the deadly diseases that threaten global citrus industry. The diseases is caused by *Candidatus liberibacter* species and transmitted by psyllid vector. The disease is also spread through grafting and use of infected plant material (budwood or rootstock). Therefore, use of certified plant materials is very important for citrus industry.

2. CTV

   Citrus Trestiza Virus is also an important viral disease that is transmitted in nursery. The disease is also vectored by aphids, in particular by *Toxoptera citrida*.

Use of certified planting material in nursery is an effective preventive measure.

3. Damping off

   This disease is most common in nursery beds where non- sterilized growing media is used. The disease is caused by many species of *Phytophthora* and most commonly by *nicotianae* species. Use of sterilized propagation media effectively prevents this disease in nursery.

4. Powdery mildew

   This fungal disease is caused by *Oidium citri* and is common under nursery screen house condition during rainy season. Sulphur dusting effectively controls the disease. Alternate use of neem oil has been found to control the disease too.

11.2 Pests

Common pests of citrus nursery are

1. Asian citrus psyllids (*Diaphorina citri* Kuyuwama)

   This is a worldwide quarantine pest that transmits huanglongbing (HLB) disease pathogen. It feeds on young leaves and tender shoots. Therefore, psyllid proof screen or net houses with double door installation is a requisite in citrus nursery.

2. Citrus leaf miner

   Leaf miner (*Phyllocnistis citrella*) is a very common nursery pest. The larvae usually feed on the underside of tender young leaves and cause leaves to twist and curve. Growth of the plant is therefore retarded significantly. Routine neem oil spray (1.5 ml per liter of water) at the time of new flushing controls the infestation.

3. Aphids (*Toxoptera citricida*)

   It is also the one of the important pests of citrus. It feeds on tender and emerging shoots and leaves. It also transmits the pathogen of CTV causing enormous economic crop loss. In nursery, this viral transmission can be checked by use of high health status planting

materials (certified) obtained from the NCR, NSC or Research Centers. However, the budwood should be registered and tested for graft transmissible diseases

4. Whitefly (*Dialeurodes citri*) (Ashmead)

   Whitefly is not a serious pest of citrus although it is common in low altitude citrus nurseries. It feeds on sap of the plant and excretes honey dew on which sooty mould develops It is also a suspected vector of Citrus chlorotic dwarf. They are known to transmit viral diseases.

5. Red scales (*Aonidiella sp*.)

   Red scales can be a problem in screen houses with higher percent shading.. Effective monitoring for their presence is important. They can be effectively controlled by neem oil spray (1.5 ml per liter of water).

12 Supply of seedlings

Currently, the National Seed Center is the only authorized supplier of the grafted citrus seedlings. The Regional Seed

Center in Bhur, Sarpang, produces grafted citrus seedlings. Seedlings may be sold directly by NSC or supplied by agencies under department of agriculture. However, the movement of citrus seedlings must be accompanied by the Movement Permit issued by BAFRA only for certified nursery stock (issued at the time of inspection). Alternately, the movement permit can be issued base on invoice if the nursery stock (produced by registered nursery) is already certified

13 Certification process

Citrus certification process is inevitable if citrus industry is to sustain. The purpose and principles remain the same although procedures may differ slightly from country to country depending on resources availability. Three main programs under certification process include:

1. Quarantine program

   Restrict and regulate the movement (introduction) of citrus species between countries or districts to minimize the risk of entry of disease pathogens.

2. Clean stock program

   The National Citrus Repository at Mithun (RDSC-Tsirang) will house the registered foundation trees. The trees will be regularly tested for graft transmissible disease to ensure their high health status.

3. Certification program

   Certification program ensure the safe production of high health status citrus seedlings, their distribution and tracking of the movement of planting material produced by registered nurseries. The steps involved are very similar to the existing seed certification systems however additional steps are to inspect nursery production process and to conform to certain minimum standard establish by Citrus Board. However, a separate certification guidelines prescribing the minimum standards is required for citrus certification.

All in all, prior to starting a citrus nursery, the following requirements need to be fully adhered to

a. All citrus nurseries must be registered with BAFRA (MoAF), Department of Agriculture and Department of Trade and Industry.
b. Approval for nursery sites must be obtained.
c. A distance of at least 1 km should be maintained from commercial orchards to citrus nurseries
d. It should be fenced with gateways for restricted entry.
e. There should be wind breaks of sufficient heights and density to keep away from psyllids blown by wind.
f. The surrounding must be free from rutaceous hosts (citrus family and relatives).
g. All propagation house structures should be enclosed with insect proof screen net and meet the minimum standard.
h. Must maintain the visitors' log.

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15 Annexure

2015

15.1 Annex-1: Rootstock Record Register

Sl. No.	Batch/Lot No.	Date of collection	Location / Institution	Variety	Quantity (kg)	Tree No.	Row No.	Tree position	Collector Name	Initials

15.2 Annex-2: Budwood Record Register

Sl. No.	Date of collecti	Batch No.	Location / Institution	Variety	Tree number	Row No.	Tree positio	No. of	No. of	Collector Name	Initials

	on						n	bud stick	buds	2015	

15.3 Annex-3: Propagation Register (Budding or grafting)

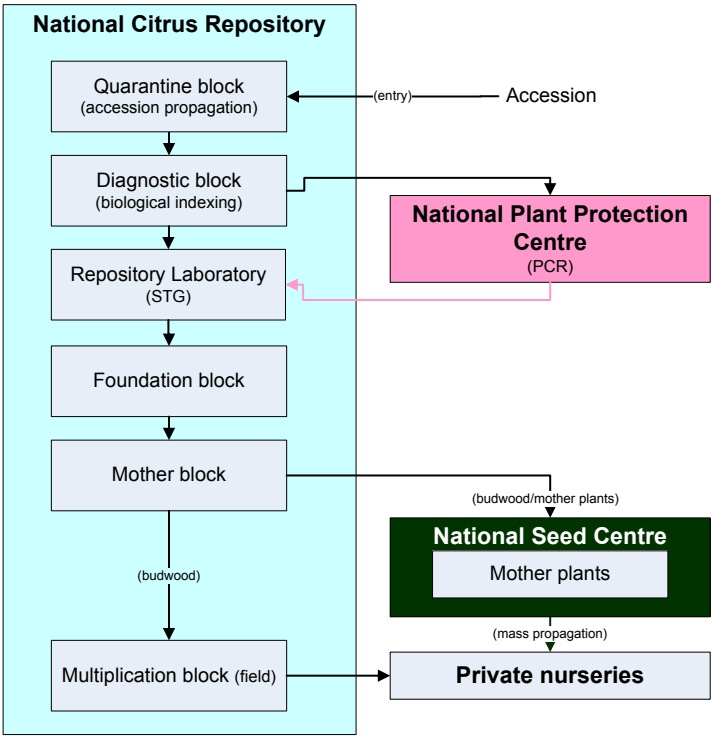
Date	Batch Number	Scion wood	Scion wood batch No.	Root stock	Root stock Batch No.	No. of seedlings	Name of Nursery man	Initials	Supervisors Initials	Inspectors Remarks

									2015	

15.4 Annex-4: Nursery Stock Register

Sl. No. (1)	Date (2)	Opening stock (3)	Purchase/Transfer stock (4)	Total stock (3+4)	Issues (5)	Wastage (6)	Closing stock (6+7)	Remarks

15.5 Annex. 5: Schematic representation NCR protocol and linkage with agencies (NPPC, NSC and private nursery)



16 Indices

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