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Dierama luteoalbidum: Liquid culture provides an efficient system for the *ex situ* conservation of an endangered and horticulturally valuable plant

L.A. Madubanya, N.P. Makunga [1], C.W. Fennell *

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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Abstract

An efficient and reliable protocol for the *in vitro* propagation of *Dierama luteoalbidum*, an endangered and horticulturally important plant is described. *D. luteoalbidum* seeds were germinated *in vitro* on full-strength solid (Murashige and Skoog, 1962) medium following decontamination. Hypocotyl explants obtained from the seedlings formed multiple shoots on MS medium supplemented with 0.5 mg L^{-1} BA (4 shoots being initiated per explant) while an increase in the BA concentration (1–2 m gL^{-1}) and addition of NAA (1 mg L^{-1}) increased the incidence of callus. After 6–8 weeks, shoots were reduced to meristemoids when transferred to a liquid-shake MS medium supplemented with 0.5 mg L^{-1} BA for mass propagation. These formed secondary shoots after 3–4 weeks on solid MS medium containing 0.5 mg L^{-1} BA. Rooting of the plantlets occurred readily but was significantly promoted by adding 6–8% sucrose. Shoots left undisturbed on the same medium for 6 months responded by forming corms. The addition of paclobutrazol (5–10 mg L^{-1}) reduced the corm induction period to 3 months. Microplants transferred to a peat: compost: bark mixture (1:1:1) (v/v/v) in the greenhouse had a survival rate of 100%. All acclimatized plantlets formed corms after 6 months following the application of 1% (v/v) Kelpak — a seaweed concentrate.

Keywords: Corm induction; Kelpak; Liquid medium; Paclobutrazol; Sucrose

1. Introduction

Dierama belongs to the Iridaceae, within the Ixioideae subfamily (Goldblatt, 1971). There are about 44 species, distributed from the southern Cape northwards through southern and eastern Africa, with the greatest number of species in KwaZulu-Natal (Hilliard and Burtt, 1991). The distribution of *Dierama* populations is always restricted to moist grasslands and shows a marked structural uniformity in the genus (Hilliard and Burtt, 1991). KwaZulu-Natal also has the widest range of flower colour and only in the midlands can *D. luteoalbidum*, which has a cream to white coloured perianth, be found (Fig. 5A). It is not to be confused with *D. argyreum*, which is also white-flowered, although often tinged with pink or mauve (Hilliard and Burtt,

Table 1
The effect of various concentrations of BA and NAA on organogenesis using hypocotyl explants of *Dierama luteoalbidum*

BA (mg L^{-1})	NAA (mg L^{-1})	Mean number of shoots per explant	Root formation	Callus formation
0.0	0.0	1.7±0.4 b	+	−
0.5	0.0	4.2±0.9 a	+	−
1.0	0.0	3.8±0.9 a	−	+
2.0	0.0	2.7±1.0 ab	−	+
0.5	1.0	1.4±0.3 b	−	+
1.0	1.0	1.6±0.4 b	−	+
2.0	1.0	1.7±0.5 b	−	+

+ and − indicate presence or absence.
LSD (5 %)=1.3.
Treatments with different letters are significantly different at $P<0.05$.

Abbreviations: AC, Activated charcoal; BA, Benzyladenine; FW, Fresh weight; GI, Growth index; NAA, Naphthaleneacetic acid; rpm, Revolutions per minute.

* Corresponding author.
E-mail address: fennel@ukzn.ac.za (C.W. Fennell).
[1] Present address: Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

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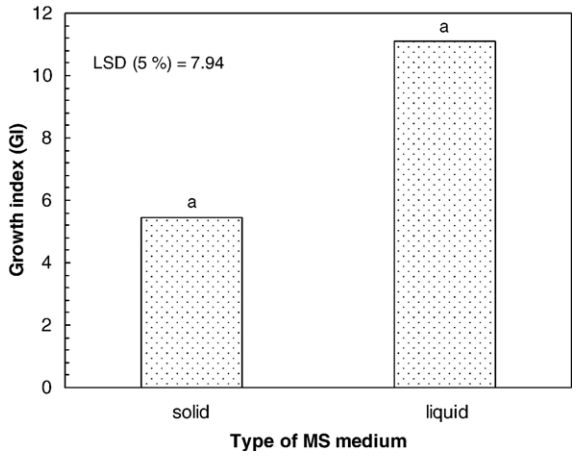

Fig. 1. The growth index of *D. luteoalbidum* shoots in solid and liquid MS medium containing 0.5 mg L^{-1} BA after 8 weeks.

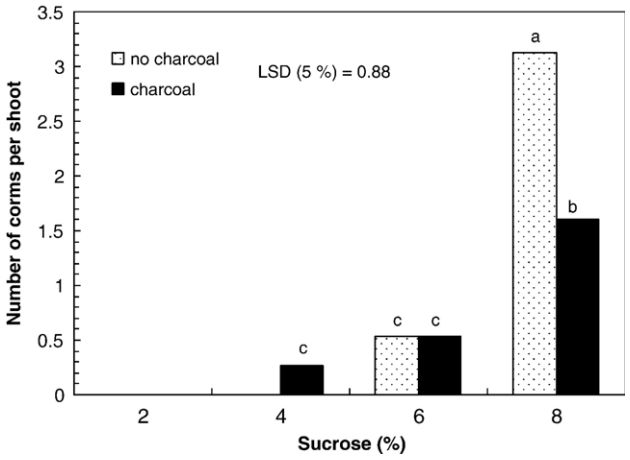

Fig. 3. The effect of sucrose and activated charcoal on *in vitro* corm formation of *D. luteoalbidum* after 6 months.

1991). All *Dierama* species have corms that do not completely go dormant, as in *Crocus* and *Gladiolus*, and the leaves are evergreen. The corms are not edible but are harvested for medicinal purposes. The long, tough leaves are used as cordage and/or as a source of fibre (Hilliard and Burtt, 1991). *D. luteoalbidum* is one of five endangered *Dierama* species endemic to KwaZulu-Natal that are threatened due to grassland transformation by agriculture and forestry (Scott-Shaw, 1999). Annual propagation by means of corms or seeds is not a feasible option for these plants as it is extremely slow. Even though the *Dierama* seeds are not reported to be recalcitrant (Hilliard and Burtt, 1991), propagation is further complicated by the susceptibility of the seeds to attack by the wild beetle, *Urodon lilii*. Tissue culture techniques were thus applied with the intention of rapidly propagating this endangered species with horticultural potential.

2. Materials and methods

Seeds of *Dierama luteoalbidum* Verdoorn were collected at Pevensey (2929 DC), Underberg (South Africa) by Mr Rogan Roth in December 2001 and were stored at room temperature. All the tissue culture media used in this study were decontaminated by autoclaving at 121 °C and 103 kPa for 20 min. When gelling agents were added to the medium the pH was adjusted to 5.8 with KOH prior to autoclaving. To initiate *in vitro* cultures, seed decontamination was carried out as follows: a one-minute dip in 70% (v/v) ethanol followed by a 15-min soak in 3.5% (w/v) sodium hypochloride. The seeds were then rinsed several times with sterile distilled water before aseptically transferring them to 80 × 25 ml tubes containing 10 ml of Murashige and Skoog (MS) (1962) medium, solidified with 2 g L^{-1} Gelrite (Labretoria, South Africa). The cultures were grown in the light (16-h photoperiod) and at a temperature of ±25 °C. Cool white fluorescent lamps (L75W/20X Osram, USA, Code F961T12) provided a light intensity of 71 μmol m^{-2} s^{-1}. 28 days after the seeds had germinated and elongated to about 6 cm, the hypocotyl explants were placed on solid MS medium supplemented with various concentrations of BA (0–2 mg L^{-1}) and NAA (1 mg L^{-1}) for multiple shoot formation, for 6 weeks. The multiple shoots were trimmed to about 2 cm and then separated into single shoots. Each shoot was then immersed in 40 ml of liquid MS medium supplemented with 0.5 mg L^{-1} BA

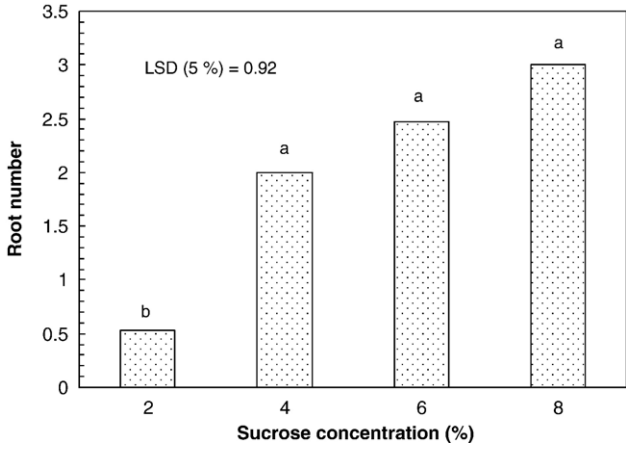

Fig. 2. The effect of sucrose on the production of *D. luteoalbidum* roots after 3 months on solid MS medium.

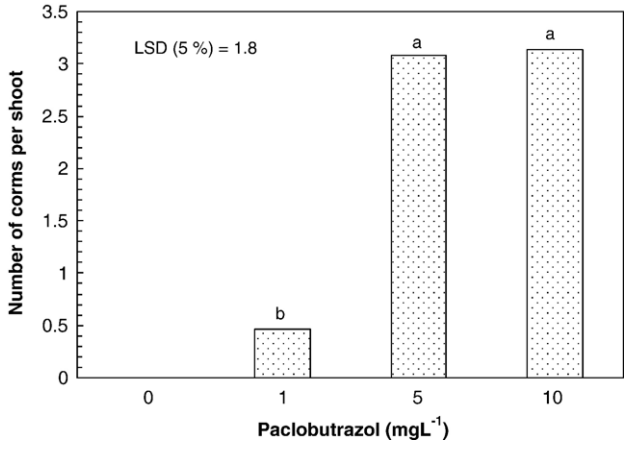

Fig. 4. The effect of various levels of paclobutrazol on *in vitro* corm formation of *D. luteoalbidum* after 3 months.