MICROPROPAGATION METHODS FOR BLUEBERRIES AND TAMARILLOS

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Abstract. Methods are outlined for the micropropagation of high-bush blueberry (*Vaccinium corymbosum*) and tamarillo (tree-tomato) (*Cyphomandra betacea*). These methods should have immediate application for the rapid propagation of new cultivars.

In the case of blueberry, multiplication is achieved by cutting the shoots which develop in culture into single node segments. A multiplication rate of about 5 fold every 6 weeks has been achieved. Small shoots about 2 cm long can be easily rooted in seedling flats under high humidity conditions.

In the case of tamarillo, multiplication is achieved by a combination of enhanced auxiliary bud development and adventitious buds arising from the swollen stem base. A multiplication rate of 6 to 8 fold every 2 weeks has been achieved. Rooting can be carried out either in sterile culture or directly in a seedling flat.

HIGH-BUSH BLUEBERRY (*VACCINIUM CORYMBOSUM*)

Plant material. Stock plants of a number of high-bush blueberry cultivars were grown in a greenhouse with supplementary lighting in winter to extend the daylength to 15 hrs. Shoots were collected at the end of a flush of growth.

Disinfestation. The shoots were cut into 6-node sections and surface sterilized in 0.6% sodium hypochlorite with 0.1% Multifilm X77 (a non-ionic detergent) for 30 min. The tissue was then rinsed three times in sterile distilled water.

Culture medium. For most of this work the culture medium contained ¼ strength Murashige and Skoog materials with full strength Linsmaier and Skoog vitamins (100 mg/l m-inositol, 0.4 mg/l thiamine-HCl), sucrose (30 g/l), NAA (0.02 mg/l) and IPA (isopentenyl adenine, 5 mg/l). The medium was solidified with 6 g/l Davis (NZ) bacteriological agar and sterilized for 15 min at 15 psi (121°C).

Procedures. The surface-sterilized shoots were cut into single-node sections using flame-sterilized secateurs. Four sections were planted in each 100 ml jar containing 25 ml medium. The jars were covered with thin sheets of sterilized, high density polythene held on with a rubber band.

After 4 to 6 weeks the axillary buds on about 50% of the nodes had produced a shoot 2 to 4 cm long with 6 to 10 leaves. These shoots were excised, cut into sections of 1 to 3 nodes and replanted on fresh medium of the same composition in petri dishes. The original cultures were kept and one or two new shoots usually grew from the base of the original shoot. The

new cultures in petri dishes produced axillary shoots which were cut into sections after 6 to 8 weeks. This procedure can be repeated indefinitely giving a proliferation rate of about 5 fold per subculture.

Rooting. *In vitro* proliferated shoots growing in petri dishes could be easily rooted in a peat mix under shaded conditions with high humidity. The basal end of the shoots about 2 cm long were dipped in Seradix 2, (a commercial rooting powder containing 0.3% IBA), planted in a seedling flat containing fine pumice-peat (50:50) and kept under high humidity and two layers of 50% shade cloth. After two weeks about 90 to 95% of the shoots had rooted. The trays were then transferred to intermittent mist for one week after which they were placed on a greenhouse bench.

Subsequent growth. After rooting had taken place the plants were watered twice weekly with a complete nutrient solution (½ strength Hoagland's). When the shoots had attained a height of 7 to 10 cm, the plants were transplanted to propagating tubes.

Comments.

(1) Using these procedures approximately 700 rooted plants can be grown from a single node explant in 9 months.

(2) These procedures have not been successfully used with the following cultivars: Atlantic, Jersey, Dixie, Stanley, Burlington, Berkeley, Blueray, and Ivanhoe.

TAMARILLO (*CYPHOMANDRA BETACEA*)

Plant Material. Stock plants were grown in a greenhouse. Axillary buds with a section of stem attached were cut off with a scalpel.

Disinfestation. The bud segments were surface sterilized as described for blueberry.

Culture Medium. Full strength Murashige and Skoog mineral salts were supplemented with Linsmaier and Skoog vitamins, sucrose (3%), and BA (benzyladenine at either 0.3 or 3.0 mg/l). The media were solidified with 6 g/l Davis (NZ) bacteriological agar and autoclaved for 15 min at 15 psi (121°C).

Procedures. The sterilized bud segments were trimmed under a dissecting microscope to give an explant cube of approximately 2 mm containing the bud. These explants were placed on a medium containing BA at 3 mg/l to induce bud break. This concentration of BA suppresses shoot elongation but axillary buds continue to proliferate. In addition some adventitious buds arise from the swollen base of the explant.

After about 4 to 6 weeks the proliferating bud clusters were cut into segments, each containing several buds, which were replated on to fresh medium. Bud proliferation rates of 6 to 8 fold every two weeks have been recorded.

Transfer of bud segments to 100 ml jars containing 25 ml of a medium with reduced BA concentration (0.3 mg/l) allowed shoot elongation to occur. Shoots of 2 to 3 cm developed over 4 weeks and were suitable for rooting either *in vitro* or directly in peat mix.

Rooting. All shoots formed roots within 8 days on transfer to a medium containing 1 mg/l IBA. After another 2 weeks these shoots were transferred to a seedling flat containing pumice-peat (80:20) and placed under intermittent mist for one week.

Alternatively, the base of the unrooted shoot can be dipped *into Seradix 1, then planted directly into pumice-peat (80:20)*, and placed under intermittent mist. Roots develop within 3 weeks but plant growth lags behind that of plants rooted in sterile culture. However, the advantage of this method is the avoidance of one sterile transfer step.

Subsequent growth. After transfer to the greenhouse bench, the flats were watered twice weekly with a complete nutrient solution (½ strength Hoagland's). Plants rooted in sterile culture attained a height of 10 cm within 4 weeks of transfer to the seedling flat, whereas plants rooted directly in seedling trays took about 10 days longer to reach the same size.