Deflasking Micropropagated Plantlets

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INTRODUCTION

Micropropagation is the multiplication of plants under sterile conditions. Plant parts, including cell, bud, stem, and leaf, can be used as explant sources. The desired piece of tissue is placed on an appropriate culture medium and induced to produce shoots and roots under controlled laboratory conditions. Practical applications include the rapid multiplication of plants, the multiplication of otherwise difficult to propagate plants, and the propagation of rare and endangered species. In addition, plants in culture are easy to import and export as the small, lightweight containers are easy to handle, and contain no soil.

DEFLASKING

The following method is used by Redlands Greenhouses with excellent success, particularly for herbaceous and tropical foliage plants.

Hygiene. Sanitation is of vital importance when transplanting from tissue culture. As the plants have previously been kept in a pathogen-free environment, they are highly susceptible to fungal and bacterial agents. Prior to deflasking, we disinfect the propagation area with Hibitane. Tools and equipment are also sanitised.

Media. Our standard propagation medium consists of peat and perlite (1 : 1,v/v). This medium is usually mixed by hand, 3 cubic metres at a time. No nutrients are added at this time. Dolomite lime is used to bring pH to 6.0.

Containers. Two types of continaers are used.

- Fifty-milliliter plastic tubes packed into wire trays which hold 100 tubes;
- Plastic liners for standard seedling trays 30 × 26 cm, each having 48 cells.

Sterilisation. Once the medium is packed into the containers, the trays are stacked four high and treated for 48 h with methyl bromide under a tarpaulin. The stack contains 60 trays holding a total of 6,000 tubes. Once aired, the trays are taken to the propagation room.

Equipment Needed for Deflasking. Clean basin, warm water (approximately 40°C), scalpel, atomiser bottle, dibble stick, disinfectant, and clean newspaper are needed.

Method. Sanitise the scalpel and dibble stick in a container of disinfectant. Place clean newspaper on the work bench and fill the basin with tepid water. After opening the flask, gently extract the plants and place them in the warm water. In most instances the culture medium (agar) will adhere to the root system. We prefer

to wash off all agar. Preferably this process should be carried out under running water in a trough. This should eliminate any chances of cross-infection should the agar or plants be contaminated. This facility is not available in our propagation area, so we use the basin method. Any contaminated flasks are left until last, again to avoid risk of cross-infection. As a precaution, fungicide or disinfectant may be added to the water.

Trials. In 1984 we conducted trials with *Spathiphyllum* to ascertain possible benefits of leaving the agar, which may still have some nutritional value, intact on the roots. There was no difference in the growth of roots or plants with or without agar. We did find, however, that the plants were easier to process when completely bare rooted.

Once the agar has been removed, place the plants on the newspaper. Some division may be necessary at this stage. Quite often with tissue culture, two or more plantlets are joined on one piece of callus tissue with some roots projecting from the callus pad. These plantlets are easily divided by using a sharp scalpel and slicing cleanly between the plant crowns. We do endeavour to leave a root piece on each plant if possible. This method of division has not proven detrimental to the subsequent growth of plants. The root system can also be trimmed if it is too extensive, as it is often difficult to insert plants with long roots into the medium. Again this seems to have no detrimental effects as the roots of tissue-cultured plantlets are rather ineffective at absorbing moisture for some days after transplanting. The plants are too fragile to be inserted into the medium without the use of a dibble stick.

At no time during the transfer stage should the plants become stressed. They have been used to a high humidity environment and deteriorate rapidly under normal glasshouse conditions. Spraying the deflasked plants with water from an atomiser will alleviate this problem until they can be placed in a humid environment. Tools are disinfected after each flask and clean newspaper and water are used for each flask.

Grading. The plantlets should be graded at planting, so as to avoid problems of varying plant sizes at the potting-on stage.

Environmental Conditions. Under controlled laboratory conditions, the relative humidity remains at 100%. Because of this, the plantlet leaves are immature, with no protective waxy cuticle and their stomata remain open, leaving the plant unable to control water loss. Therefore, high humidity conditions are essential during the first two weeks to avoid stressing the newly transferred plants.

To achieve a humidity of at least 95%, we at Redlands Greenhouses have designed polythene tents under which the plants are reestablished. The frame is constructed from rigid PVC tubing and suspended by wire from the framework of the glasshouse. Opaque plastic is draped over these structures to form a tent, 8.9 × 1.2 × 1.6 m. The tents are situated inside the propagation glasshouse on benches that are heated with hot water. These benches are covered with black plastic which extends to the concrete floor. This arrangement helps trap heat and the plastic is easily sanitised between crops. The opaque plastic of the tent extends past the bench tops to keep in the heat and to allow some sealing.

Misting nozzles, controlled by a time clock, run the full length of the benches inside the tents. The plants are misted for 8 sec every 10 min. The nozzles are

regularly cleaned and checked for blockages to ensure that there are no dry spots. Inverted wire trays are placed over the trays containing the plants. On top of these trays, newspaper is spread, one-sheet thick. This newspaper:

- Reduces light intensity and so prevents the plants from being burnt. Light intensity in most greenhouses is far higher than it is in tissue culture labs.
- Ensures that heavy water droplets do not damage the plants and helps avoid over wetting.
- Increases the humidity level around the plantlets and keeps it constant.

Because fungi and bacteria thrive under these conditions, it is very important to inspect the plants every morning. Any diseased material should be promptly removed.

Hardening-Off. The newspaper can be removed after 7 days. The following week the sides of the tent can be raised during the hottest part of the day, and lowered again at night. This procedure is continued, with the sides being raised for extended periods each day, until the plants have hardened-off sufficiently to allow the tent to be raised permanently. The hardening-off time varies between species, but it usually takes 3 weeks. Misting can also be reduced during this stage.

After-Care. Once established, the plants are transferred to the general indoor hardening- off section to grow until they are ready for potting on. During this time they are liquid fed through the watering system and will benefit from two to three grains of nutricote per tube. Some species, e.g. *Platycerium*, will continue to multiply during this stage. The smaller plants are easily removed for replanting.

CONCLUSION

No doubt many nurseries have their own methods of re-establishing tissue-cultured plants. This method works well for us and ensures a high survival rate.