RELEVANCE TO NEW ZEALAND

Initial investigations suggest lotus root may be successfully produced in Northland and other warmer areas of New Zealand. The wide diversity of lotus as a food, medicine, and ornamental along with the increasing Asian presence in New Zealand would suggest that there may be opportunities for supplying both the local market as well as providing export opportunities.

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Acceleration of Blueberry Selections from Tissue Culture©

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INTRODUCTION

When micropropagated plants are first removed from culture vessels they are vulnerable to abrupt changes in environmental conditions (Kyte and Klein, 1983). The plants are extremely tender, with soft leaves and poorly developed stomata for water regulation (Zobayed et al., 1999). Transpiration losses are high and the plants wilt readily if a sudden drop in relative humidity is imposed, as for example when the lid of the vessel is removed too quickly. The plants may also be more sensitive to pathogen attack. Unless in vitro plants are acclimatized and handled carefully, losses can be high.

Factors to consider when transplanting micropropagated plants to the greenhouse have been well documented (Preece, 2001), but individual crops have specific requirements and nurseries generally develop their own methods through trial and error. HortResearch uses shoot-tip micropropagation with blueberries (*Vaccinium* sp.) to bulk-up plants of elite selections for evaluation. Although we have developed a protocol for multiplication of the plants in the laboratory (Miller and Rawnsley, 2001), reliable exflasking techniques are still being developed. In this work we investigated factors that affect survival and growth of in vitro blueberry plants after exflasking, with particular emphasis on economics of the system.

MATERIALS AND METHODS

Four different blueberry cultivars were used in this trial: two commercial production types, *Vaccinium corymbosum* 'O'Neil' and Selection A; and two ornamental home-garden types: Selection B and Selection C.

Shoot tip micropropagation was carried out as previously described (Miller and Rawnsley, 2001) in the HortResearch tissue-culture laboratory at Ruakura, Hamilton. When ready, the tubs were removed from the lab and allowed to acclimatise to room temperature for 10 days.

The trial was started in Autumn 2002. Plantlets of each selection were exflasked on four different occasions through the year: April, August, November, and February. At the time of exflasking the plants were evenly sized within each cultivar with an initial dry weight ranging from 0.005 to 0.14 g.

The plantlets were removed from the tubs and any agar adhering to the base of the stem was washed off with water before inserting the plantlets with the callus still attached into cell trays containing a 1 peat and 1 perlite (v/v) propagation mix. Two different cell sizes were used: a medium cell, 42 mm diameter with 60 cells per tray; and a small cell, 19 mm diameter with 273 cells per tray. No roots had formed on any of the plantlets at the start of the trial. Once planted, the trays were placed in a fog tent with under-tray heating.

Fertiliser requirements were provided by applications of the liquid foliar feed, Nitrosol® organic blood and bone, 8N-3P-6K (Rural Research Ltd, N.Z.). A 0.5% suspension of Nitrosol® was applied either weekly or every 21 days using a small spray bottle. Treated plants were sprayed to run-off while control plants received no additional nutrients.

After 15 weeks the surviving plants were counted and graded. They were then removed from the trays and the roots were cleaned of potting mix. All plants were then oven dried at 60 °C for 48 h. Once dry, the plants were weighed and the change in dry weight was calculated to determine the accumulation of vegetative matter and root growth over the 15-week trial period.

RESULTS

Plant Survival. We were able to exflask blueberry plantlets successfully at any time of the year, although best survival over all cultivars occurred during November (late spring, southern hemisphere). However, the plants did not necessarily grow well at this time of the year and with three out of the four selections used in this trial, the biggest increase in dry weight occurred when plants were exflasked in August (late winter, southern hemisphere). For example, we measured 95% survival of selection A in November, compared with 88% in August, but the plants were measurably smaller in November (0.39 g) than they were in August (0.69 g).

Optimum Cell Size. Traditionally we have used 42-mm round cells to exflask tissue-cultured plants. These work well, as the plant plugs can be transplanted straight into a 1- or 2.5-L pot to continue growing and skip the "tube" stage. The disadvantage is that there is only 60 cells per tray and they take up a large table area in the greenhouse. When these were compared with a 19-mm square tray containing 273 cells, we found that the small cells were more economic for large numbers of plants or if rooted plants were to be exported within a short space of time. In the 15-week trial period, the majority of plants in the 19-mm cells from each of the four selections used in our work filled the plug and roots could be seen extending from the bottom of the cell. They were at a good stage for potting-on and would have started to deteriorate if left much longer. In the 42-mm cell the root systems of the plants had established well and were visibly larger than those in the 19-mm cell, but had yet to form a compact plug. These plants could have stayed in their trays for at least another 3 weeks.

Nutrition. Response to fertiliser treatment was variable, but in general all selections responded well to weekly applications of Nitrosol®. Control plants without fertiliser were consistently smaller than treated plants, with smaller leaves and

significant reddening of the foliage. Growth of the unfertilised plants terminated after 15 weeks, in both cell sizes and this observation was consistent for plants exflasked in April, August, and November. Data from the February exflasking was not available when this report was presented, but early indications from appearance of the plants are that this trend will be continued, and growth will terminate in unfertilised plants before the end of the trial.

CONCLUSION

An understanding of the unique requirements of tissue culture exflasks is essential for successful establishment of plants in the greenhouse. In this work, a number of factors have been identified which affect survival and growth of blueberry plants. Quality of the plants that come from the laboratory has an influence on quality of the in-vivo plantlets (Kyte and Klein, 1983), but ultimately success of the exflasking process depends on skill of the grower. Good techniques for acclimatizing micropropagated blueberry plants are essential if the effort spent developing cultures to the exflasking stage are to be of benefit (Armstrong, 2001). With care and understanding, large numbers of healthy clonal blueberry plants can be produced economically through tissue culture.

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Acclimatizing Tissue Culture Plants: Reducing the Shock©

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INTRODUCTION

Many tissue culture milestones have been achieved over the past 50 years (Gamborg, 2002), with micropropagation established as a commercially viable form of vegetative propagation since the 1970s. Although many species are propagated using this technology there are still many more species that are either recalcitrant or cannot be cost-effectively propagated by tissue culture. Consequently, the commercial application of tissue culture propagation is restricted to mass propagating, high value, superior genotypes, and/or high health lines.

Plant tissue culture relies on growing microbe-free plant material in a sterile environment, in conjunction with defined media containing nutrients, growth promoters, and a carbohydrate source. Typically plant tissue culture is carried out on