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The Basics of Plant Tissue Culture

Micro propagation or plant tissue culture (TC) has become more common in the nursery industry and its use is becoming more widespread as new plant varieties are brought onto the market in large numbers. Plant tissue culture can be a complicated and technical process that should be, generally speaking, left to the experts. The de-flasking of tissue cultured plantlets for propagating is a much simpler procedure that you can do in your production nursery. As with any process, there is a right and wrong way and there are many things that can impede success. In this Nursery Paper, Industry Development Officer for Victoria, Robert Chin, will introduce you to the basics of the plant tissue culture process, focusing on de-flasking, and the dos and don'ts to ensure that you maximise your returns and produce a saleable crop.

The Basics of Plant Tissue Culture

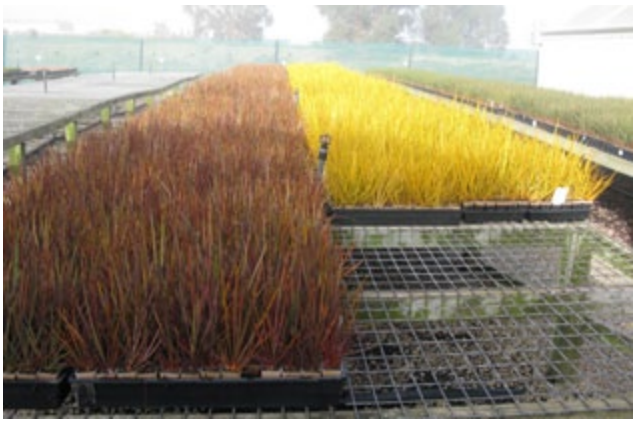
There are some production nurseries that are experienced at de-flasking tissue cultured plantlets and some that need to improve their processes. It is fair to say that the best results are obtained when it is understood that:

- All plant species are different and require their own specific treatment
- Individual tissue culture laboratories do things differently therefore the same plants from various laboratories can have unique requirements
- Hygiene is paramount (staff, facilities, containers & growing media)
- There can be high levels of losses if not done well
- De-flasking is not "do it and forget" – plants need to be monitored and conditions and treatment changed as they go through the growth cycle
- You can do something once and it works however the next time you do it exactly the same (apparently) you may get different results

De-flasking can be complicated initially but once mastered it can become an invaluable tool for your production nursery and an important part of your propagation process.

What is Tissue Culture?

Micro propagation is the practice of rapidly multiplying stock plant material to produce a large number of young plantlets, using modern plant tissue culture techniques. Plant tissue culture is a process used to propagate particular plants, in-vitro, on artificial growing media under sterile conditions, to produce clones (identical plantlets) of those parent plants. In short, with this form of propagation you have the potential to produce large numbers of identical plants (clones) in a short timeframe. This makes it a very desirable multiplication technique capable of producing large numbers of clones, from a small motherstock base, for quick entry into the market.



De-flasked plants hardening off at Greenhill's Propagation Nursery

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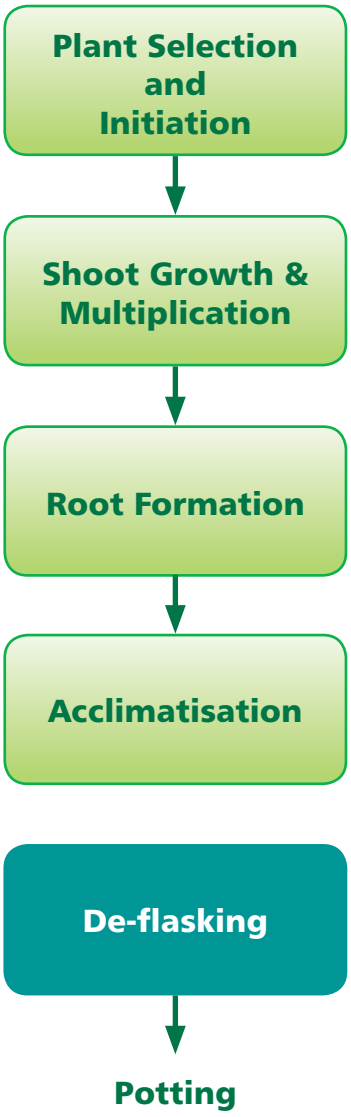
Steps in the Process

There are a number of steps in the process of tissue culturing plants as identified below. Most production nurseries will only be concentrating on the de-flasking component therefore, in this Nursery Paper we will concentrate on this step within the process.

The diagram below shows the major steps in the process of tissue culture:



Major Considerations when De-flasking Tissue Cultured Plantlets.

Tissue cultured plantlets are young plants, therefore, as with any living organism, they require the most attention. Given the right conditions de-flasking will be successful however, the difficulty is that many growers cannot always produce those ideal conditions. Things can go wrong, and when this happens in tissue culture the consequences can be significant with major crop losses.

1) Light Levels

As with other forms of plant propagation, it is important to get the levels of light correct. Inadequate light levels do not allow the plants to produce enough energy through photosynthesis and too much light can burn the plantlets and is a waste of energy. In Australia, light levels are measured in Lux. All good propagators must have an appropriate light meter.

Light levels from between 7,000 and 20,000 Lux will be ideal for most propagation. Ambient light levels in Australia can be 40,000 Lux, therefore shading using screens or shade cloth is normally required. Light levels can be adjusted as the plantlets develop with shade levels required being typically 50-80% of ambient light levels.

Inside a small TC lab – some new initiations. Note the lighting and sterile conditions

2) Humidity

Humidity levels are critical to achieving successful results in de-flasking. Humidity levels will need to vary depending on a range of factors however, what is most important is that you vary the humidity levels as your plants move through the growing cycle. Typically higher levels are needed at the start and they get decreased as the plants become more established. If the humidity level is too high the plants are more susceptible to rotting due to increased fungal/bacterial diseases and if they are too dry desiccation will become an issue and the plantlets will dry out.

Humidity levels vary from 90% (initially) to 70% (when hardening off).

Humidity tunnel at Australian Roses – this allows good control of humidity levels

3) Growing Media

New growing media is essential as this offers the best point of disease freedom. Different growing media mixes are used by individual growers but normally a modified propagation mix is used. Not all growers use fertiliser in the initial propagation growing media and those that do, use a 'mini' controlled release fertiliser (CRF). Most growers use a mix of peat and perlite. Some production nurseries mix their growing media themselves and others buy it pre-mixed. Recipes varying between growers.

4) Hygiene

Not enough can be said about the importance of hygiene in de-flasking tissue cultured plantlets. It should be noted that at every stage of the process, attention should be paid to ensuring that these young and susceptible plantlets are not infected by plant pathogens (diseases). Ensure that:

- All tools/implements that come into contact with the plantlets are sterile
- The de-flasking station is separate from growing areas
- Working surfaces (benches/tables) and containers are clean and sterile/disinfected
- Growing media is new and pathogen free
- Staff involved use high hygiene principles including the wearing of gloves at all times

5) Conditions of Plantlets on Arrival

Whilst you would expect that when you order TC plantlets they would arrive in prime condition, this is not always the case. Sometimes they can be damaged in transit or they are not quite ready to de-flask when you receive them. Either way they might not be ready to de-flask straight away and may need to be stored for a period of time in the appropriate growing area. Always immediately un-pack and sort your shipment of young plantlets.

If plantlets are not ready to de-flask (e.g. roots are not developed enough) you will need to grow the plantlets on in your greenhouse until they reach appropriate development. If they are damaged in transit you may need to pay extra attention to them when you de-flask and take care not to cause further injury. Some plantlets can be de-flasked without obvious root initiation. In either instance you should pay careful attention to these plantlets as they begin establishment.

If there is obvious contamination (disease) in the containers you may want to dispose of that container or de-flask the plantlets and keep them isolated from other plants until a diagnosis is determined.

6) Nutrition

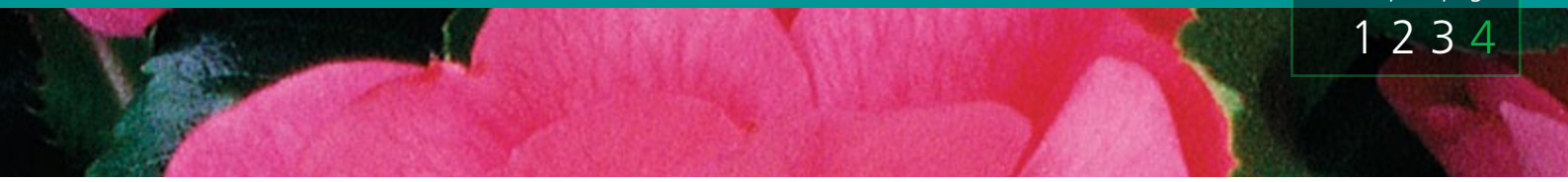
Many growers (not all) use a controlled release fertiliser, normally a 'mini' formulation in the growing media when planting out. Trace elements and some supplements are also commonly used.

Liquid feeding is often applied once the plants start hardening off.

It is important to note that de-flasking tissue cultured plantlets should be approached in the same manner as any other growing/propagation procedure. Attention to hygiene, the climate, growing conditions and management is important.

Some TC plants at Majestic Selections – ready for de-flasking

Siew Teoh of NMIT regularly teaches tissue culture to nursery people.



What the experts do

It is an understatement to simply state that different production nurseries do different things in the area of de-flasking. Here is some information from three experts in the area of de-flasking and their various techniques.

(See table right)

As with all areas of plant propagation there is a lot of variabilty in de-flasking tissue cultured plantlets and each grower needs to trial the process to establish the most appropiate technique. Tissue culture is a developing trend as a way of multiplying plants and this will increase particularly for new plant varieties that are well suited to this process. If you decide to de-flask plants supplied from tissue culture laboratories you need to be aware that there are a range of factors to consider. You can do this and get positive results but if done poorly you can incur high financial losses. Ensure that you do your research and have the right conditions and practices in place. Talk to other people that are de-flasking successfully before you start and experiment with small orders before 'jumping in feet first'.

Factor	Greenhill's Propagation	Mansfield's	Majestic Selections
Time taken	3 months	2-3 months	10-12 weeks
Ideal Temperature – ambient	27-29°C	De-flasked at room temp – must be less than 32°C	24-28°C
Ideal Temperature range – bottom heat	24-27°C	18-20°C Use plastic covers over trays	24-28°C
Humidity Range	65-100%. Start high and reduce over time – say 1 week.	Individual lids on trays 85% + 1 week Open vents then remove lids	75-80% then to 60-75%
Light levels – Range	Restricted – less than 70% and increased slowly	Variable – light levels relative to daylight	70-80% shade
Growing Media	Peat 30% + Perlite 70%	80% course and 20% fine perlite + 10% peat	Coco-peat 90%-10% perlite
Nutrition	Not normally required	Fertiliser in the mix CRF Mini	CRF at mixing Foliar feed weekly after first week
Watering	Initial watering with fungicide drench – after 7 days – hand watered (light) and every 2-5 days depending on season	Water initially and then to heat benches Water + fungicide drench and then 1 week	Not in first week then as required
Dont's	Allow to dry out Keep it covered when not working on them Planted too deep – causes rot	Winter – be careful rot easier – don't grow as fast	Allow to dry out – do it quickly Don't plant plantlets upside down Observation
Do's	Hygiene – gloves and working areas/surfaces Hand mist whilst working on them Roots need to be well in the mix to develop and stop them drying out	De-flask asap on arrival. Don't leave plants exposed without planting for more than 15 minutes Hygiene, hygiene, hygiene	Sterile Only Plant with forceps Trim if required Observation Botrytis
Upon arrival	Immediately un-pack and sort Store in greenhouse until ready to go (roots)	Dictated by ambient temperature Check for contamination	Check for contamination
Storage	Store in polystyrene boxes at say 9°C	Only if necessary– store in cooler area (fridge at say 12°C)	In light area, 24°C un-opened for up to 2 weeks
Others	Fungicide drench when watering. Tepid water to wash-off when de-flasking. Soft touch /gentle when handling – size/softness Variability is the norm – even between – don't assume the same – even from different labs.	Some get dipped in hormone Some get initial support roots (if too many – back to the callus) Anti-transpirant (Envy) applied when opening containers TC plantlets are not as delicate as people think	When washing off they use tepid water with a trichoderma and foliar feed mix. Don't need to get all agar off

Further information and acknowledgements:

- Harman & Kester et al: 2001. Plant Propagation: Principles and Practices. 7th Edition.
- NGIA, 2006. NIASA Best Management Practise Guidelines.

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