

# RE-ESTABLISHING PLANTLETS FROM TISSUE CULTURE: A REVIEW

A.J. CONNER AND M.B. THOMAS

*Department of Horticulture,  
Lincoln College,  
Canterbury, New Zealand.*

**Abstract.** The literature pertinent to the re-establishment of tissue-cultured plants *in vivo* is reviewed. The difficulties associated with survival and growth of tissue cultured plants after transplantation are attributed to the poor control of water loss from the plants and their necessity to switch from heterotrophic to photoautotrophic nutrition. Aspects discussed include: the possibility of transplanting directly from stage II shoot proliferation cultures and rooting *in vivo*; the importance of stage III culturing for preconditioning plants prior to transplantation; the optimum sizes of propagules and substrate preferences for transplantation; stress reduction, disease prevention, and the importance of humidity, temperature and light levels during transplantation. The relative merits of numerous approaches to transplantation by various workers for many species are discussed. Suggestions of areas in which further work is urgently required are given, along with some recommendations and general guidelines for the re-establishment of tissue cultured plants *in vivo*.

## INTRODUCTION

Plant tissue culture has become very important in horticulture for the rapid clonal propagation of plants (53-57). In recent years this technique for plant propagation has surpassed the mere stage of laboratory research and resulted in the establishment of many laboratories throughout the world to mass produce a wide variety of plants (especially ornamentals) on a commercial scale. Although there has been considerable research to optimize the nutrient medium and culture conditions for numerous plant species/cultivars, there has been a general lack of research examining problems associated with the re-establishment of tissue-cultured plants *in vivo*. This is unfortunate because the ultimate success of plant tissue culture as a commercial means of plant propagation depends on the ability to transfer plants out of culture on a large scale, at low cost, and with a high survival rate.

In this paper we review the literature relevant to the transfer of plants from tissue culture. Our intentions are to identify those areas in which further research is urgently required for the commercial exploitation of plant tissue culture and to provide some suggestions and general guidelines for the large scale re-establishment of tissue cultured plants *in vivo*.

## PROBLEMS ASSOCIATED WITH TRANSPLANTING FROM TISSUE CULTURE

A sound knowledge of general nursery propagation meth-

ods is very important when attempting transplantation from tissue culture. However, it should be realised that plant propagation via plant tissue culture differs from conventional nursery practice in several key aspects, an understanding of which is necessary to maximise survival rates when re-establishing cultured plants *in vivo*. The plantlets used in tissue culture are usually much smaller and held in more precisely controlled environments than nursery seedlings or cuttings. Furthermore, the plantlets are cultured under aseptic conditions on nutrient media containing exogenous sugars and plant growth regulators. A third key aspect, especially relevant to transplanting, is that in tissue culture plantlets are grown in very high humidities, about 100 percent.

Due to the very precise conditions under which plantlets are cultured *in vitro*, carefully controlled hardening-off procedures are necessary for survival when transplanting from tissue culture. Even when supposedly gradual hardening-off procedures are carefully followed, poor survival rates are frequently reported.

Tissue cultured plants are difficult to transplant for two main reasons; firstly, their heterotrophic mode of nutrition and secondly, their poor control of water loss.

The plantlets used in tissue culture are very small and their growth requires an exogenous sugar (usually 2 to 3% sucrose) in the culture medium. Although the plantlets may appear "fully functional" physiologically, they are unlikely to be actively photosynthesizing — simply because it is unnecessary. Even if chlorophyll is present in the leaves, it is probable that the enzymes responsible for photosynthesis are inactive or absent. The transition from the heterotrophic state to the photoautotrophic state during the transplantation of *Brassica oleracea* Botrytis group plantlets from tissue culture has been studied by Grout and associates (30,31). They found that in culture there were very low levels of photosynthesis (estimated through the activity of the Hill reaction,  $^{14}\text{CO}_2$  uptake and net  $\text{CO}_2$  exchange in light and dark), despite the green appearance of the plantlets (although chlorophyll levels were also low). Active growth in culture was therefore dependant on the exogenously supplied carbon source. When such plants are transplanted, there is an immediate necessity for the regenerated plants to assume a fully photoautotrophic nutrition. Even seven days after transplantation of *B. oleracea* Botrytis group plants from tissue culture, there was still no net  $\text{CO}_2$  uptake (ie:  $\text{CO}_2$  released in respiration was greater than that taken up by photosynthesis) (30). A net uptake of  $\text{CO}_2$  was not achieved by these plants until 14 days after transplantation, at which stage they had become fully photoautotrophic and could sus-

tain normal growth. Therefore, poor development of the photosynthetic system in tissue culture may be a major factor causing newly transplanted plants to be vulnerable to any form of environmental stress.

There have been several investigations examining the problems associated with the water relations of plantlets on their transfer from tissue culture. The leaves of tissue-cultured *Prunus insititia* plants have smaller palisade cells, larger intercellular spaces and lower stomatal frequencies compared with transplanted plants (13). Such leaf anatomy is characteristic of leaves grown in high relative humidities and is also more sensitive to water stress (24). In tissue-cultured plants, poor vascular connections between the shoots and roots may reduce water conduction. Such morphological abnormalities have been reported in tissue cultured *Brassica oleracea* var. *botrytis* plantlets, where no water transfer from roots to shoots could be detected (29).

Scanning electron microscopy studies have revealed a considerable reduction or absence of epicuticular waxes on the leaves of plantlets produced in culture compared with greenhouse grown plants (28,29,31). There was an increase in the density of structured epicuticular waxes occurring in response to a gradual decrease in humidity during the hardening-off process. For *Brassica oleracea* Botrytis group this was also associated with an increase in both the weight of epicuticular waxes and the water contact angle, and a decrease in the rates of water loss from plant tissues (28,29,31). Because the wax component of cuticles determines the rate and extent of water diffusion through the cuticle (48), the extreme susceptibility of tissue-cultured plants to wilting on transplantation was initially attributed to a severe reduction during culture in epicuticular wax formation and possibly a deficiency of wax within the cuticle (28,29,31). However, more recent evidence suggests that this may be due to stomatal as well as cuticular phenomena.

A considerably higher rate of water loss from excised leaves of tissue-cultured plants than plants transferred to a greenhouse has been demonstrated in *Malus domestica* (12), *Prunus insititia* (13) and *Solanum laciniatum* (18). In these experiments, the tissue-cultured leaves lost more than 50 percent on their moisture content within 30 minutes for *P. insititia* and 70 minutes for *S. laciniatum*, compared with 90 and 140 minutes respectively for transplanted leaves. Electrolyte leakage and ethylene/ethane analyses have indicated cell injury in *P. insititia* leaves at 50 percent water loss (42). The rates of water loss and stomatal closure have been examined in excised leaves of *M. domestica* plants during acclimatization from culture to the greenhouse (12). The results demonstrated

that the high rates of water loss from tissue-cultured leaves are attributable to slow stomatal responses. With five days acclimatization at 30 to 40 percent relative humidity, these leaves had regained normal stomatal functioning. Microscopic examination of detached *S. lacinatum* leaves (initially fully turgid) over a 16 h period, demonstrated that leaves of transplanted plants had closed all of their stomata within 30 minutes of detachment, whereas half of the stomata from tissue-cultured leaves were widely open after 16 hours (18). Measurements of water loss from *P. insititia* leaves with either the adaxial, abaxial, both or neither surfaces coated with silicon rubber have clearly shown that the water loss occurs solely through the abaxial surface (25). All the stomata on these leaves are located on this surface (12).

Therefore, it appears that the rapid wilting of tissue-cultured leaves immediately after transplantation can be attributed to the inability of their stomata to close, rather than reductions in wax components of leaf cuticles. This is because the leaf cuticles are primarily effective in controlling water loss only after stomatal closure. Nevertheless, later acclimatization involving cuticular development is no doubt important once the stomata are fully functional, and is probably essential before transplanting plants from a greenhouse into the field. The reason why tissue-cultured plantlets have inactive stomata and poor cuticular development remains to be shown. This probably relates, to some extent, to the culture environment (especially the very high humidity in culture vessels); however, the culture medium (particularly the plant growth regulator component) may also have an important influence. Once such casual factors are clearly understood, then possible treatments to help avoid the wilting response during transplantation can be examined.

The problems associated with transplantation from tissue culture may be alleviated to some extent during the final stages of tissue culture as discussed later in this paper.

#### TRANSPLANTATION FROM STAGE II CULTURES

Four important stages of plant tissue culture have been defined by Murashige (53,56) for use in plant propagation:

- Stage I — establishing an axenic culture,
- Stage II — the multiplication of propagules,
- Stage III — the preparation of propagules for transfer to soil and
- Stage IV — the re-establishment in soil.

Although propagules of some cultivars from stage II have been directly transplanted out of tissue culture, difficulties are fre-

quently encountered with some cultivars and may be overcome by a preparatory step (stage III) just prior to transplantation.

Transplantation from stage II cultures involves the rooting of individual shoots after their removal from tissue culture, i.e.: treating them as micro-softwood cuttings. In fact, shoots from *Vaccinium ashei* cultures have proved to be easier to root than conventional softwood cuttings (46). A distinct advantage of such early transplantation for commercial plant propagation is that it by-passes the need for stage III culturing. Cost analyses for propagating plants via tissue culture indicate that labour comprises over 65% of the total tissue culture production costs and that stage III requires a considerable labour input for subculturing with usually no increase in the number of propagules (6,5,22). Substantial savings per unit plant produced from tissue culture have been calculated for *Begonia rex* (22), *Brassica oleracea* (6) and *Ficus elastica* (22) if stage III is eliminated.

Successful rooting and growing on of tissue-cultured shoots after their transplantation from culture has been achieved in a wide variety of species. In several species pretreatment of the bases of excised shoots with auxins has promoted rooting. However, such hormone dips can occasionally be toxic to the delicate tissues of cultured plantlets, e.g.: *Vaccinium* cultivars (15). Debergh and Maene (21) recommend that shoots with fragile leaves and stems be pretreated with an aqueous auxin prior to transplantation. They have developed two successful techniques involving the prolonged use of weak auxin solutions (2 mg l<sup>-1</sup> IBA). Isolated shoots were either soaked for ten days in 2 mm of the auxin solution (especially successful for *Begonia* × *tuberhybrida*) or planted directly into an artificial substrate (rockwool) previously saturated with the auxin solution. Using the latter method, the auxin concentration is gradually lowered by appropriate misting or irrigation. This promotes root elongation after the auxin-induced root initiation. The rate of leaching can be easily controlled for the species in question.

A partial tissue culture technique for ferns has been developed by Knauss (41) which is essentially similar to transplantation from stage II cultures. This involves the culture of fern gametophytes which are macerated in a blender and then spread over a soil mix. From the fragmented gametophyte tissue, large numbers of sporophytes develop (41).

#### IMPORTANCE OF STAGE III CUTTINGS

Even though it may be possible to transplant a species from stage II cultures, a short stage III period may not only increase survival rates (e.g.: *Asparagus officinalis* (34)), but also

markedly improved the vigour of the transplants (e.g.: *Brassica oleracea* (4)), and uniformity in growth of the plantlets (e.g.: *Chrysanthemum* × *morifolium* (23)). However, with other species it is not possible to transplant directly from stage II shoot proliferation cultures and a preparatory step (stage III) is obligatory. The objectives of stage III are to fulfill one or more of the following (53-56).

- a) the division of shoots and their individual rooting,
- b) rendering the plantlets capable of photoautotrophic growth,
- c) fulfillment of dormancy requirements, and
- d) attempt to confer some resistance to moisture stress and microbial infection.

The length of the stage III culture period may be relatively short. After transplantation, survival rates of *Dracaena surculosa* (Syn. *D.godseffiana*), *Scindapsus aureus* and *Syngonium podophyllum* were maximized with only seven days of stage III culture, whereas *Cordyline terminalis* required 14 days (52).

1. **Formation of Roots.** When inducing roots on shoots cultured *in vitro* it is advisable not to let the roots grow too long as this increases the probability of root damage during transplantation. Also roots frequently die after transplantation and new roots must then develop *in vivo* if the plants are to survive (20,21). Unfortunately this is usually accompanied by a cessation in plant growth (21). When rooting *in vitro* is necessary, it is preferable to transplant the plantlets just after root initiation and before root elongation. If stage III culturing is necessary to provide objectives other than rooting, then the culture medium used may not necessarily be the one which initiates root formation the quickest, but rather the one which initiates root formation just as the other objectives of stage III are fulfilled. For rooting *Citrullus lanatus* plantlets, Barnes (9) used a liquid medium with a vermiculite substrate for support and aeration. This resulted in a significantly superior root system with numerous lateral roots and extensive root hairs compared to rooting on an agar based medium (9). There was also less damage to the roots and higher survival rates on transplantation out of culture. The rooting of *Malus* spp. has also been very successful under similar conditions (69). To aid vascular connections between shoots and roots, it is important that the roots are initiated directly from the shoots and not from callus at the shoot bases. Therefore, any unorganized tissue should be removed from the shoot bases during their subculture onto a rooting medium. When roots arose from such callus in *Salpiglossis sinuata*, plantlets failed to survive on transplantation (38).

**2. Induction of photoautotrophic growth.** The stress experienced by tissue-cultured plantlets immediately after transplantation is undoubtedly reduced if their mode of nutrition is switched from heterotrophic to photoautotrophic growth prior to transplantation. Therefore, whenever the other stage III objectives are possible under photoautotrophic conditions, the stage III culture medium need not contain an exogenous carbohydrate. For example, *Solanum laciniatum* shoots readily rooted on MS mineral salts (17), whereas rooting on such inorganic media has not been successful for *Saintpaulia ionantha* (62).

**3. Fulfillment of dormancy requirements.** The possibility of having to fulfill dormancy requirements of plantlets before their transfer out of culture has been discussed by Murashige (53-56). He suggests that unique temperature regimes may be necessary in the stage III culture of plants with bulbs, corms, tubers or other similar storage organs and possibly other plants adapted to a temperate climate. Apparently the necessity of pre-exposure to low temperatures is especially important for propagules lacking foliage (56). To prevent dormancy in the apical buds of *Prunus insititia* after transplantation from tissue culture, it has been necessary to provide a two-month chilling pre-treatment at 0°C before potting and/or a post-potting spray of 200 mg l<sup>-1</sup> GA<sub>3</sub> (37). There was also a strong tendency for plantlets of *Gladiolus* and other bulbous plants to become dormant and form resting corms or bulbs, both in culture and when planted into soil (39). However, this dormancy could be overcome by a cold treatment at 5°C for 3 to 4 weeks (39). The necessity of stratification prior to transplantation has also been recognized by Hilderbrandt (36) for cormlets of some *Gladiolus* × *hortulanus* cultivars. Maintaining cultures at low temperatures is also useful as a means of storing plantlets until sufficient numbers accumulate for transplantation on a mass scale and/or when a suitable market exists for plants. Rooted plantlets of *Fragaria* × *ananassa* have been stored for several months under refrigeration prior to transplantation (11). Transplanted plantlets of *Rubus* spp. have even been stored at 4°C under low light intensity for 14 months with losses less than 5% (69).

**4. Tolerance of moisture stress and pathogens.** The hardening of plantlets to improve their tolerance of moisture stress and pathogens can be achieved to some extent in stage III tissue cultures by increasing the agar concentrations and/or the light intensity. A switch from liquid to agar-based medium during the final culture phase reduced wilting of *Brassica oleracea* Botrytis group plantlets after their removal from culture (31). Such a change has also allowed the successful transplantation of *Syngonium podophyllum* (52). Increasing the agar

concentrations from 1 to 1.4 percent for the rooting phase created considerably drier culture conditions and resulted in higher survival rates for many herbaceous perennials after transplantation (68). Such changes no doubt improve the water relations of the plantlets by promoting stomatal functioning and/or cuticular development. Promoting cuticle formation can also help in creating a barrier to pathogen infection (48).

Increasing light levels by 3 to 10x during the final culture phase is known to improve survival and/or growth after transplanting in bromeliads (53). Higher light intensities for many species including *Carica papaya* (67), *Citrullus lanatus* (9), various ferns (14) and *Ficus* spp. (47) have helped. Higher light intensities not only increase cuticular development (48), but may also assist in promoting photosynthetic activity. In *Asparagus officinalis*, higher light intensities also induced the differentiation of cladophylls (34). Plants with cladophylls had a considerably higher survival rate after transplantation. It was suggested that cladophylls may have helped to establish photoautotrophic growth (34).

**5. Potential problems with stage III culturing.** It is important to realize that stage III culturing can have some subtle detrimental effects on transplantability. For example, although rooting of *Hosta decorata* has been possible on a range of NAA concentrations, high survival rates on transplantation were only possible for those plantlets rooted on a medium containing no or low NAA levels (58). A similar response has been reported in *Rosa* hybrids where the concentrations of plant growth regulators and the inorganic salts on which the plantlets were last cultured, greatly influenced subsequent transplantability (33).

A related problem involves the early transplantation of plantlets. For example, if various bromeliads are transferred into soil too soon, a residual effect of the culture medium results in continued axillary shoot proliferation (51). Unfortunately, this is undesirable in many plants, including bromeliads (51) and woody species to be used as rootstocks, where single-stemmed plants suitable for grafting are required (37). The tendency for weak multi-stemmed plants to develop after transplantation in *Prunus insititia* rootstocks has been overcome with a two month chilling treatment at 0°C prior to transplantation and/or spraying with 200 mg l<sup>-1</sup> GA<sub>3</sub> subsequent to transplantation (37). When light intensities are increased during stage III culturing to assist hardening-off, it is noteworthy that such treatments are also known to reduce rooting to *Asparagus officinalis* (7,34), *Gerbera jamesonii* (59) and *Saintpaulia ionantha* (10). Therefore, when rooting and exposure to higher light intensities are important during stage

III culturing, it may be necessary to start the cultures under low light for a short period (1 to 2 weeks) to initiate root development, then subsequently increase the intensity.

#### PROPAGULE SIZE FOR TRANSPLANTATION

From studies reporting survival rates for different sized propagules after transplantation from tissue culture, it appears that propagule size must be over a certain minimum to maximize survival rates. In addition, Leech (45) found that the initial mean shoot height of *Pinus taeda* plantlets surviving transplantation was 2.6 cm, compared with 1.4 cm for those which died. He also noted that, among the surviving plants, those with larger initial heights tended to have greater initial shoot growth for the first 17 weeks after transplantation. Greater uniformity in plant growth after transplantation was achieved if *Chrysanthemum* × *morifolium* plantlets less than 1 cm high or with poor roots were not potted up (23). Well rooted plants have also been reported to improve acclimatization of *Malus* spp. (69), and survival rates of *Acacia koa* (60) during transplantation.

#### STRESS REDUCTION DURING TRANSPLANTATION

The placement of culture vessels in a greenhouse for several to 10 days before plantlet removal has occasionally been recommended to allow for some acclimatization to greenhouse light and temperature regimes prior to transplantation. This has been successful for *Prunus avium* and *Prunus insititia* (40). However, a potential problem with such procedures as heat accumulation within the enclosed culture vessel due to a "double greenhouse effect". This can be overcome by removing the closures of culture vessels provided the plantlets are adequately watered to prevent wilting. The possible introduction of microbial contaminants at this stage is considered unimportant. Takatori *et al.* (64) flushed the culture vessels daily for one week with half strength Hoagland solution to slowly dilute and remove the unused sugar and other organic constituents of the culture medium, thereby promoting the switch to photoautotrophic growth.

Although leaving a gelled, sucrose-containing culture medium intact around the roots has been reported to help reduce stress when transplanting *Lactuca sativa* plants from tissue culture (43), this practice is not advisable. When removing plants from tissue culture it is important that all the culture medium be thoroughly washed from around the roots. Any traces of culture medium will be rapidly colonized by microorganisms after removal from axenic conditions. Severe problems associated with damping off, etc., could result from any

active microbial growth around the very tender root tissues of cultured plants. The gelled culture medium should only be left intact if it contains no organic constituents. Rooting can occasionally be achieved on such media, e.g.: *Solanum laciniatum* (17), and transplanting with gelled media intact may help to prevent damage to the delicate roots and assist in acclimatization to the soil mix.

#### SUBSTRATE PREFERENCES FOR TRANSPLANTATION

The nature of soil mixes used for transplantation can influence both survival rates and subsequent growth. Different species appear to do best with different substrates, so no general guidelines can be given for all plants. However, it is reasonable to expect that soil mixes in which a species is usually grown for conventional vegetative propagation will be suitable to use for transplantation from tissue culture. A thin layer of sphagnum moss over the surface of the planting mix improved both the survival and rooting of *Rhododendron* spp. (3).

For most plants it is important that the soil mix be porous enough to prevent water-logging conditions. Good aeration after transplantation from tissue culture is known to be important for the survival and growth of *Grevillea* hybrids (27), *Rhododendron* spp. (3) and *Rubus idaeus* (61). The pH of the soil mix may also be important. For example, plantlets of *Fragaria* × *ananassa* transplanted into peat do best at a pH between 5.5 and 7.0 (19). During re-establishment *in vivo* the addition of fertilizer to the soil mix greatly improved the health and survival of *Rhododendron* spp. (3). In other studies commercial fertiliser preparations have been routinely added to soil mixes, or plantlets have been irrigated with the inorganic salts of the tissue culture medium or full or half-strength Hoagland solution (38,45,66). Williams and de Lautour (66) found a slow-release fertilizer to be just as effective as half-strength Hoagland solution. The application of nutrients as foliar sprays may have the added benefit of helping to prevent desiccation. Plantlets of *Carica papaya* have been sprayed with 0.1% Hyponex (N:P:K = 7:6:19) after transplantation from culture (67) whilst those of *Pyrus communis* were fertilized once a week by spraying with an atomized solution of 0.2% fertilizer (N:P:K = 20:20:20) (44).

The hardening-off of tissue-cultured *Acacia koa* plants was more successful in non-sterile Hoagland solution compared with various soil mixes (60). This assisted with the initiation of photoautotrophic growth and the development of functional roots (especially when light was excluded from the root system) (60). Such procedures should prove useful for

those plants which can tolerate their roots being continually submerged in liquid, as this may assist them to overcome the water stress problems associated with transplantation.

To assist tissue-cultured plants in acclimatizing to soil mixes, they may be aseptically planted into culture vessels containing sterilized soil mix for a short period. This has been very successful for the carnivorous plants *Cephalotus follicularis* (1) and *Pinguicula moranensis* (2). Such a step can be easily incorporated into the stage III tissue culture period. *In vitro* rooting in a liquid medium using a soil mix for support and aeration has been readily achieved for *Citrullus lanatus* (9) and *Malus* spp. (67). To promote the switch to photoautotrophic growth in *Coffea arabica*, Herman and Hass (35) aseptically transferred rooted plants into culture vessels containing a sterile soil mix saturated with the inorganic salts of the tissue culture medium.

#### DISEASE PREVENTION DURING TRANSPLANTATION

Unsterilized soil mixes have occasionally allowed successful transplantation of tissue-cultured *Brassica oleracea* (28). However, this practice resulted in serious microbial infection problems for *Lactuca sativa* (43). Although plants may have some genetic capacity to resist disease, when removed from tissue culture their small size, poorly developed cuticles and soft, immature tissues makes them very vulnerable to pathogenic attack. The principles and methods of disease prevention during plant propagation outlined by Baker (8) and McCully and Thomas (50) should be adhered to when transplanting from tissue culture. Disinfected soil mixes and containers plus good hygiene and sanitation are important.

Fungicides have also been frequently used to guard against pathogenic attack when transplanting tissue cultured plants. This may involve treating the soil mix itself and/or the plantlets either before or after transplantation. Jiffy 7 expandable peat pellets have been soaked in  $0.3 \text{ mg l}^{-1}$  Terrazole before transplanting *Daphne*  $\times$  *burkwoodii* (16). *Artemisia dracunculus* var. *sativa* and *Acalphya wilkesiana* were dipped into an aqueous captan mixture (26,63) and *Trifolium* hybrids were washed with 0.045 percent benomyl prior to transplantation (65).

#### IMPORTANCE OF ENVIRONMENTAL CONDITIONS DURING TRANSPLANTATION

As with conventional cutting propagation, success in growing on tissue-cultured plants essentially relies on the maintenance of turgid plantlets until growth begins. A small

loss of turgidity can slow plantlet growth or reduce survival rates. The most important environmental factors are humidity, temperature, and light.

1. **Humidity.** In tissue culture, plantlets are grown in very high humidities (ca. 100 percent). Due to their poor control of transpiration, a gradual change from very high to low humidity is especially important, otherwise plantlets rapidly wilt and become excessively desiccated, from which they are unable to recover, and eventually die. For example, the relative humidity must be maintained above 90 percent for at least 15 days after transplanting *Fragaria* × *ananassa* (19) and above 80 percent for 14 days for *Saintpaulia ionantha* (10).

High humidity can be maintained with the use of intermittent mist and/or humidity tents. With intermittent mist the moisture content of the growing medium can become excessively high and result in abnormal O<sub>2</sub> and CO<sub>2</sub> levels (22). Waterlogged soil mixes are known to inhibit the growing on of tissue-cultured *Grevillea* hybrids (27), *Rhododendron* spp. (3) and *Rubus idaeus* (61). Poorer growth and/or survival of tissue-cultured plants under intermittent mist compared with humidity tents have been reported for *Begonia rex* (22), *Citrullus lanatus* (9), *Nephrolepis exaltata* (22) and *Saintpaulia ionantha* (22). In addition, the leaves of tissue-cultured *Sinningia speciosa* became dotted with small black necrotic spots when grown-on under intermittent mist compared with healthy leaves under humidity tents (32). Therefore, it is recommended that freshly transplanted plants from tissue culture be placed under closed tent-like structures designed to maintain high relative humidities. The use of capillary mats under tents enables very high humidity levels to be maintained. Plants can be gradually hardened by allowing progressively more air flow through the tent as new shoot and/or root growth appears.

The spraying of plantlets with antitranspirants and/or waxes immediately after transplantation may also help, to reduce wilting and promote survival and may, to some extent, overcome the necessity for high humidity chambers. Survival rates of 95 percent have been attained for *Anigozanthos* spp. and *Macropidia fuliginosa* when plantlets were sprayed with 1 percent (v/v) 'Acropol' (a poly-vinylacetate antitranspirant) and placed directly on greenhouse benches, compared with only 80 percent survival for plantlets in high humidity chambers (49).

2. **Temperature.** As most plants show optimum growth at even, moderate temperatures between approximately 20°C and 27°C (22), such conditions are recommended when transplanting tissue-cultured plants. Lower or higher temperatures and/

or drastic fluctuations may result in uneven growth. Root development may be hastened by the use of bottom heat, especially when ambient temperatures are low.

If humidity tents are used, then temperatures within them should be monitored. This is because excessively high temperatures may occur during high light intensities and when the ambient temperatures are high. Extra shade or ventilation could be used to overcome this problem.

3. **Light.** It is generally recognised that freshly transplanted plants from tissue culture show greater growth and higher survival rates if they are initially placed under low light and gradually moved to higher light intensities. Light intensities of approximately  $60-130 \mu \text{ E m}^{-2} \text{ sec}^{-1}$  have been recommended for plantlets when they are initially removed from tissue culture (22). For the rapid growth of *Acacia koa* Skilmen and Mapes (60) found that plants removed from tissue culture required light intensities of at least  $100 \mu \text{ E m}^{-2} \text{ sec}^{-1}$ .

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