MICROPROPAGATION OF *DAPHNE* × *BURKWOODII* TURRILL

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Abstract. A method for the rapid propagation of *Daphne* × *burkwoodii* is described involving meristem-tip culture, shoot proliferation, root initiation and transfer of plants to potting medium. The place of this method in a clean stock programme is described and results with *Daphne odora* are compared.

The virus diseases of *Daphne spp.* have been extensively studied by Milne and his students at Massey University (2). Three viruses were found in *D.* × *burkwoodii* and at least ten in *D. odora* cultivars. Nurseries in New Zealand need high-health propagating material free of known virus diseases and the New Zealand Nursery Research Centre in conjunction with the Plant Physiology Division has initiated a programme to produce this material. Last year the ways in which meristem-tip culture and thermotherapy might be used to produce high-health stock were outlined (1). In this paper the development of techniques suitable for meristem-tip culture of *D.* × *burkwoodii* to be used in conjunction with thermotherapy will be described.

MATERIALS AND METHODS

Young shoots of *D.* × *burkwoodii* were collected in late spring from two plants growing in gardens in Palmerston North. Micro-dissection and subsequent transfers were carried out under sterile conditions. Both apical and axillary buds were dissected using sterile needles and microknives made from thin chips of non-stainless razor blades held in a pin vice.

Media consisted of Murashige and Skoog (MS) salts (3) supplemented with myoinositol 100 mg/l, thiamin, 0.4 mg/l, sucrose 30 g/l and varying amounts of naphthaleneacetic acid (NAA) and kinetin (K) as indicated. Bacteriological Agar (Davis Gelatin Co., Christchurch, N.Z.), 6 g/l, was added to all media. Plastic petri dishes were used for the establishment of the cultures and early shoot proliferation and glass jars or tubes for further shoot proliferation and rooting of cuttings.

Incubation of cultures was carried out under a fluorescent light bank with an irradiance of 11 W m^{-2} PAR (about 2,500

lux) in a room maintained at 25 ± 2°C, with a photoperiod of 16 hours.

RESULTS AND DISCUSSION

Establishment of cultures. The shoot-tip, consisting of the meristematic dome and up to three primordial leaves, was excised and place on agar medium in a petri dish. The size of these explants was 0.2 - 0.5 mm wide and up to 1 mm long.

A series of 16 media with NAA and K at concentrations of 0, 0.1, 0.3 and 1.0 mg/l was tested. It was found that NAA was undesirable in the establishment phase, whereas K at 1 mg/l was most suitable. Within three weeks, shoots with somewhat thickened leaves up to 1 cm long developed on this medium (Fig. 1). If left on this medium, however, normal leaves rarely developed. The shoots were therefore transferred to a medium containing 0.1 mg/l NAA and 0.3 mg/l K on which one to several shoots with normal leaves and elongated internodes developed. Within four to six weeks these shoots had 15 to 20 leaves.

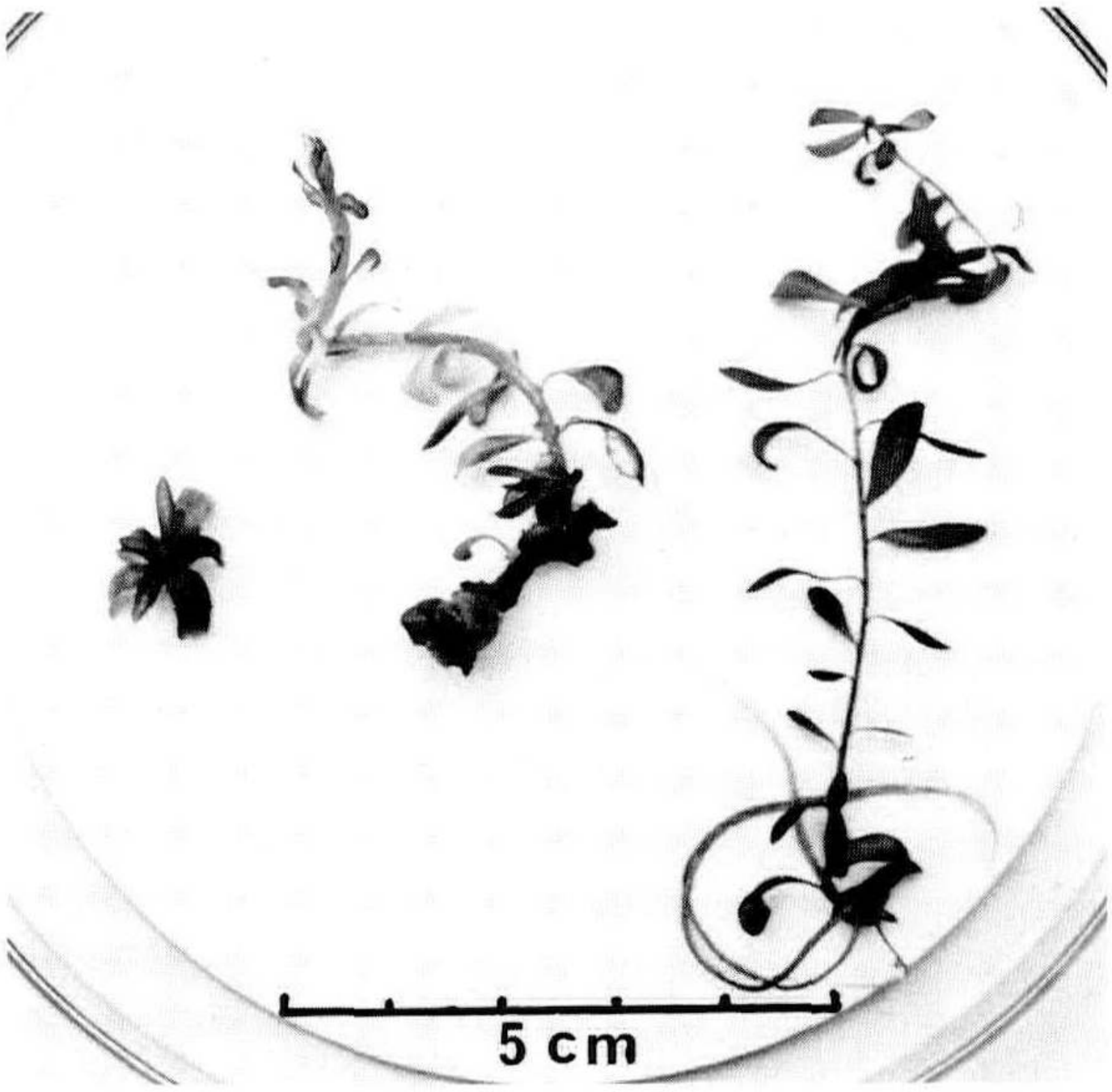

Figure 1. Stages in the micropropagation of *D* × *burkwoodii*.
Left – shoot-tip after 3 weeks on 1 mg/l K.
Centre – a single shoot growing from a 4-node section on 0.1 mg/l NAA and 0.3 mg/l K.
Right – rooted cutting after a tip section was dipped in 100 mg/l NAA and then grown on a medium without hormones.

Shoot multiplication. In order to increase shoot number, elongated shoots were cut into 4 node sections, then placed on fresh 0.1 NAA/0.3 K medium. New axillary shoots appeared,

usually from the upper nodes and each of these shoots was re-divided after a further four weeks to give 3 or 4 sections. On the basis of a 5-fold increase in 4 node sections per month, it would be possible to produce several thousand sections within six months.

Rooting of cuttings. Rooting of shoot-tips produced in culture proved to be difficult. Low levels of NAA were without effect, whereas higher concentrations induced callus and distorted roots which did not develop further. In order to induce normal roots, tip sections were dipped into a solution of 100 mg/l NAA in 50% ethanol before being placed on a medium without hormones; roots began to appear after three weeks.

Subsequent trials tested steeps in aqueous solutions of NAA at 100 or 200 mg/l for 5-60 minutes. A 10 minute steep in 200 mg/l NAA adjusted to pH 5.7 appeared to be satisfactory, and rooting has been induced in up to 7 out of 10 cuttings in some trials.

Transfer to potting medium. Great care needs to be taken during the transfer of plantlets from tissue culture, in which they are fed with a full nutrient medium under sterile conditions, to a potting medium in which they are exposed to a harsher environment and must rely on their own photosynthesis for sugars. When plantlets were transferred to Jiffy 7 peat pots and placed under intermittent mist, most of the plants died. A gradual hardening process was required. The procedure we have adopted has resulted in approximately 90% survival of the rooting cuttings.

The rooted plantlets were rinsed to remove the agar and were planted in Jiffy 7 pots soaked in a 0.3 ml/l solution of Terrazole (25% emulsifiable concentrate). The plants (and the Jiffy 7) were covered with an inverted 600 ml jar and placed under the light bank in the culture room. After about 4 days the edge of the jar was raised to reduce humidity and the plant was left for a further 3 days. The plants were then transferred to a propagating house with intermittent mist. After a further week, root tips were visible on the outside of the Jiffy 7 and the plants were transferred to a growing-on area in the glasshouse.

Health status of plants. Approximately 2/3 of the plants derived from shoot-tip culture of *D* × *burkwoodii* have been found to be free of rod viruses, using the electron microscope. Glasshouse-grown plants still infected with rod virus did not have any obvious virus symptoms. Since attempts to transmit this virus mechanically to the indicator plant *Chenopodium*

quinoa were unsuccessful (K.S. Milne, personal communication), the virus is probably Daphne Virus S. However, further tests are required to positively identify this virus and to ascertain whether other viruses might be present.

Work on Daphne odora. Parallel experiments have been carried out to ascertain the media requirements for *D. odora*. An auxin was required for the establishment phase and we found 1 mg/l NAA with 0.3 mg/l K was satisfactory, although growth of the shoot-tips was much slower than for *D.* × *burkwoodii*. After the initial establishment of *D. odora* shoot-tips, shoot growth was best on a medium with NAA reduced to 0.3 mg/l and 0.3 mg/l K.

Root induction on stem tip cuttings from tissue culture of *D. odora* 'Leucanthe' has been successful using the same procedure described for *D* × *burkwoodii*. To date, no indexing for virus has been done on shoot-tip culture derived plants of *D. odora*.

Problems. With *D. burkwoodii* the shoot-tip sometimes died and growth was then continued by an axillary shoot. With *D. odora*, the basal leaves on a shoot often senesced rapidly and the base of the shoot died. No cause has been found for either of these problems, but when they occurred green tissue was transferred to fresh medium and shoot growth continued.

The future. These techniques have already been applied to several daphne cultivars following thermotherapy. The first of the rooted plantlets from this experiment (carried out in conjunction with the N.Z. Nursery Research Centre) will shortly be transferred to Jiffy 7's. It is hoped that this experiment will yield a number of plants free of all viruses known to infect daphne species. If so, further plants will be propagated using rapid micropropagaiton techniques and will be released to the trade through the Nursery Research Centre.

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LITERATURE CITED

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