Tissue Culture Techniques with New Zealand Blueberry Selections©

Shirley A. Miller and Emma K. Rawnsley
Horticulture and Food Research Institute of NZ.(HortResearch) Private Bag 3123, Hamilton

INTRODUCTION

Techniques for propagation of blueberries (*Vaccinium corymbosum*) through tissue culture have been well documented (Cohen, 1980; Eck, 1988; Lyrene, 1980; Zimmerman and Broome, 1979). The plant breeding and improvement programme for blueberries at HortResearch uses tissue culture (micropropagation) alongside conventional propagation techniques to provide clones of elite germplasm for experimentation. We are constantly refining the techniques, as new methodology becomes available and believe that micropropagation can be readily applied to commercial operations and used for the dispersion of new cultivars. At the present time we have 19 blueberry selections in culture, most of which are ornamental cultivars, suitable for the home garden.

MATERIALS AND METHODS

Effects of Light on Growth of In-Vitro Plants. For this experiment, mother plants of five blueberry selections were maintained in a clean glasshouse environment for several weeks before the start of spring growth. Four of these were ornamental selections and the other a highbush selection suitable for commercial production. From each plant, shoots with fresh lateral growth were selected and removed. All leaves were trimmed off, taking care not to damage the buds in the leaf nodes. The shoots were cut into short lengths, rinsed briefly in 95% ethanol, then sterilized for 30 min in 10% hypochlorite solution. Sterilised shoots were placed directly onto initiation media in tubs (Cohen, 1979) with five to seven shoots per tub. These were then sealed, labelled, and incubated in a growth room at 25°C with lighting provided by cool-white fluorescent tubes at 350 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 16 h per day.

Once initiated, any shoots arising from the buds were excised, cut into two- to three-bud segments and transferred to elongation media (Cohen, 1979). These were incubated as before, except that half of the cultures were placed on shelves with no additional lighting, hence received only indirect light from the surrounding shelves. Light levels on the unlit shelves were 2 $\mu E \cdot m^{-2} \cdot s^{-1}$.

All tubs, with buds or shoots at any stage of development, were checked on a weekly basis to detect any fungal or bacterial contamination. Shoots under the different lighting regimes were measured after 6 weeks, and colour of the plants was recorded.

Ex-Flasking Environment Plants of the Southern highbush blueberry variety cultivar *Vaccinium* 'Oneal' were ex-flasked in Nov. 2001, and planted into cell trays of three different sizes: small (14 cm^3), medium (43 cm^3), and large (68 cm^3). Half of the plants were dipped in Seradix® (Aventis Agriculture, U.S.A.), root forming hormone (0.1% β-indole-3-butyric acid) before planting. Half of the plants were left without Seradix® (β-indole-3-butyric acid) (Aventis Agriculture, U.S.A.) as controls. The growing medium was a perlite and peat (1:1,v/v) mix and the trays were placed on benches in a propagation house under fog, or mist or inside a humid chamber with no extra water allowed to reach the foliage.

Plant establishment and survival were measured and growth was monitored over 6 weeks. Plant height was measured at the end of the experiment and number of shoots per plant counted.

RESULTS AND DISCUSSION

The technique described here has been used to initiate a total of 19 new selections from the blueberry breeding programme. We have used five of these selections to investigate the effects of light on growth and development of the plants in vitro.

The positive affects of light on tissue-cultured blueberries have been reported previously, and it has been shown that red light stimulates shoot growth while far red improves root formation (Read et al., 1988). We found that leaves and stems of all selections tested developed a red coloration when incubated under high light intensity. By contrast, plants under low light intensity remained green throughout the experiment. Plant height was also affected and three out of five of the selections grew taller under low light conditions than those under high light (Table 1).

Table 1. Effects of light on gowth of micropropagated blueberries

	Plant height (mm)	
	High light	Low light
Ornamental A	24.6 + 8.5	24.1 + 9.4
Ornamental B	21.3 + 7.6	21.7 + 7.9
Ornamental C	15.7 + 6.8	24.7 + 10.1
Ornamental D	6.4 + 2.6	20.9 + 13.2
Commercial highbush	25.5 + 7.3	35.2 + 11.0

Research has shown that plants derived from tissue culture have a bushier growth habit, more flower buds per plant, and as a consequence of this greater yields (El-Shiekh et al., 1996; Read et al., 1987). A further advantage of tissue culture is its suitability for varieties that are hard to root. Moreover, because so many plants can be derived from each bud, nurseries need to maintain fewer stock plants.

In preliminary trials, we found that plants of the cultivar 'Oneal' ex-flasked in November were affected by the size of the cell trays more than they were by the environment in which they were maintained, though these measurements were quite varied and need to be repeated next season to confirm the results.

Placing plants in a humid chamber, without allowing extra water to land on their leaves, resulted in plants of the best appearance, with more shoots per plant. There were fewer losses when plants were ex-flasked into medium or large cell trays, rather than small ones. Seradix® (β-indole-3-butyric acid) had no measurable affect on rooting ability.

Despite the apparent advantages of tissue culture, it is not the preferred method of commercial propagation of blueberries in New Zealand or Australia at the present time. In New Zealand, most plants are propagated by softwood cuttings. These are taken in November through to March, with rooting normally occurring within 6 to 8 weeks. By contrast, in Australia, hardwood cuttings are preferred. They are more reliable in the Australian climate and give a better strike rate than softwood cuttings.

Aside from the high set-up costs, one of the most commonly cited disadvantages of tissue culture is the production of genetic variants, or plants that look and behave differently to the parent. Understandably this has resulted in a certain amount of mistrust in the product. For this reason, we have tended to concentrate our efforts to date on ornamental blueberry selections which are destined for the home garden market, rather than commercial use. More research is needed to investigate the production of genetic variants in micropropagated plants. Until this issue is resolved, conventional propagation techniques will remain the mainstay of the industry.

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