Plant Cell, Tissue and Organ Culture **60:** 151–154, 2000.
© 2000 *Kluwer Academic Publishers. Printed in the Netherlands.*

Research note

In vitro propagation of the endangered orchid, *Geodorum densiflorum* (Lam.) Schltr. through rhizome section culture

S.S. Sheelavantmath, H.N. Murthy*, A.N. Pyati, H.G. Ashok Kumar & B.V. Ravishankar
*Plant Tissue Culture Laboratory, Department of Botany, Karnatak University, Dharwad 580003, India (*requests for offprints; Fax: 0836–747884; E-mail: karuni@bgl.vsnl.net.in)*

Received 9 September 1999; acceped in revised form 11 April 2000

Key words: rhizome culture, orchid

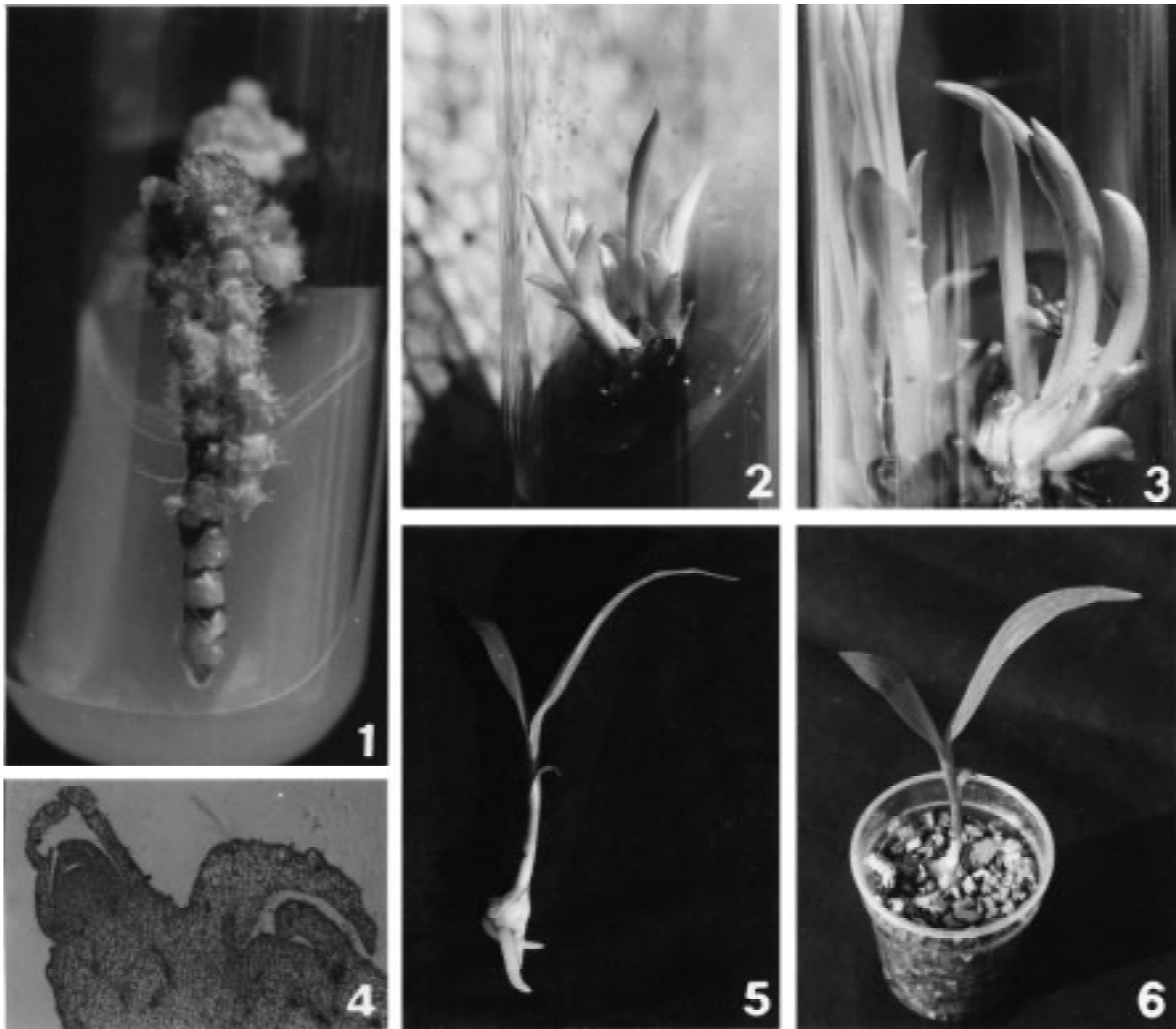
Abstract

Micropropagation of the endangered terrestrial orchid, *Geodorum densiflorum* (Lam.) Schltr. was achieved through rhizome section culture from *in vitro* seed- derived rhizomes. Rhizome sections were cultured on Murashige and Skoog (MS) and Knudson C(KC) media supplemented with various growth regulators and 0.1% activated charcoal. The rhizome sections responded on MS medium. Naphthaleneacetic acid (NAA) at 2.0 μM stimulated rhizome growth. However, benzyladenine (BA) at 5.0 μM induced multiple shoots within four weeks of culture and inhibited rhizome growth. The regenerated shoots rooted on MS only or with NAA at 1.0 μM. Well-developed plantlets were transferred to community pots and then to a greenhouse where the plants have been acclimatised.

Abbreviations: BA – benzyladenine; KN – kinetin; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; KC – Knudson C medium; MS – Murashige and Skoog's medium; NAA – 1-naphthaleneacetic acid; PLB – protocorm like bodies;

Orchids are experiencing a steady decline in tropical countries due to destruction of natural forest areas. It is essential to take measures for the conservation and propagation of these endangered orchid species. *Geodorum densiflorum* (Lam.) Schltr. is an endangered terrestrial orchid (Datta et al., 1999) appearing above the ground only during the rainy season. The introduction of an asymbiotic seed germination method by Knudson (1946) and shoot tip culture by Morel (1960) has helped in developing methods for orchid propagation. *In vitro* culture of seeds of *Geodorum densiflorum* (Lam.) Schltr. resulted in development of protocorms. These protocorms transformed into rhizomes, instead of shoots and roots. The rhizomes continue to grow and ramify without the formation of other organs for a long time and occasionally shoots are formed from rhizome apices. The present studies were focused on induction of organogenesis from rhizome section cultures of *Geodorum densiflorum* (Lam.) Schltr.

Plants of *Geodorum densiflorum* (Lam.) Schltr. were collected from Khanapur, Belgaum District, Karnataka State, India and were maintained in a greenhouse, Department of Botany, Karnatak University, Dharwad. To obtain sufficent capsules, the flowers were hand pollinated. Capsules were harvested as soon as they turned green to yellowish green (about 30 days after pollination). The capsules were surface sterilised in 0.1% mercuric chloride for 8 minutes followed by three washes in sterilised distilled water. The seeds were scooped out from sterilised capsules and cultured on Knudson C medium (Knudson, 1946) with growth regulators and adjuvants. The seeds germinated into PLBs (protocorm like bodies) on KC medium with 10% v/v coconut water. The PLBs developed into rhizomes. Four-week-old rhizomes were used as explants. Rhizomes were initially cut at nodal regions into small pieces and then internodal regions were longitudinally cut into 5–10 mm length and cultured on MS (Murashige and Skoog, 1962) or KC



Figures 1–6.
Figure 1. Proliferation of rhizome on MS medium with 2 μM NAA.
Figure 2. Development of multiple shoots from rhizome section on MS medium with (2 μM) BA.
Figure 3. Development of multiple shoots on MS medium with 5 μM BA after 12 weeks of culture.
Figure 4. Section through rhizome showing development of shoot primordia.
Figure 5. Rooting of shoots on MS medium with 1 μM NAA.
Figure 6. Transplanted plantlet.

(Knudson, 1946) media containing 2% sucrose, 0.8% agar, supplemented with 0.1% charcoal and cytokinins [benzylaminopurine (BA), kinetin (KN)] and auxin [1-naphthaleneacetic acid (NAA)] at different concentrations of 0.5μM, 1.0 μM, 2.0 μM, 5.0 μM, 10.0 μM and 20.0 μM. The pH of the medium was adjusted to 5.7 prior to autoclaving at 121 °C and 1.2 kg cm^{-2} for 20 min. Explants developed multiple shoots and simultaneously exuded phenolic compounds into the medium and, to overcome this explants were subcultured to the MS medium containing 0.1% activated charcoal. On fresh medium the shoots developed further. The well-developed shoots were transferred for rooting to MS basal medium and MS supplemented with auxins indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) at the concentration of 0.5 μM, 1.0 μM, 2.0 μM and 5.0 μM. The cultures were maintained at 22 $\pm$ 2°C under cool white fluorescent light (40 μmol m^{-2} s^{-1}) with a 12-h photoperiod . The experiments were repeated 3 times and the results are based on 12 replicates; the results are represented as means $\pm$ the standard error. For

Table 1. Effect of MS medium with different concentrations of NAA and BA on rhizome section culture of *Geodorum densiflorum* (Lam.) Schltr

Medium	No. of explants cultured	% of cultures responded	No. of shoots/explant	Shoot length (mm)	Rhizome length (mm)
MS + NAA-concentration					
MS + 0.5 μM	12	33.3	–	–	11.0 ± 0.28
MS + 1.0 μM	12	50.0	–	–	11.66 ± 0.40
MS + 2.0 μM	12	100.0	–	–	25.08 ± 0.31
MS + 5.0 μM	12	83.3	–	–	17.9 ± 0.23
MS + 10.0 μM	12	83.3	–	–	8.9 ± 0.09
MS + 20.0 μM	12	66.6	–	–	4.25 ± 0.16
MS + BAP-concentration					
MS + 0.5 μM	12	16.6	2.0 ± 0.0	9.5 ± 0.35	–
MS + 1.0 μM	12	33.3	3.0 ± 0.0	11.25 ± 0.42	–
MS + 2.0 μM	12	83.3	3.83 ± 0.06	14.0 ± 0.27	–
MS + 5.0 μM	12	100.0	8.20 ± 0.06	24.8 ± 0.07	–
MS + 10.0 μM	12	66.6	3.20 ± 0.09	21.18 ± 0.35	–
MS + 20.0 μM	12	50.0	2.30 ± 0.13	6.75 ± 0.12	–

Values are means ± standard error

structural evaluation rhizomes were fixed in FAA (90 ml of 70% ethanol, 5 ml formaldehyde, 5 ml glacial acetic acid), dehydrated in a tertiary-butyl alcohol series, embedded in paraffin, and cut into 6 μm serial sections using a microtome. Sections were stained in Amido Black B.

The explants cultured on KC medium did not show any response and turned brown in 6–8 weeks. However, the explants cultured on MS medium responded well. The morphogenetic response of the explants to BA or NAA is shown in Table 1. The explants cultured on medium containing NAA showed further growth but did not show evidence of organogenesis (Table 1, Figure 1). The explants cultured on medium containing kinetin did not respond at all. The explants cultured on medium containing BA responded very well and developed multiple shoots in 4–6 weeks (Figure 2). However, the growth of rhizome retarded on BA-containing medium. Shoots first appeared as small, green protuberances on the rhizome surface and eventually developed into shoots. The frequency of the shoot regeneration and number of shoots per explant increased with increasing concentrations of BA up to 5.0 μM. High frequency of shoot regeneration (100%) coupled with maximal number of shoots (6–8 shoots/explant) was recorded at 5 μM BA (Figure 3). However, a further increase in BA (10 μM and 20 μM) did not increase shoot induction (Table 1). After initiation of shoot buds the explants after 2–4 weeks of culture released phenolics into the medium and hindered further growth of shoot buds. To overcome this, the explants were transferred to fresh medium containing 0.1% activated charcoal and, on subculturing shoot buds developed further into shoots after another 2 weeks. The developing shoots (in groups) were subcultured onto fresh MS medium for further growth at an interval of 4 weeks. Histological sections of rhizomes showed development of multiple shoots directly from the rhizome without intermediate protocorm stage or callusing (Figure 4). Similar, shoot organogenesis from rhizome explants has been reported for several *Cymbidium* species (Paek et al., 1990) *Cymbidium kanran* (Chung et al., 1985), *Cymbidium faberi* (Hasegawa et al., 1985), *Cymbidium naveomagenatam*, *C. goeringii* (Paek and Kazoi, 1998) and *Cymbidium forrestii* (Paek and Yeung, 1991).

Shoots developed very well after 14 weeks. At this stage, they were transferred to rooting medium. Two to three healthy roots developed from shoots after four weeks of subculture on MS basal medium. Of the three auxins IAA, IBA and NAA tested for inducing roots, IAA and IBA did not show any response. However, NAA at low concentrations induced root development in 4 weeks. NAA at 1.0 μM induced 100% rooting

with 3–4 roots per explant (Figure 5). Higher concentration of NAA inhibited rooting. The rooted plantlets were transplanted to small plastic pots containing vermiculite and weaned (Figure 6). At 4-day intervals half-strength MS liquid medium was added to the pots. The plants were transferred to the greenhouse after 4 weeks, where they have acclimatised.

This simple and efficient procedure for regenerating a large number of plantlets from rhizome cultures of *Geodorum densiflorum* (Lam.) Schltr. could be used for large-scale propagation and *ex situ* conservation of this orchid species.

References

Chung JD, Chun CK, Kim SS & Lee JS (1985) Factors affecting growth of rhizomes and organogenesis of Korean native *Cymbidium kanran*. Kor. Soc. Hort. Sci. 26: 281–288

Datta KB, Kanjilal B & Desarker D (1999) Artificial seed technology: Development of protocol in *Geodorum densiflorum* (Lam) Schltr. An endangered orchid. Current Science 76: 1142–1145

Hasegawa A, Ohashi H & Goi M (1985) Effects of BA, rhizome length, mechanical treatment and liquid shaking culture on shoot formation from rhizome in *Cymbidium faberi* Rolfe. Acta Horticulturae 106: 25–40

Knudson L (1946) A nutrient for the germination of orchid seeds. Amer. Orchid Soc. Bull. 15: 214–217

Morel GM (1960) Producing virus free *Cymbidium*. Am. Orchid Soc. Bull. 29: 495–497

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Planatarum 15: 473–497

Paek KY & Kozai T (1998) Micropropagation of temperate *Cymbidium* via rhizome culture. Hort. Technology 8: 283–288

Paek KY & Yeung EC (1991) The effect of 1-napthaleneacetic acid and N^6-benzyladenine on the growth of *Cymbidium forrestii* rhizome in vitro. Plant Cell Tiss. Org. Cult. 24: 65–71

Paek KY, Shim GB & Kim JJ (1990) Effect of natural products and BAP exposing periods on organogenesis of temperate *Cymbidiums* using rhizomes. Kor. Soc. Hort. Sci. 31: 74–80