

LEARNER GUIDE

Plant Propagation

Level 3



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- Tissue culture propagation in citrus involves growing plants from a micro-portion of plant material in-vitro in a laboratory.
- Propagation tools that are commonly used are budding knives, budding tape, pruning shears, a sharpening stone and sterilisation liquid.
- Propagation tools must be sterilised to prevent the development of pathogens.

3. **Successful and Unsuccessful Propagation**

- Indicators of successful propagation are trueness-to-type, freedom from pathogens and pests, and the health of the plant.
- Indicators of unsuccessful propagation are deviations from type, diseased plants, mixed cultivars and inferior plant quality.
- Environmental conditions must be maintained to ensure successful propagation.



<p><u>Propagation</u></p> <p>Propagation in citrus production refers to the multiplication of plant material that is of a specific cultivar and variety, and that possesses more desirable characteristics, such as yield, fruit size and shape and internal quality.</p>
<p><u>Dormancy</u></p> <p>Dormancy refers to the ability of certain plant-parts, such as seeds, to suspend metabolic processes until ideal environmental conditions occur.</p>
<p><u>Metabolic Processes</u></p> <p>Metabolic processes refer to organic chemical processes inside a cell that enable life.</p>
<p><u>Humidity</u></p> <p>Humidity, also referred to as <i>relative humidity</i>, is the amount of water vapour in the air at a given temperature, and is expressed as a percentage. This means that at 20% relative humidity, 20% of any given volume of air will consist of suspended water molecules.</p>
<p><u>Respiration</u></p> <p>Respiration refers to the process during which the plant takes up oxygen (O₂) and releases carbon dioxide (CO₂).</p>
<p><u>Photosynthesis</u></p> <p>Photosynthesis refers to the chemical reaction that takes place when the plant takes up CO₂, which combines with water molecules in the plant to produce carbohydrates, which is food for the plant. O₂ is released during this process.</p>
<p><u>Ovule</u></p> <p>The ovule is a small structure inside the ovary of a seed plant that contains the female reproductive cells inside the embryo sac, and which develops into a seed after fertilisation.</p>

Embryo

The embryo is a plant in its earliest stage of development before an organism becomes self-supporting. Once the embryo begins to grow out from the seed, or germinate, it is called a seedling.

Meiosis

Meiosis is a type of cell division in organisms that reproduce sexually and results in cells with half the number of chromosomes of the original cell.

Rootstock

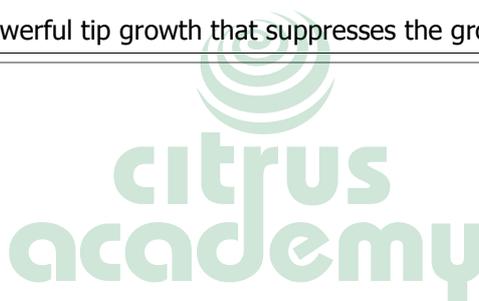
Rootstock means the root or part of a root used for plant propagation. In reference to grafting, the rootstock is that part of a grafted plant that supplies the aboveground plant parts.

Grafting

Grafting refers to any process of inserting a part of one plant into or onto another plant in such a way that they will unite and grow as a single unit.

Apical Dominance

Apical dominance refers to powerful tip growth that suppresses the growth of lateral buds.



Chapter 1

After completing this chapter, the learner will be able to:

Demonstrate an understanding of the function of environmental requirements for propagation within a specific agricultural production context

1. Introduction

Controlling the environmental factors to imitate nature to a certain extent during propagation is essential for a successful outcome. In nature, plants develop slowly to reach maturity. Citrus propagation aims at emulating the natural process and enhancing it.

This requires an understanding of the natural environment in which citrus plants develop and an understanding of variations of these conditions. It is also necessary to have a thorough understanding of the metabolic processes of plants because environmental conditions determine how successfully plants are able to complete these processes.

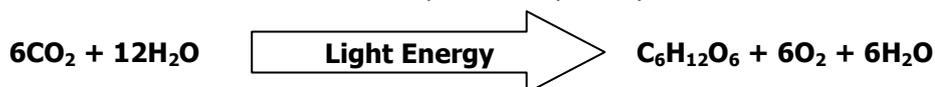
2. Metabolic Plant Processes

Plant growth is a result of a series of biochemical reactions that take place in the plant. It is essential to understand the principles of two of these processes, namely photosynthesis and transpiration, to better understand the influence of environmental conditions on propagation.

2.1. Photosynthesis

At germination, red light triggers the emergence of the radicle, or root, which in turn initiates the elongation of the hypocotyls (stem) and the development of leaves. After leaves emerge, chlorophyll and other compounds are synthesised through absorbed energy. Chlorophyll is the main pigment responsible for photosynthesis. Photosynthesis occurs throughout the lifespan of the plant, and is the initial step of growth after seed germination.

The formula below summarises the process of photosynthesis:



During photosynthesis, the plant absorbs CO_2 through the leaves and green cells, and water through the roots. With the heat generated in the plant through light and plant molecule movements, the plant manufactures sugars (carbohydrates), oxygen, and water molecules. Kinetic energy is released and used to activate particles in the plant.

2.2. Respiration

The reverse reaction of photosynthesis is referred to as respiration, where oxygen (O_2) from the air is used and carbohydrates in the plant are broken down into carbon dioxide (CO_2), which is released in the air. Water molecules are diffused from the leaf surface into the surrounding air. The diffusion of water molecules, in the form of water vapour, is the core of the transpiration process.

Transpiration is driven by light intensity, water vapour in the air, and the moisture level in the growth medium or soil. In the plant, transpiration is expressed through turgid cells, opened stomata, which are small openings on the surface of the leaf, and CO_2 diffusion into leaves. The

rate of transpiration is closely related to the amount of water vapour in the surrounding air and the amount of water vapour diffused to the surface of the leaf.

When the moisture in the surrounding air increases, plant transpiration decreases. Conversely, when the moisture in the surrounding air decreases, the transpiration rate increases. In other words, the transpiration rate increases in dry air environments, and decreases in moist air environments. The quantity of moisture that is contained in the surrounding air is influenced by the ambient temperature. Warm air contains more water vapour than cold air.

Light intensity influences transpiration through leaf surface temperature. Higher leaf surface temperatures result in an increased transpiration rate. At temperatures above 30°C however, the stomata on the leaves of citrus plants begin to close, and the rate of photosynthesis and transpiration decreases.

The water available to the roots of the plant also influences the rate of transpiration. Less water results in stunted growth with short internodes and small leaves, resulting in a reduced surface area for photosynthesis and transpiration. As the rate of water absorption decreases, the plant struggles to find the necessary moisture to support metabolic processes. Transpiration uses the little water available, resulting in the wilting of the plant. At extreme wilting point, no food (energy) is manufactured as there is virtually no photosynthesis taking place, and the plant is forced to use up all its reserves to survive.

At the other extreme, excess water results in increased cell-size and a normal transpiration rate occurs initially. However, under anaerobic conditions, being conditions where the roots are drenched in water, the plant is starved of oxygen and suffocates it in the process. Such conditions are also conducive to the development and spread of pathogens like rhizotocnia (damping off) and phytophthora (root rot).

3. Environmental Factors for Propagation

The environmental conditions that need to be controlled for plants to grow successfully are categorised in three classes, being:

- ***Atmospheric conditions***, including light, water, humidity, temperature, and gas exchange
- ***Biotic conditions*** refer to conditions in the root-zone with regard to bacteria, fungi and viruses, to insects, and to weeds.
- ***Edaphic conditions***, including physical soil properties (texture, porosity, temperature) and chemical soil properties (pH, mineral nutrients, gas exchange), and container properties

Citrus plants respond to the environmental conditions through growth rate. Where factors are well managed, better growth is achieved, while retarded growth and even loss of plants may result from an environment that is not well managed.

Every environmental factor plays a specific role, both on its own and in interaction with other factors. In this section, each factor is discussed with the assumption that all other factors are favourable.

3.1. Atmospheric Conditions

Citrus is a sub-tropical species, and its propagation is more successful in a climatic environment that resembles the sub-tropics more closely.

The time of the day and season of the year are factors the propagator has to consider when planning to propagate citrus. For instance, the best time for budding is when the temperature is between 25°C and 30°C, and when the process of photosynthesis is active with opened stomata, turgid cells, abundant sugars for growth, and CO₂ diffusion is increasing.

3.1.1. Light

Light manipulation is a very important factor in propagating citrus. Seed germination is activated with increased light intensity. The bud-take rate is influenced by the sap flow, and rates of transpiration and photosynthesis in the plant, which in turn is influenced by light intensity.

Two light sources are used, being the sun (solar radiation) and artificial lights. Understanding solar radiation is important before planning any manipulations. Intensity alone is not sufficient if the quality of the light is not also considered.

The sun radiates up to 10,000 lux of light on a clear, sunny day and about 3,200 lux on an overcast day. A fluorescent light discharges 1,400 lux. In nature, light intensity decreases with the presence of clouds or dust in the atmosphere. Propagation structures that draw light primarily from the sun experience the same fluctuations in intensity. Fluctuations in intensity trigger different responses from the plant in terms of its growth rate. High light intensity causes more chlorophyll to be synthesised and a greater gross rate of photosynthesis.

Light quality refers to wavelength, measured in nanometres (nm), and is associated with the colour of the light as perceived by the human eye. Light is classified in eight bands, ranging from ultraviolet (<280 nanometres) to near infrared (>1,000 nanometres) and includes the visible range, being violet, blue, green, yellow, orange, and red light. Fluorescent plant lights used in germination rooms are rich in red light (610-700 nanometres) and deficient in the far red (700-730 nm). Enzymes and proteins are activated when subjected to light and initiate radicle emergence and elongation, and ultimately seedling growth.

Beyond the germination room, solar radiation is the main source of light, with quality closer to 700nm where CO₂ absorption is at its optimum. At this spectrum, coupled with higher light absorption rates, light indirectly affects the rate of transpiration as enzymes are denatured at higher leaf-surface temperatures. The accumulation of starch – which absorbs less water than sugar – in the leaf cells results in stomata closing and a reduced transpiration rate.

A lower light absorption rate adversely influences the transpiration rate. In this case, sugars are not transformed to starch, but maintain turgid leaves for a longer time leading to a reduced starch level in the plant. With starch being the form of carbohydrate that is used in respiration, this results in a reduced photosynthesis rate.

3.1.2. Humidity

Relative humidity, or the water vapour level in the ambient air, regulates the gross transpiration rate of plants. The level of humidity, or relative humidity (rh), is generally below 80% in the sub-tropics where citrus originates from. Ideal conditions for propagation requires a high rh, being between 80% and 95% and occasionally reaching 100%.

In an environment where the water vapour level is high, it takes longer for the water molecules on the leaf surface to evaporate, meaning the exchange factor is lower. Where relative humidity is low, the transpiration rate increases.

These fundamentals are important for the manipulation of rh in the propagation environment. Seed germination requires high humidity around the seed, where respiration is normal, allowing the seed to absorb more water molecules, which in turn hydrolyses insoluble foods to soluble forms. As respiration increases the radicle emerges and elongates, but if it decreases the storage life of the seed is longer, meaning that the hydrolysis of stored foods does not take place, and thus no germination takes place.

Seedling growth and development also depends on the transpiration rate because this directly relates to photosynthesis. Plants require an environment where the processes of transpiration, respiration and photosynthesis can take place at normal rates. In such an environment, CO₂ is rapidly diffused into leaves and transformed in carbohydrates that are used for growth. The same applies to budded seedlings.

For cuttings, high humidity ensures that leaves do not lose their turgidity, and that they continue with photosynthesis and respiration while roots are developing. A more detailed process will be discussed in propagation structures.

The figure below shows the variations in humidity relative to the time of day and the temperature.

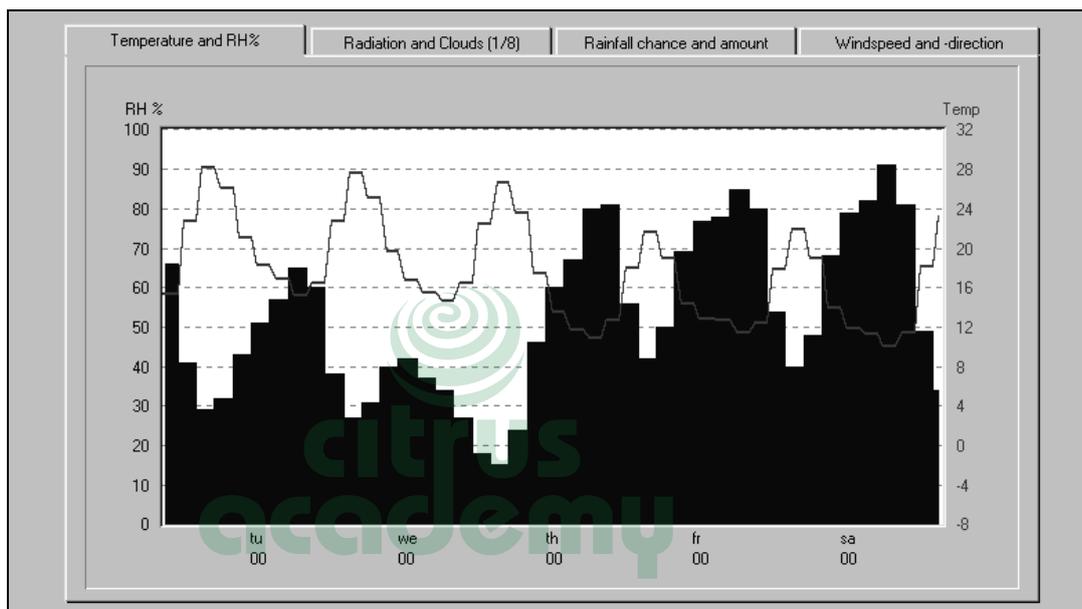


Figure 1.1: Letsitele Weather Records – Week 28/11/2004
(Courtesy of Du Roi QMS)

3.1.3. Temperature

From seed germination to the healing of the bud union, temperature plays a major role in propagation of citrus plants. Germination requires relatively high temperatures, with a range of between 27°C and 32°C, with an average of 29°C being considered optimal.

Within this range, the propagator has to identify the temperature at which the processes of photosynthesis and respiration are optimal. This point, identified as 29°C in general, makes large quantities of carbohydrates (sugars) available for plant growth and development. Cell division and elongation is at an optimum and vigorous growth is expressed through large and healthy leaves, thicker stems, and denser, healthy roots.

Higher temperatures, in the region of 35°C or more, reduce the rate of photosynthesis, exhausting stored reserves of carbohydrates. The increase in temperature also alters molecular structures of enzymes and proteins.

If the temperature falls below 12°C, growth is affected because the physical properties of water in the plant are altered, becoming less fluid and reducing the rate of molecular movement in the plant. This translates to a reduced rate of compound translocation and catalytic reactions which can lead to plant death.

Extended exposure to temperatures below 10°C causes cold injuries to plants, mainly to new shoots. This is the reason why citrus cannot be propagated without costly protective measures in areas where frost occurs.

3.3. Edaphic Conditions



Edaphic

The term edaphic means the effect of soil characteristics, especially chemical and physical properties, on plants and animals. For the purpose of this section, edaphic conditions refer to the properties of the growth medium, whether it is soil or another substance.

Not all plants are grown in soil, with some grown in soilless media, such as pine-bark which is commonly used for container grown seedlings in South Africa. In addition to pine-bark, there are other media that can be used in different combinations. One can consider using sawdust, peanut shell, river sand, composted organic material, and ash mixed in different proportions that allow optimum root development. However, it is crucial for the propagator to establish the chemical and physical properties of the growth medium and that the medium is free from harmful pathogens.

Should plant material be used as a medium for plant propagation, it has to be well composted to avoid competition from micro-organisms. Otherwise these micro-organisms use up nutrients, especially nitrogen, to continue with the composting process, which results in reduced availability of nutrients for plant growth. Beyond the competition, there are organic acids, also referred to as tannins, found in plant materials that have to be allowed time to get to an inactive stage.

The properties of the growth medium are important because they determine what can be grown in the medium, and they give an indication of the possible behaviour of the growth medium, and as a result the plant, under certain conditions relating to air, water and temperature.

3.3.1. Physical Growth Medium Properties

Generally, physical soil properties refer to the texture, structure, depth, stratification and aeration of the soil. In terms of propagation, physical growth medium properties refer to the texture, structure and aeration of the soil. Soil depth and stratification are controlled in the nursery environment.

Water and air are components of the growth medium that are kept in a specific balance to allow optimum plant growth. The balance comes into play in relation with the solid particles of the medium. In a container, the mix should have enough air to supply the roots, enough water for physiological and metabolic processes to take place, and solid particles to anchor the roots.

The texture and particle size of the medium greatly influence root development. Pine-bark presents a coarser texture with irregular surface and structure, which determines the direction in which the roots will grow and the ease with which they will spread into the container. Because of its coarseness, and other chemical properties that will be discussed later, pine-bark is known to be less effective in holding water for a long time, which has to be taken into account in irrigation management.

A complementary property in this regard is the size of particles making up the growth medium. A growth medium with small particles tends to compact easily, making the soil less permeable and therefore impeding the movement of roots and water. Growth medium with small particles could also have excessive water holding capacity, which has the potential to promote disease development, such as root rot, and starve the plants of air and nutrients.

Larger particles on the other hand have a reduced water holding capacity. The bigger the particles, the less water can be held for uptake by plants.

In germination rooms, temperatures may also rise out of range. In this case, lights are the most probable cause, and the propagator must carefully select certain lights to switch off temporarily. Where citrus is propagated through cuttings, bottom heating might be required if basal temperatures are not within the required range. Bottom heating can be avoided by timing the propagation period.

The humidity level often drops below the required level in germination rooms or in tunnels or greenhouses. Wetting the floor assists in building up the relative humidity, and plants, if already transplanted as single units, are mist-sprayed with a knapsack sprayer at least every 10 minutes, depending on the temperature.

Aeration, or ventilation, is used at night to remove excess CO₂ in the tunnels and to replenish the O₂ levels. However, this could be problematic in the winter periods, as the ambient air temperature outside the structure may be too cold and could cause damage to the plants. The propagation structure must be aerated carefully to reach the required range, taking all factors into consideration.



Chapter 1

- Environmental conditions impact on the growth rate of plants, by mostly impacting on two metabolic processes, being photosynthesis and respiration, or transpiration.
- During photosynthesis, the plant absorbs CO₂ and water, and uses heat to manufacture sugars (carbohydrates), oxygen, and water molecules.
- During respiration O₂ from the air is used to break down carbohydrates in the plant into energy that is used by the plant, CO₂ that is released into the air, and water molecules that are dispersed from the surface of the leaf.
- When the moisture in the surrounding air increases, plant transpiration decreases and light intensity influences transpiration through leaf surface temperature.
- Three classes of environmental conditions impact on plant growth, being atmospheric conditions, biotic conditions and edaphic conditions.
- Atmospheric conditions refer to light, humidity, temperature, water and aeration.
- Seed germination is activated by increased light intensity and light impacts on the rate of photosynthesis and respiration. Fluorescent lights are used in germination rooms to promote seed germination, and natural light is used in tunnels and shade houses.
- Relative humidity impacts on the transpiration rate of plants.
- Temperature impacts on photosynthesis and respiration rates. Germination requires relatively high temperatures, with a range of between 27°C and 32°C, with an average of 29°C being considered optimal.
- Water plays a role in keeping the plant cells turgid, as a catalyst in biochemical reactions in the plant, and in the translocation of manufactured compounds.
- Aeration in the leaf and root areas allows gaseous exchange that ensures that growth and development take place normally.
- Biotic conditions refer to bacteria, fungi and viruses in the rhizosphere, insects and weeds.
- Certain types of bacteria, fungi and viruses in the root-zone are beneficial and have a positive impact on the growth of the plant, while others are non-beneficial and have a negative impact.
- Insects are also classified as beneficial and non-beneficial. Examples of beneficial insects are *Aphytis lingnanensis*, *Chilocorus nigritus*, and *Cryptolaemas montrouzieri*, while non-beneficial insects include red mites, red scale, mealybugs, aphids, leafminer, and thrips.
- Weeds negatively impact on the growth of seedlings because they compete for water and nutrients.
- Edaphic conditions refer to physical and chemical properties of the growth medium in the root-zone, and the properties of the contained in which the plant is grown.
- Soilless media that are also used in nurseries include pine-bark, sawdust, peanut shell, river sand, composted organic material, and ash.
- In terms of propagation, physical growth medium properties refer to the texture, structure

and aeration of the soil. Soil depth and stratification are controlled in the nursery environment.

- Chemical growth medium properties are those characteristics that cannot be seen or felt, but influence reactions that take place in it and include the pH, salinity, and potential gas exchange reactions.
- Container properties are determined by the material used for the containers in which plants are grown.



Complete activities 1 and 2 in the **Learner Workbook**.



Electronic control systems that can be linked to a computer are also available for the monitoring of humidity and temperature. These generally come with additional options such as light intensity sensor, heating or cooling commands, and ventilation and dehumidification commands. As such, they provide the ultimate control mechanism to ensure optimum conditions for plant growth and development.

For citrus propagation, the recommended humidity level in enclosed structures is in the region of 80%, with an approximate 10% variation.

2.3. **Water**

Moisture levels are measured using soil moisture sensors which convert pressure, or water tension, in the soil to centibars of suction. Tensiometers may be used to measure available soil moisture in the root-zone. They are placed in the soil at different depths for better representation of the moisture front in the soil. Lengths of tensiometers range from 15cm to 180cm for different rooting depths.

If citrus seedlings are grown in containers in a citrus nursery, the containers do not provide enough depth for long and thick devices such as Irrometers. Although a 15cm Irrometer could be used, it is more appropriate to use hand-held probes.

Irrigation water represents a high risk area for a citrus nursery with regard to the presence of harmful pathogens, such as fungi and nematodes. To ensure that the water is not contaminated and free of harmful pathogens, the water must be treated.

Treatments begin with the flocculation, being the precipitation of silts and organic material, of the water stored in a reservoir. Flocculation makes use of aluminium sulphate based products, such as AluFloc. The amount of AluFloc that is used depends on the amount of soil particles and other organic material in the water. The flocculated, or clear, water is then filtered using a sand filter to remove any silt or organic material that was not precipitated successfully.

Any remaining propagules and harmful pathogen spores, such as Phytophthora and Pythium, are eradicated through chlorination at 2-3ppm preferably using chlorine gas, or otherwise sodium hypochlorite (Jik) or granular chlorine (HTH).



Propagules

Propagules are the shoots, seeds, or other parts that plants use to spread or propagate, either sexually or vegetatively.

Monitoring water quality requires a constant monitoring of the level of free chlorine available in the irrigation water. This can be done by using a swimming pool kit, or more sophisticated methods, such as Hanna Instruments HI9011. The presence of pathogen spores can only be detected through laboratory tests which should be done on a regular basis.

Chemical water quality factors are monitored by using pH and EC meters, which are combined in some devices. For improved nutrients uptake, the pH of the solution has to be between 5.8 and 6.2 with a maximum of 6.5. pH levels outside this range impair nutrient uptake. Critical levels are below 4.5 and above 7.5.

Electrical conductivity (EC) is used to monitor the salinity in the irrigation water, which may cause build-up in the root-zone. Excessive salinity may lead to death of seedlings. Levels above



Chapter 2

- Biotic and edaphic conditions are not likely to change quickly, but atmospheric conditions can change in a short space of time and must therefore be monitored.
- Light is not generally monitored in citrus nurseries, but can be measured by a light-meter.
- Humidity and temperature is monitored using a battery operated Hygro-Thermograph meter.
- Tensiometers and irrometers are used to measure soil moisture content, while laboratory analyses are used to monitor water quality.
- Unforeseen changes in atmospheric conditions are mostly due to changes in weather conditions.
- Records are kept of atmospheric conditions and of all actions that are taken in the nursery.



Complete activity 3 in the **Learner Workbook**.



**citrus
academy**



Chapter 3

After completing this chapter, the learner will be able to:

Demonstrate an understanding of the general propagation procedures and select appropriate procedures within a specific agricultural production context

1. Introduction to Budding

Budding is a form of grafting, which is a vegetative plant propagation method where a scion bud from a selected tree of the desired cultivar and variety and with desirable traits is inserted into a rootstock seedling to produce a tree.

The term budding therefore refers to bud grafting, meaning a technique of grafting using a single bud as the graft scion where each part, the scion and the rootstock, have distinct roles to play.

2. Rootstock Selection

Healthy rootstock seedlings are produced and raised through germination. The choice of the rootstock depends on the following factors:

- Predictable performance in environmental conditions in the orchard, which include the water quality, and the physical and chemical properties of the soil, such as soil pH and salinity;
- Resistance or tolerance to diseases;
- Compatibility with the scion;
- The market entrance timing in relation to time to maturity; and
- Internal fruit quality

The characteristics of the most commonly used rootstocks are shown in the table below.

	<i>Rough-lemon</i>	<i>Swingle Citrumelo</i>	<i>Carrizo Citrange</i>	<i>X639</i>	<i>C-35</i>	<i>MXT</i>
Disease Factor						
Exocortis	Tolerant	Tolerant	Sensitive	Susceptible	Susceptible	Susceptible
Tristeza	Tolerant	Tolerant	Tolerant	Tolerant	Tolerant	Tolerant
Phytophthora	Susceptible	Tolerant	Tolerant	Susceptible	Tolerant	Tolerant
Citrus nematodes	Susceptible	Tolerant	Susceptible	(Not known)	Tolerant	Tolerant
Soil Factor						
Poor drainage	Susceptible	Tolerant	Sensitive	Sensitive	Tolerant	Sensitive
High clay content	Sensitive	Intermediate	Intermediate	Sensitive	Tolerant	Intermediate
High sand content	Tolerant	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
High chlorides	Tolerant	Intermediate	Sensitive	Intermediate	Sensitive	Sensitive
High pH	Tolerant	Sensitive	Sensitive	Tolerant	Sensitive	Sensitive

	<i>Rough-lemon</i>	<i>Swingle Citrumelo</i>	<i>Carrizo Citrange</i>	<i>X639</i>	<i>C-35</i>	<i>MXT</i>
Drought	Tolerant	Intermediate	Intermediate	Sensitive	Sensitive	Sensitive
Replant	Sensitive	Tolerant	Intermediate	Sensitive	Tolerant	Intermediate
Tree Performance						
Tree growth rate	Vigorous	Moderate	Moderate	Moderate	Slow	Moderate
Final tree size	Large	Medium	Medium	Medium	Small	Medium
Cold hardiness	Poor	Good	Good	Good	Good	Good
Longevity	Fair	Good	Good	Fair	Good	Good
Yield per tree	High	Good	Good	Good	Good	Good
Fruit quality	Low	Good	Good	Good	High	High
Rind colour development	Intermediate	Late	Early	Early	Intermediate	Intermediate

Table 3.1: Characteristics of Commonly Used Rootstocks

Adapted from *Citrus Rootstocks: The Choice You Have* – Louis A. Von Broembsen

Rootstocks are produced through seed propagation. Seeds are bought from certified sources or collected from the fruit of rootstock variety trees that are grown specifically for this purpose. The seeds are sown either outside in sand seedbeds, or, more commonly in South Africa, in seed-trays that are kept in a controlled environment in germination rooms. All seed trays are clearly marked with water-proof identification labels stating the variety, date sown and source code.

Due to various reasons that lead to losses, about double the quantity of seeds required for rootstock to be budded, is sown. Reasons for losses include:

- Death of seedlings due to diseases which result from excess water in the growing medium, such as damping-off and phytophthora.
- Off-types, which are seedlings that are not genetically true-to-type.
- Albinism, which is a condition that expresses itself at the seedling stage if not treated properly.
- Seeds that have been sown deeper and take longer to germinate, which lead to the considerable risk of seedlings emerging with defects associated with reduced levels of chlorophyll.
- The medium used is also a potential cause for losses. Compacted media makes radicle development more difficult because there is less space for the radicle to navigate freely. A larger number of seedlings produced will have bench roots, which are tap roots that are distorted and bent instead of being straight.

The best seedlings are transplanted into trays with individual cells filled with bark mixed with a slow release fertiliser. These seedlings are raised to become rootstocks for budding. The seedlings trays are normally kept in tunnels where the environment can be regulated.



Figure 3.1: Carrizo Rootstock in Cavities

Three to four months after transplanting from the germination tray into the seedling trays, the seedlings are transplanted into bigger containers, such as four litre polyethylene bags and placed in shade-cloth structures to grow and develop for budding.

At this stage, losses may occur through selection, as more seedlings may display unwanted traits that have been hidden previously. These seedlings are referred to as off-types.

Management at this stage is concerned with creating an environment conducive to transpiration and photosynthesis taking place at a good rate. Each batch of seedlings retains its identity label.

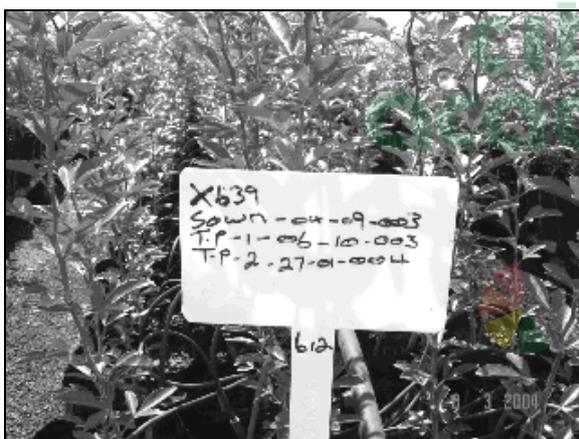


Figure 3.2: X639 Rootstock Labelling

Budding takes place about four to six months after the seedlings are transplanted.

The bud itself comes from a twig cut from the selected cultivar with desired traits. The twig constitutes the bud-wood and is referred to as the **scion**. The bud-wood must have enough food reserves for the bud to survive before it is taken over by the rootstock cambium.

3. **Bud-Wood Selection**

All bud-wood that is used in certified citrus nurseries in South Africa is provided by the Citrus Foundation Block (CFB) which is situated in the Eastern Cape. The CFB falls under the management of Citrus Research International (CRI). The bud-wood is certified to be true-to-type, meaning that the type and cultivar of the bud-wood is guaranteed. The bud-wood is also certified to be free of all known graft-transmissible diseases, such as exocortis, except for a mild strain of tristeza virus that is specifically inoculated into the bud wood source plants and acts as a protection factor against severe strain infection.

The selection of the bud-wood plays an important role in achieving success. The selection has to be done at the right growth stage. Scion material is collected from healthy and young twigs that are

actively growing. The shape of the bud-wood is not clearly prescribed. Some propagators use buds from well rounded bud-wood sticks, while others prefer using those from slightly angular shaped sticks.

4. **Budding Procedure**

The ultimate goal of the propagator is to produce a successful union between the scion and the rootstock. To ensure a successful union, the propagator must aim at maximum contact between cambiums of the rootstock and scion. To this end, tissues must be matched correctly, and care must be taken to ensure that they are bound and remain in contact.

Budding in nurseries is carried out at a time of year that promotes the success of the operation and subsequent union. The most ideal time is in late spring and summer when the sap flow is optimal in the rootstock. There must be clear signs of growth on the rootstock, such as new leaves and shoots.



Figure 3.3: Bud-wood from the Foundation Block

An inverted T-cut is made on the stem of the rootstock at a height of about 20cm above the growth medium. For the sake of uniformity, the propagator ensures that the height of budding is the same throughout.

The depth of the cut is shallow, being just deep enough to lift the bark. The thickness of the stem is important because it has to lend itself to manipulation. The thinner the stem, the more difficult it is to bud onto.



Figure 3.4: The Inverted T-Cut: Vertical Cut (left) and Lateral Cut (right)

The scion is inserted into the T-cut and pressed down firmly to ensure that the tissue comes into contact. The joint is wrapped and sealed to allow the wounds to heal.

After inserting the bud into the rootstock, it has to be wrapped using a polyethylene plastic strip to maximise the contact between the rootstock and the bud, and keep water out as it might infect and rot the bud.

7. Other Propagation Methods – Shoot Tip Grafting

In citrus propagation, shoot-tip grafting is the method used to eliminate any viruses that could be hosted in the plant material. Viruses tend to move more slowly in the plant in the new cells multiplying in the meristem of new buds, and one is therefore able to graft the virus free portion of the meristem of a bud onto a virus free rootstock.

Under laboratory conditions the procedure is as follows:

- Cleaning the growing point
- Rootstock preparation
- Grafting

7.1. Cleaning Growing Point of Material of Viruses

- Pick a 1cm to 10cm tip of a new shoot and place in a Petri dish to prevent it from drying out.
- Break off the large leaves under a stereo microscope.
- Sterilise for 5 minutes in a 7.5% Jik solution
- Rinse twice in sterile distilled water (SWD)
- Transfer to a third rinse solution until needed. If kept overnight, remove SWD and store at 4°C.

7.2. Rootstock Preparation

- Place a germinating rootstock seedling into a test tube with an artificial growing medium.
- Once the rootstock has etiolated, remove it from the test tube.
- Cut off the top to give about 5cm long stem and remove the cotyledons (seed leaves).
- Trim roots so that it can be easily replaced in the tube.
- Make a horizontal cut into the cortex approximately 10mm below the top.
- Cut down from above this cut to form a chip 1mm to 2mm long.
- Check that the bark comes freely away and then press it back to prevent drying out.

7.3. Grafting (STG)

- Under a stereo microscope, break off the remaining leaves of the shoot tip of the cultivar until the meristem dome is visible.
- Cut off the top 0.15mm of the meristem.
- Transfer to the stem of the rootstock in an upright position on the cambium.
- Plant the grafted plantlet into liquid medium in a test tube and label it with necessary details.
- Transfer the test tube to a growth room and monitor twice weekly.

- Remove rootstock outgrowths regularly.
- The grafted material should start to grow out after two to three weeks, about 5mm long with small leaves.



Chapter 3

- Budding is a form of grafting, where a bud from a selected tree is inserted into a rootstock seedling to produce a tree.
- The choice of the rootstock depends on predictable performance in environmental conditions in the orchard, resistance or tolerance to diseases, compatibility with the scion, market entrance timing in relation to time to maturity, and internal fruit quality.
- Rootstocks are produced through seed propagation and because losses of seedlings occur due to a variety of reasons, double the amount of seed is sown.
- Rootstock seedlings are planted in seedlings trays and then transferred to larger containers three to four months later.
- Rootstocks are always labelled carefully with the cultivar, planting date and transfer dates.
- Budding takes place about four to six months after the seedlings are transplanted.
- All bud-wood that is used in certified citrus nurseries in South Africa is provided by the Citrus Foundation Block.
- To promote a successful union between the scion and the rootstock, maximum contact between the cambiums of the rootstock and the scion is required.
- There must be signs of active growth in the rootstock and the bud-piece from which the scion is taken.
- An inverted T-cut is made on the stem of the rootstock and the bud is inserted and secured with a polyethylene plastic strip.
- Sanitation and hygiene is essential for preventing contamination, and especially cutting tools must be sterilised properly.
- Budding knives and pruning shears are the main tools that are used during budding.
- Shoot tip grafting is another propagation method that is used to eliminate viruses in the plant material.



Complete activities 4 and 5 in the **Learner Workbook**.

- **Sweeping rakes** are used for cleaning in and around the nursery or shade-house.
- **Leather gloves** are used for certain operations such as staking to prevent injury while pushing the stake into the bag.

2. **Health, Hygiene and Safety**

In general, issues of health, hygiene and safety are addressed by the Occupational Health and Safety Act (OHSA). The health and safety of all persons working within and around the nursery has to be considered against all hazards that can be found in this environment.

As stated in the OHSA, there must be an anticipation, recognition, evaluation and control of conditions arising in or from the workplace, which may cause illness or adverse health effects to persons.

Personal protective clothing is the second line of defence after a sound health and safety policy. Protective clothing includes vinyl gloves, overalls, masks, and gumboots.

Other aspects to be included are a washing point for disinfection and cleaning in general, a dumping site for unwanted and used items, and specific disposal methods of used items

3. **Storage of Tools**

Tools used for propagation are stored in areas with controlled entrance and use. Access to these must be coordinated with a tools register that records the use of each tool and its condition before and after use. Gloves, overalls, masks, and gumboots are stored in locked cupboards. Wheelbarrows, sweeping rakes, watering cans, spades, and hosepipes are kept in a storeroom.

The propagator must ensure that tools are grouped according to their use and kept in suitable areas in terms of hygiene and safety.

Chapter 4

- The tools and equipment used commonly in South African citrus nurseries are seed trays, seedling trays, watering cans, knapsacks, soil augers, jugs, spades, planting containers, mesh panels, hose pipes, stakes, collection bags, wheelbarrows, tying machines (tapeners), rakes, and leather gloves.
- Health and safety is addressed by the Occupational Health and Safety Act (OHSA), which prescribes that conditions that may threaten the health and safety of workers must be anticipated, recognised, evaluated, and controlled.
- Tools and equipment must be stored in clean and dry facility that is kept safe and secure.

Complete activities 6 and 7 in the **Learner Workbook**.



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