Pre- and Post-Flasking Factors Affecting Establishment of *Cordyline* 'Purple Tower' from Tissue Culture©

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The establishment of *Cordyline* 'Purple Tower' plants from tissue culture was studied in two experiments. Experiment I examined the affect of early ex vitro nutrition, particularly N and P, on establishment and subsequent growth, and Experiment II examined the affect of high sucrose Stage III rooting (tissue culture) media on survival and plantlet morphology. Early ex vitro nutrition had no effect on establishment. The importance of initial plantlet size was identified. Foliage growth was greatest at low to medium nitrogen (N) levels. This was 385 g N m^{-3} which is equivalent to 70 g N m^{-3} per month. Added P reduced root dry weight but foliage growth was unaffected. High sucrose (6%) increased microcutting sucrose content and survival and reduced height and shoot quality at transplanting. Shoot quality and height for plants from high sucrose recovered to equal those from low sucrose (3%) after 19 days.

INTRODUCTION

Cordyline 'Purple Tower' has dark purple leaves, and is a strong growing hybrid from *C. banksii* in the Agavaceae (Metcalf, 1987). There are 15 *Cordyline* species with five, including *C. banksii,* endemic to New Zealand (Metcalf, 1987). The species *australis, banksii,* and *fruticosa* (syn. *C. terminalis*) are often raised by tissue culture. In 1990, 600,000 *C. fruticosa* were grown in the Netherlands using micropropagation, making it the eighth most important pot plant in Europe (Pierik, 1991). The micropropagation of *C. fruticosa* has been most frequently described. This species was found to have high bud production and a high percentage of viable in vitro buds at 24°C during the day and 18 to 24°C at night (Hvoslef Eide, 1993).

In tissue culture, sucrose is primarily used as an energy source for developing explants. But large amounts of sucrose reduce chlorophyll and bisphosphate carboxylase activity thus lowering the plantlet's photosynthetic rate. These changes are irreversible and continued growth of the plantlet is dependent on the formation of new leaves (Roberts et al., 1990). The extra sucrose increases the osmotic potential of the medium, which can produce plantlets with high intracellular solutes. These plantlets remain turgid and are better able to survive transplanting to another environment (Fallon and Phillips, 1988; Conner et al., 1993).

The differing osmotic potential of varying sucrose concentrations affects the morphology of plantlets. Low sucrose (<60 g $liter^{-1}$) concentrations produce long, fibrous, thin, and weak roots in asparagus, whereas at 60 g $liter^{-1}$ sucrose and above, storage roots developed (thick and short) (Conner and Falloon, 1990). High sucrose concentrations resulted in a substantial reduction of shoot growth. The combination of storage roots and reduced shoot growth at 60 g $liter^{-1}$ provides ideal plantlets for establishment. Conner and Falloon (1990) suggested the osmotic effects provide an important trigger for root formation, but that the nutritional effect of high sucrose

stimulates the development of storage roots. Samyn (1995) suggested that sucrose at 30 to 40 g liter^{-1} rather than 10 to 20 g liter^{-1} encouraged medium-sized adventitious shoots, which separated easily into true-to-type explants.

The acclimatization of Stage III plantlet can also be enhanced by reducing the humidity (approximately 35%) of the in vitro environment, by adding a desiccant to the vessel or by cooling the vessel and uncapping for 1 week prior to transplanting, or by using plant growth regulators, such as paclobutrazol (Roberts et al., 1990).

Ex Vitro Nutrition. The effects of early ex vitro nutrition are variable; the response seems to be species specific. Rahman (1988) found with *Artocarpus heterophyllus* that low amounts or an absence of nutrients for the first 20 days gave greater growth and higher survival of ex vitro plantlets. Improved plantlet survival through low nutrients has been shown for *Homalomena* 'Emerald Gem' (Matysiak et al., 1995). Roux et al. (1989) increased the growth of micropropagated strawberry plants with Osmocote fertilizer at Day 28 of acclimatization. Osmocote (15-6-12) at 600 g m^{-3} improved the health and survival of rhododendron cuttings (Anderson, 1978).

METHODS AND MATERIALS

Plant Material. Explants of *Cordyline* 'Purple Tower' were obtained from Lifetech Propagation Laboratories, Auckland, New Zealand. They were in a Stage II multiplication medium containing MS salt and vitamins (Murashige and Skoog, 1962), plus 4 g liter^{-1} Fe EDDHA, 1 mg liter^{-1} BAP, 30 g liter^{-1} sucrose, and 6 g liter^{-1} bacteriological agar. When ready, the explants were transferred to bottles (10 per bottle) containing 50 ml of Stage III medium (similar to Stage II but with 1 mg liter^{-1} IBA added to promote root initiation). Cultures were incubated at 25^{o}C under cool white fluorescent lamps (62 µmol m^{-2} s^{-1} for Stage II; 28 µmol m^{-2} s^{-1} for Stage III) with a photoperiod of 16 h light and 8 h dark.

EXPERIMENT I.

Experimental Design. Treatments consisted of two factors (N and P) each at five levels replicated six times in randomized blocks.

Ex vitro Culture. Plantlets were removed from culture on 25 July 1997 and their roots gently rinsed in lukewarm water to remove any agar before being transplanted singly into 85-mm square tubes (0.5 liter). The tubes were filled with a mix of pumice and peat (3 : 2, v/v) with a base fertilizer mix of 892 g m^{-3} Osmocote (37% K), 1000 g m^{-3} of dolomite, 1 kg m^{-3} of Aglime, and 300 g m^{-3} of 'Micromax'. Osmocote formulations (5-6 month) of 23% N and 18% P were used to provide the desired treatment rate as shown in Table 1. The plantlets were placed inside a polythene tent with bottom heat of 21°C. They had a 16 h photoperiod, with 4 h of supplementary lighting from 10 PM to 2 AM supplied by a 400 W mercury vapour lamp, 1 m above the polythene tent. An intermittent misting for 5 sec at 50-min intervals (8 AM to 8 PM) and 60-min intervals (8 AM to 8 PM) maintained humidity within the tent. After 3 weeks they were transferred to an open table in a fiberglass automatically ventilated greenhouse. The air temperature was kept above 16°C. Plants were watered manually when required.

Data Measurements. The explants were assessed at the start and on six subsequent occasions and harvested after 15 weeks. The plantlets were rinsed of agar, dabbed dry, and weighed. The number of functional leaves was recorded. Plantlet shoot

height was measured from the top of the medium to the point where the two uppermost leaves branched.

Experiment II. Fifty explants were transferred to each of two Stage III mediums containing either 3% (30 g liter^{-1}) or 6% sucrose on 17 Oct. Average sucrose content was measured colorimetrically after enzymatic bioanalysis as described by Sekin (1978). The explants were removed from the sucrose mediums on the 8 Oct.; their roots gently rinsed in lukewarm water, then transplanted singly into 70-mm square tubes (0.3 liter). The tubes were filled with the same medium and base fertilizers as in Experiment I with 1674 g m^{-3} Osmocote (23% N) and 917 g m^{-3} Osmocote (18% P) added. The plantlets were stored by size into six blocks; each block contained ten plants from each sucrose treatment. The environmental conditions were the same as for Experiment I. Plantlets remained in a polythene tent in the greenhouse and were measured weekly for one month then harvested. Plants were given one of five values depending on leaf growth and color: 5 = deep purple and healthy; 4 = light purple or green; 3 = chlorotic; 2 = necrosis of the upper portion; 1 = rotting or dead.

RESULTS

Experiment I. The plantlets initial fresh weight strongly influenced their chance of surviving transplanting. Those heavier than 680 mg established well, the lighter plants were more variable. Plants higher than 16 mm tended to have a greater establishment rate. Initial plantlet weight proved to be a useful covariate when analysis of variance was done on the other taxa.

Nutrition had no effect on the initial establishment of the plantlets but affected growth after 15 weeks. The number of leaves per plant did not vary by treatment (data not shown), but added N (Table 1) increased leaf area and shoot height. The response was greatest at 385 g m^{-3} (equivalent to 70 g N m^{-3} per month). Leaf number and shoot height (Table 1) increased most from the nil to the lowest rate of added N, which was 192.5 g N m^{-3} or 35 g N m^{-3} per month. Additional amounts of N had less effect. Shoot dry weight data (Table 1) showed a similar response. Additions of P did not affect foliage growth. However both N and P additions reduced root dry weight. Nitrogen uptake increased with the amount of nitrogenous fertilizer applied (Table 1), while foliar P did not depend on the amount applied. There were no significant interactions between N and P (Fig. 2).

Experiment II. Doubling the Stage III media sucrose content from 3% to 6% increased ($P < 0.05$) the plantlet sucrose content from 11.8 to 15.0 g per kg of dry matter. Significantly more of the high-sucrose treated plantlets had survived after 19 days in the tubes (65% cf. 37.5%).

The high-sucrose plantlets were shorter (base height to the tip of the highest leaf) (38 cf. 43 mm) and had poorer quality shoots (3.7 cf. 4.1) (both $P < 0.05$) at de-flasking. Treatment had no effect on initial fresh weight, and leaf number and root rating at each observation. At the last harvest (27 days) treatment had no effect on either plantlet weight (fresh or dry), or the number and length of roots. Non-significant data is not presented (Fig. 2).

DISCUSSION

Experiment I. Considerable variation exists in tissue culture populations (Karp and Bright, 1985). This variability shows when transplanting to ex vitro environments.

A normal establishment figure from tissue culture is 75%. In this experiment 76% of the plantlets grew.Varying the early ex vitro nutrition did not affect establishment. In contrast, Rahman (1988) found that early ex vitro nutrition inhibited growth of jackfruit, while Fisher et al (1993) found early ex vitro nutrition increased growth of asparagus.

Plantlet size affected establishment, with plants above 680 mg establishing well. Other research agrees with this finding, suggesting that plantlets have a minimum size above which establishment improves (Conner et al., 1992; Fisher et al., 1993; Fisher et al., 1996). Conner and Thomas (1981) reported examples of *Pinus taeda*, *Acacia koa*, *Malus* species, and chrysanthemum showing this effect.

During subculturing, after 59 days on root-inducing media, only 43% of the plants had started to produce roots. This is longer than in current commercial practice. This may have been because of exudation. Exudates are often phytotoxic and inhibit growth and development (Hartmann et al., 1997). Plantlets were moved to new

Table 1. Effects of ex vitro nitrogen (N) and phosphorus (P) on the growth of *Cordyline* 'Purple Tower'

Treatment[1]	Leaf area (cm2)	Shoot height (mm)	Shoot weight (g)	Root weight (g)	Nutrient Uptake[2]
N					
0	138.0	91.3	1.30	0.62	1.46
193	216.7	121.7	1.98	0.43	2.38
385	254.6	138.3	2.54	0.47	2.62
577	204.3	117.2	2.03	0.34	2.73
770	241.3	130.0	2.35	0.36	2.85
Significant contrasts:	L**,Q*	L**,Q**	L***,Q*	L***,Q#	..
P					
0	193.4	114.5	1.90	0.50	0.27
83	207.1	122.5	2.05	0.44	0.39
165	219.5	120.1	2.18	0.46	0.39
247	196.4	112.8	1.82	0.39	0.36
330	238.8	128.5	2.24	0.42	0.40
Significant contrasts:	ns	ns	ns	L*	..

[1]Treatment refers to the main effect of N or P at various rates, gm^{-3}, in the medium.

[2]Plant percentage N or P.

*** = P < 0.001, ** = P < 0.01, * =P < 0.05, # = P < 0.10, ns = not significant, (L) linear or (Q) quadratic contrast.

Figure 1. A plant grown from an explant of *Cordyline* 'Purple Tower', a hybrid of *C. banksii*.

Figure 2. Explant of *Cordyline* 'PurpleTower'. Thick roots and stunted shoot growth occurs with high media sucrose levels (6 %).

mediums when exudates were seen in order to reduce possible phytotoxicity.

Once *Cordyline* 'Purple Tower' had established, foliar growth increased with added N (Table 1). The leaf area, shoot height, and dry weight were all maximized at 385 g N m^{-3} which is equivalent to 70 g N m^{-3} per month from slow-release fertilizer. This is a low to medium rate, which is possibly expected for small plantlets from tissue culture. Indeed, additional growth did not occur with added P, optimum root growth occurred at nil P. Tissue N content increased in proportion to fertilizer N but this plant seemed quite tolerant of highest level of N. However this plant may be less tolerant of or responsive to added P since root growth declined with increasing P level.

The differing initial size of the explants increased the variance of many of the variables. Its use as a covariate to find significant treatment effects was necessary. Perhaps more precise nutritional responses could have been demonstrated had fully established plantlets been used. Rahman (1988) found this with jackfruit where the response to nutrition after 20 days of root development was greater than in the initial period.

One of the advantages of ex vitro root development is that the roots are acclimatized to the soilless media. In vitro roots often do not function correctly, with poor nutrition and water uptake (Grout and Aston, 1977) and poor vascular connections (Thomas and Conner, 1981).

Experiment II. Increasing Stage III media sucrose from 3% to 6% caused plantlet sucrose content to increase. This improved plantlet survival when deflasked and pricked-out into soilless media. Sufficient sucrose provides plants with the energy necessary to cope with the stresses of acclimatization especially before photosynthesis begins (Roberts et al., 1990). At any given stress level high sucrose plantlets should survive longer before exhausting their energy supply.

Increasing sucrose content of the media from 3% to 6% will increase the osmotic potential from 2 to about 6 atmospheres (Weast, 1976). At 5 to 6 atmospheres the medium withholds water from the plantlets, decreasing plantlet turgor pressure and cell expansion. But meristems have a greater potential for water than other plant tissues thus allowing development to continue (Paleg and Aspinall, 1981; Pallon and Phillips, 1989). Thus it was expected that root formation would be higher at 6% sucrose than at 3%. This was not so in this experiment. It is possible that the plantlets would have responded differently had they been left longer in the sucrose media, allowing more adventitious root formation.

Plantlet morphology was altered by sucrose. High sucrose tended to decrease total shoot height and quality. Later the plants grew out of this influence.

CONCLUSION

The early establishment of *Cordyline* 'Purple Tower' was not affected by the amount of N or P in the planting-out medium. Establishing plantlets responded to moderate levels of N, equivalent to 70 g N m^{-3} per month, with improved foliage growth, but P suppressed root growth, and needs to be at low levels. The initial size of plantlets had a significant influence on establishment and if too variable can mask the effect of other factors. It is, therefore, suggested that studies on establishment make allowance for the influence of plantlet initial size. High sucrose levels in Stage III culture were advantageous and produced plantlets that established well ex vitro.

LITERATURE CITED

Anderson, C.E. 1978. Rooting of tissue cultured rhododendrons. Comb. Proc. Intl. Plant Prop. Soc. 28:135-139.

Conner, A.J., **D.J. Abernethy**, and **P.G. Falloon.** 1992. Importance of in vitro storage root development for the successful transfer of micropropagated asparagus plants to greenhouse conditions. N.Z. J. Crop Hort. Sci. 20:477-481.

Conner, A.J. and **P.G. Falloon.** 1990. Osmotic verses nutritional effects when rooting asparagus minicrowns on sucrose media. Acta Hort. 271:100.

Conner, A.J. and **M.B. Thomas.** 1981. Re-establishing plantlets from tissue culture: a review. Comb. Proc. Intl. Plant Prop. Soc. 31:342-357.

Conner, A.J., **Z. Xinrun**, and **A.R. Wooding.** 1993. Micropropagation of oca on a high sucrose medium promotes starch accumulation and plant establishment in soil. N.Z. J. Crop and Hort. Sci. 21:91-93.

Fallon, K. M. and **R. Phillips.** 1988. Responses to water stress in adapted carrot cell suspension cultures. J. Expt. Bot. 40(215):681-687.

Fisher, K.J., **B.R. Mackay**, and **M.A. Nichols.** 1993. Early nutrition of micropropagated asparagus transplants. N.Z. J. Crop Hort. Sci.21:59-66.

Fisher, K.J., **B.R. Mackay**, and **M.A. Nichols.** 1996. The establishment of asparagus clones out of tissue culture. Acta Hort. 415:249-256.

Francois, L.E. and **R.A. Clark.** 1979. Boron tolerance of twenty-five ornamental shrub species. J. Amer. Soc. Hort. Sci. 104(3):319-322.

Grout, B.W.W. and **M.J. Aston**.1977. Transplanting of cauliflower plants regenerated from meristem culture. I. Water loss and transfer related to changes in leaf wax and to xylem regeneration. Hort. Res. 17:1-7.

Hartmann, H.T., **D.E. Kester**, **F.T. Davies, Jr.**, and **R. Geneve**. 1997. Plant propagation, Principles and practices. 6 ed. Regents/Prentice Hall, New Jersey.

Hvoslef Eide, A.K. 1993. Influence of environmental conditions on *Cordyline fruticosa* (L.) A. Chev. Mother plants and on subsequent growth of in vitro explants. Gartenbauwissenschaft. 58(2):89-94.

Karp, A. and **W.J. Bright.** 1997. On the causes and origins of somaclonal variation. Oxford Surveys of Plant Molecular and Cell Biol. 2:199-234.

Matysiak, B., **J. Nowak**, and **A. Kano.** 1995. Acclimatisation of ex vitro *Homalomena* 'Emerald Gem' as affected by nutrient solution concentration and CO_2 enrichment. Acta Hort. 399:157-160.

Metcalf, L. J. 1987. The cultivation of New Zealand trees and shrubs. Reed Methven Publishers Ltd. Auckland.

Miller, L.R. and **T. Murashige.** 1976. Tissue culture propagation of tropical foliage plants. In Vitro. 12(12):797-813.

Murashige T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

Paleg, L.G. and **D. Aspinall.** 1981. The Physiology and biochemistry of drought resistance in plants. Academic Press, Sydney.

Pierik, R.L.M. 1991. Micropropagation of ornamental plants. Acta Hort. 289:45-53.

Radin, J.W. and **M.P. Eidenbock.** 1984. Hydraulic conductance as a factor of limiting leaf expansion of phosphorus deficient plants: Role of hydraulic conductivity and turgor. Plant Physiol. 69:771-377.

Rahmam, M.A. 1988. Effects of nutrients on the growth and survival of in vitro *Artocarpus heterophyllus* Lam. Plantlets after transfer to ex vitro conditions in the glasshouse. J. Hort. Sci. 63(2):329-335.

Roux J.M., **E. Parisot.**, and **J. Marchal.** 1989. Mineral nutrition of micropropagated strawberry plants during acclimatization ex vitro. Fruits. Paris. 44(5):275-279.

Roberts, A.V., **E.F. Smith**, and **J. Mottley**. 1990. The preparation of micropropagated plantlets for transfer to soil without acclimatisation. In: W. Pollard and J. M. Walker (eds.). Method in molecular biology. Vol 6. Plant Cell Tissue Culture. Humana Press. Clifton, New Jersey.

Samyn, G. 1995. Influence of sucrose concentration in the growth medium on adventitious and axillary shoot production and on the stability of the chimeral *Cordyline fruticosa* (L.) Chev. Cultivar 'Rosa'. Bulletin des Recherhes Agronomiques de Gembloux. 30(1-2):67-75.

Sekin, S. 1978. Enzymatic determination of glucose, fructose and sucrose in tobacco. Tobacco Sci. 23:75-77.

Weast, R. C. (ed.). 1976. CRC Handbook of chemistry and physics, 57th ed. CRC Press. Cleveland, USA.