

considered essential, since the seeds later are scarified in concentrated sulfuric acid. If the seeds are moist, the water:acid interaction can create enough heat to kill the embryo. In our treatments, seeds were extracted from fresh fruit, disinfested, and then placed directly onto tissue culture medium. Since germination occurred within a few days without cool-moist stratification, it may be that drying bramble seeds tends to induce dormancy. Dale and Jarvis (2) studied this phenomenon and reported that freshly harvested acid-scarified red raspberry seeds from 4 cultivars averaged 75% germination in a peat/sand mix when the seeds were not dried excessively. Lasheen and Blackhurst (5) showed germination inhibiting substances to be present in relatively high concentration in the blackberry endosperm, lower in the testa, and much lower in the embryo. It appears that early seed coat damage or removal facilitates germination before inhibitors can be translocated to the embryo.

Because the need for stratification can be eliminated by either in vitro or potting mix germination, the relative merits of each system must be considered individually. Tissue culture is a labor intensive operation. The germination pretreatments of Lundergan and Carlisi (6) and Dale and Jarvis (2) are less intensive and, hence, may be useful for large seed batches. Therefore, the bramble geneticist may choose to use tissue culture to germinate seeds of critical crosses that yield few seed (e.g., interspecific hybridizations), the other systems for more routine situations.

Bramble geneticists also may find our in vitro system useful to reduce generation time. Dale and Jarvis (2) found red raspberry seeds required 15 to 25 days for 50% of the seeds that would germinate to do so. In our experiments, all viable seeds often germinated within 8 to 12 days. This 1 to 2 week time savings, in the early spring, might be enough to ensure well-rooted seedlings capable of surviving field transplanting the same year that crosses are made.

Literature Cited

1. Brinkman, K.A. 1974. *Rubus* L., blackberry, raspberry, p. 738-743. In: C.S. Schopmeyer (ed.). Seeds of woody plants in the United States. Agr. Hdbk. No. 450. Forest Serv., USDA.
2. Dale A. and B.C. Jarvis. 1983. Studies on germination in raspberry (*Rubus idaeus* L.). *Crop Res.* 23:73-81.
3. Galletta, G.J. and R.L. Puryear. 1983. A method for *Rubus* embryo culture. *HortScience* 18(5):588. (Abstr.)
4. Kerr, A. 1954. Seed development in blackberries. *Can. J. Bot.* 32:654-672.
5. Lasheen, A.M. and H.T. Blackhurst. 1956. Biochemical changes associated with dormancy and after-ripening of blackberry seed. *Proc. Amer. Soc. Hort. Sci.* 67:331-340.
6. Lundergan, C.A. and J.A. Carlisi. 1984. Acceleration of the reproductive cycle of the cultivated blackberry. *HortScience* 19(1):102-103.
7. Moore, J.N., G.R. Brown, and C. Lundergan. 1974. Effect of duration of scarification on endocarp thickness and seedling emer-

gence of blackberries. *HortScience* 9(2):204-205.

8. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
9. Ourecky, D.K. 1975. Brambles, p. 98-129. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.
10. Quoirin, M., P. Lepoivre, and P. Boxus. 1977. Un premier bilan de 10 années de recherches sur les cultures de meristemes et la multiplication "in vitro" de fruitiers ligneux. *In Compte Rendu des Recherches, 1976-1977 et rapports de synthese. Station des Cultures Fruitières et Maraichères, Gembloux.* p. 93-117.
11. Walkey, D.G. 1972. Production of apple plantlets from axillary-bud meristems. *Can. J. Plant Sci.* 52:1085-1087.
12. Zimmerman, R.H. and O.C. Broome. 1980. Apple cultivar micropropagation, p. 54-58. In: Proceedings of the conference on nursery production of fruit plants through tissue culture — applications and feasibility. USDA, Sci. and Educ. Admin., Agr. Res. Results ARR-NE-11.

HORTSCIENCE 20(6):1049-1050. 1985.

In Vitro Propagation of *Dierama latifolium*

Yvonne M. Page and J. Van Staden

UN/CSIR Research Unit for Plant Growth and Development,
Department of Botany, University of Natal, Pietermaritzburg, 3200,
South Africa

Additional index words. tissue culture, 6-benzylaminopurine, 1-naphthaleneacetic acid

Abstract. *Dierama latifolium* N.E. Br. was propagated in vitro using corm cultures. Shoots were induced from corm explants when grown on solidified Murashige and Skoog (MS) medium supplemented with 30 g/liter sucrose, 100 mg/liter meso-inositol, and 0 or 0.5 mg/liter NAA. Shoot proliferation was not improved by the addition of BA to the initial culture medium. Multiple shoots were induced by transferring those produced in vitro, to a modified MS medium supplemented with 0.5 or 1.0 mg/liter BA. Rooting of these shoots was induced by subculturing single excised shoots, 5-10 mm in height, either a hormone-free basal medium or a BM supplemented with 0.5 or 1.0 mg/liter NAA. Utilizing this technique, about 90 plants could be produced from a single corm within 12 months. Chemical names used: 1-naphthaleneacetic acid (NAA); N-(phenylmethyl)-1H-purin-6-amine (BA).

A number of important ornamental plants have their origin in attractive indigenous South African plant species. Tissue culture techniques for some of these plants have been described over the past 15 years. Data are available concerning the in vitro regenerative capacity of *Gladiolus* inflorescence stalks (7), *Ornithogalum* stem, leaf, ovary, sepal and bulb scale pieces (2, 5), *Freesia* stem segments (1, 3), and *Lachenalia* leaves (6).

Southern Africa has about 20 species of *Dierama* C. Koch, family Iridaceae. Several of these species are attractive and have horticultural potential, particularly if hybrids could be obtained. Tissue culture was begun with the hope of eventually obtaining somatic hybrids. This paper describes an in vitro technique for propagating plantlets of *Dierama latifolium* N.E. Br. from corm explants.

Plants of *D. latifolium* were collected from their natural habitat and grown in pots. The

following procedure was used for successful in vitro sterilization of *D. latifolium* corms. The leaves and roots were removed from the corms of harvested plants. The corms were sorted, and only the most recently developed ones were selected for in vitro culture. These were scrubbed under running tap water, then immersed in a solution of 100% alcohol for 5 min. Under aseptic conditions, the material was disinfested further by immersing it in a 0.1% (w/v) mercuric chloride (HgCl₂) solution for 20 min and then rinsing twice with sterile distilled water. The corms were cut vertically into 6 to 8 explants and transferred into 80 × 25 mm tubes containing 10 ml of solidified nutrient medium. Utilizing the sterilization technique described, 98.44 ± 1.32% (P = 0.05) of the explants remained sterile within the first 2 months of culturing.

The basic nutrient medium (BM) consisted of the MS salt mixture (4) supplemented with 30 g/liter sucrose, 100 mg/liter meso-inositol, and 10 g/liter Difco Bacto agar, plus various amounts of NAA and BA. The pH of the medium was adjusted to 6.0 with NaOH before autoclaving for 20 min at 120°C. For each hormonal treatment, 6 explants were used. Treatments were repeated 4 times.

Cultures were maintained at a temperature of 25° ± 2°C. A light intensity of 0.147

Received for publication 6 Feb. 1984. The financial assistance of the C.S.I.R., Pretoria, is acknowledged. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Effect of various concentrations of NAA and BA on the growth response of corm explants of *D. latifolium*.

NAA concn (mg/liter)	BA concn (mg/liter)	Growth response ^z			
		Callus formation	Root formation	Shoot formation ^y	No organo- genesis
0	0	7.2	4.9	6.2	12.3
	0.5	5.9	4.9	9.0	10.8
	1.0	6.9	4.9	7.8	11.0
	3.0	4.9	4.9	8.6	11.5
0.5	0	6.0	7.4	8.2	8.9
	0.5	6.0	6.9	8.3	10.0
	1.0	7.1	5.8	8.2	10.2
	3.0	7.0	5.8	6.3	12.1
1.0	0	7.4	7.2	6.7	9.1
	0.5	6.2	7.8	6.9	10.4
	1.0	7.2	7.4	4.9	10.3
	3.0	7.0	8.3	7.8	8.0
3.0	0	7.1	6.1	7.5	10.4
	0.5	7.4	7.0	7.0	8.9
	1.0	7.6	6.3	6.0	9.9
	3.0	5.8	6.3	4.9	13.6
LSD .05		3.1	2.7	2.7	3.3

^zExpressed as an average value of the transformed data of the explant response recorded per treatment replicate.

^yIncludes explants which formed both shoots and roots or shoots only.

Wm⁻² was provided by cool-white fluorescent tubes for 16 hr daily.

The general growth responses of corm explants cultured on different NAA and/or BA supplemented media were recorded 10 weeks after inoculation in vitro (Table 1). These data were transformed using a logit transformation and then analyzed using the statistical analysis of variance. This analysis showed that no significant correlation existed between a callusing type of explant response and the hormonal treatments. With respect to the formation of roots, the results

indicated that a BM supplemented with auxin was necessary for rooting and that the highest percentage of explants developed roots when cultured on a BM containing 1.0 mg/liter NAA. For shoot formation, the interaction between hormonal treatment and explant response was less obvious. However, analysis of only the influence of NAA upon shooting indicated that a significantly higher number of explants formed shoots when grown on a BM devoid of hormones, or supplemented with 0.5 mg/liter NAA, rather than 3.0 mg/liter NAA. The shooting response

recorded for explants grown on a medium supplemented with 1.0 mg/liter NAA was not significantly different from that recorded for other NAA concentrations.

Neither root nor shoot proliferation was significantly influenced by the addition of BA to the BM. Of the total number of explants cultured, an average of 56 (24%) did not respond in vitro.

The subculturing of shoots produced in vitro on a BM supplemented with either 0.5 or 1.0 mg/liter BA resulted in callus and multiple shoots. The rooting of these shoots was induced by placing them, when 5–10 mm in height, on a BM devoid of hormones or supplemented with low concentrations of NAA (0.5 or 1.0 mg/liter). The utilization of this technique should yield about 90 plants from a single corm within a period of a year.

Literature Cited

1. Bajaj, Y.P.S. and R.L.M. Pierik. 1974. Vegetative propagation of *Freesia* through callus culture. *Neth. J. Agr. Sci.* 22:153–159.
2. Hussey, G. 1976. Plantlet regeneration from callus and parent tissue in *Ornithogalum thrysoides*. *J. Expt. Bot.* 27:375–382.
3. Hussey, G. 1977. *In vitro* propagation of some members of the Liliaceae, Iridaceae and Amaryllidaceae. *Acta Hort.* 78:303–309.
4. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473–497.
5. Nel, D.D. 1981. Rapid propagation of *Ornithogalum* hybrid *in vitro*. *Agroplanta* 13:83–84.
6. Nel, D.D. 1982. Rapid propagation of *Lachenalia* hybrids *in vitro*. *S. Afr. J. Bot.* 2:245–246.
7. Ziv, M., A.H. Halevy, and R. Shilo. 1970. Organs and plantlets regeneration of *Gladiolus* through tissue culture. *Ann. Bot.* 34:671–676.