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MICROPROPAGATION OF AN ENDANGERED TERRESTRIAL ORCHID *GEODORUM DENSIFLORUM* (LAM.) SCHLTR. OF KANYAKUMARI DISTRICT, INDIA

G.V. Gegi[1], Dr. B. Christudhas Williams[2] & Dr. R. Mary Suja[3]

[1]Research Scholar, Scott Christian College (Autonomous) Nagercoil – 3, Affiliated with Manonmaniam Sundaranar University Tirunelveli - 627012.

[2]Research Guide, Scott Christian College (Autonomous) Nagercoil – 3, Affiliated with Manonmaniam Sundaranar University Tirunelveli - 627012.

[3]Director, William Research Centre, Nagercoil, India.

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***Corresponding Author**
Dr. R. Mary Suja
Director William Research Centre, Nagercoil, India.
rmsuja.83@gmail.com

ABSTRACT

Geodorum densiflorum (Lam.) Schltr. is an endangered orchid with pharmaceutical importance. *In vitro* studies will immensely aid conservation measure of this orchid species. Seeds of *Geodorum densiflorum* (Lam.) Schltr. were aseptically cultured on 0.8% (w/v) agar solidified Murashige and Skoog (MS) media. Seeds germinated and formed light green globular structures directly produced tiny seedlings which underwent elongation in PGRs supplemented MS medium. The callus was friable, opaque and creamish to light yellow in colour. In the assessment of callus induction MS media supplemented with coconut water showed maximum number of PLBs (15.93 per callus) in BAP (1 mgL-1) + NAA (1 mgL-1) + 15% CW. The produced PLBs are converted into plantlets after 8 to 10 weeks of culture in only those cases regenerated on auxin and cytokinins combinations. 0.5mg/l KIN and 0.5mg/l BAP showed maximum number of shoots (4.4±0.4) and shoot length increased to 2.4±0.3cm. The combination of BAP +IAA showed maximum number of roots per plantlet (3.57) obtained from 0.5 mg/L each of BAP+IAA with charcoal supplementation and 1.0 mg/L each of BAP +IAA combination gave the highest number of roots (2.667 cm) with charcoal supplementation. Hardened plant was transferred to green house after *ex vitro* rooting techniques.

KEYWORDS: *Geodorum densiflorum*, BAP, endangered orchid, micropropagation, MS media, green house, hardening, phytochemical analysis.

INTRODUCTION

Geodorum densiflorum is a stout terrestrial herb, leaves 4 per shoot, petiole 8-10 cm long; lamina ovate to lanceolate, 25-40 cm long, 8-11 cm wide with 3 prominent ribs, deciduous, annually plicate, stalked and the petiole of all leaves forming a pseudostem enclosed together in 2 common sheating bracts. Peduncle is longer than the rachis and the distal part nodding in flower and straightening in fruit. 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.[1] *G. densiflorum* is a medicinal plant that has been traditionally used for the treatment of various diseases. The root is used as an insecticide, to treat irregular menstrual cycle in women and in wound healing.[2] The tuber and rhizome are used to treat impotency.[3, 4] and to increase sperm density.[5] The pseudo bulb is used to treat diabetes[6, 7] and carbuncles.[8] Pharmacological studies on various plant parts of *G. densiflorum* have reported its antimicrobial[9-11], antioxidant[11], cytotoxic[11,12], thrombolytic[13], analgesic and sedative[14] properties. Phytochemical investigation of the leaves and pseudobulb of orchid has reported the presence of flavonoids, terpenoids, alkaloids and steroids.

Habit dilapidation and anthropogenic activities are the major threats to the survival of terrestrial orchid. The scarcity of pollinators and poor seed setting are the major constraints in the natural propagation, leading to a continuous depletion of its natural population. Propagation from seeds is held back by low germination and survival rates due to the inept environmental conditions. As a result their wild populations are diminishing at an alarming rate.[15] The introduction of an asymbiotic seed germination method were focused on induction of organogenesis from seed cultures of *Geodorum densiflorum* (Lam.) Schltr. MS media containing 2% sucrose, 0.8% agar, supplemented with 0.1% charcoal and cytokinins [benzylaminopurine (BA), kinetin (KN)] and auxin [1-naphthaleneacetic acid (NAA)]. The pH of the medium was adjusted to 5.7 prior to autoclaving at 121C and 1.2 kg cm for 20 min. Explants developed multiple shoots and simultaneously exuded phenolic compounds into the medium and, to overcome this explants were sub cultured to the MS medium containing 0.1% activated charcoal.

MATERIALS AND METHODS

Geodorum densiflorum (Lam.) Schltr. was collected from the Kothiyar Hills of Kanyakumari District in the month of March. Juvenile shoots were acquired from the mature plants of *Geodorum densiflorum* (Lam.) Schltr. grown in the Botanical Garden. Mature capsules of *Geodorum densiflorum* were collected from the potted plants after 1 month was washed with running tap water for 30 minutes to remove the dust particles from the surface were micropropagated. The collected plants were identified using the Flora of Presidency of Madras Gamble, J. S, 1935 (Plate - 1). *In-vitro* phytochemical constituent of *Geodorum densiflorum* (Lam.) Schltr. leaf extracts was determined as per the standard procedures.[16]

Plate 1: *Geodorum densiflorum* (Lam.) Schltr.

RESULTS AND DISCUSSIONS

In *Geodorum densiflorum* (Lam.) Schltr. callus induction was observed in the MS media supplemented with coconut water. The callus was friable, opaque and creamish to light yellow in colour varied in size (small, medium and large). The callus initation was observed after 28 to 37 days and the callus induction varied from 55.8-60.6% in MS media (Table 1).

Table 1: Effect of coconut water on callus induction of *Geodorum densiflorum* (Lam.) Schltr. immature seeds on MS Media.

Supplemented medium with coconut water	Response of immature seeds in culture		
	Morphogenesis	% Callus induction	Number of days taken
Control	-	-	-
MS Media +CW 10%	++	55.8±0.92	28
MS Media +CW 15%	+	60.6±0.61	31
MS Media +CW 20%	++	59.4±0.62	37

No response, + small quantity, ++ = Moderate, +++= Large quantity of calli Data showing the mean of 10 replicates ± standard error.

Regeneration

The regeneration ability of induced callus was evaluated by sub-culturing them on MS media. The maximum number of PLBs (15.93 per callus) in *Geodorum densiflorum* (Lam.) Schltr. was noted on MS medium supplemented with BAP (1 mgL-1) + NAA (1 mgL-1) + 15% CW. The produced PLBs are converted into plantlets after 8 to 10 weeks of culture in only those cases regenerated on auxin and cytokinins combinations (Table 2).

Table 2: Influence of growth regulators (PGRs) on development of protocorm like bodies (PLBs) from immature seed callus of *Geodorum densiflorum* (Lam.) Schltr. on MS Media supplemented with 15% coconut water.

PGR Treatment (mgL^{-1})	No of PLBs from each callus	Type of response	Development of shoot buds from PLBs
MS (Control)	-	-	-
BAP (5) + NAA (5)	-	-	-
BAP (5) + NAA (2)	1.3±0.32	Greenish PLBs formed	---
BAP (2) + NAA (2)	4.7±0.36	Greenish PLBs formed	---
BAP (2) + NAA (1)	7.33±0.96	Greenish PLBs formed	-
BAP (1) + NAA (1)	15.93±0.64	Healthy green PLBs formed	Shoot buds and plantlet formation

Mean number of PLBs per 0.01g fresh weight of callus. Data showing mean of 15 replicates ± standard error (SE), - No response, ---- = No further growth.

In order to induce rapid growth, the germinated seedlings were transferred to the shoot elongation medium. For shoot multiplication different combinations of KIN and BAP ranging from 0.5 and 1.0mg/l were added to MS medium supplemented with 3% sucrose. It was observed that at 0.5mg/l KIN and 0.5mg/l BAP, maximum number of shoots (4.4±0.4) was obtained with shoot length increased to 2.4±0.3cm. As the KIN concentration increased, the number of shoots decreased (Table 3).

Table: 3 Effect of various combinations of Kinetin and BAP on shoot multiplication and shoot length.

PGR	Number of shoots	Shoot length
0.5KIN+0.5BAP	4.4±0.4	2.4±0.3
0.5KIN+1.0BAP	4.0±0.2	1.4±0.5
1.0KIN+0.5BAP	2.1±0.4	1.3±0.3
1.0KIN+1.0BAP	1.0±0.0	1.0±0.0
0.5KIN+0.5BAP	2.4±0.5	1.5±0.3
0.5KIN+0.5BAP	3.2±0.2	1.9±0.4

The combination of BAP +IAA showed maximum number of roots per plantlet (3.57) obtained from 0.5 mg/L each of BAP+IAA with charcoal supplementation and 1.0 mg/L each of BAP +IAA combination gave the highest number of roots (2.667 cm) with charcoal supplementation (Table 4).

Table 4: Combined effect of growth regulators on *in-vitro* rooting of *Geodorum densiflorum* (Lam.) Schltr. after inoculation in MS medium.

Growth hormones	Concentrations (mg/L)	Number of roots/plantlet	Length of root (cm)
		With charcoal	With charcoal
BAP+IAA	0+0	0.00d	0.00d
	0.5+0.5	3.576a	2.140b
	1.0+1.0	1.583c	2.667a
	2.0+2.0	1.700b	2.130b

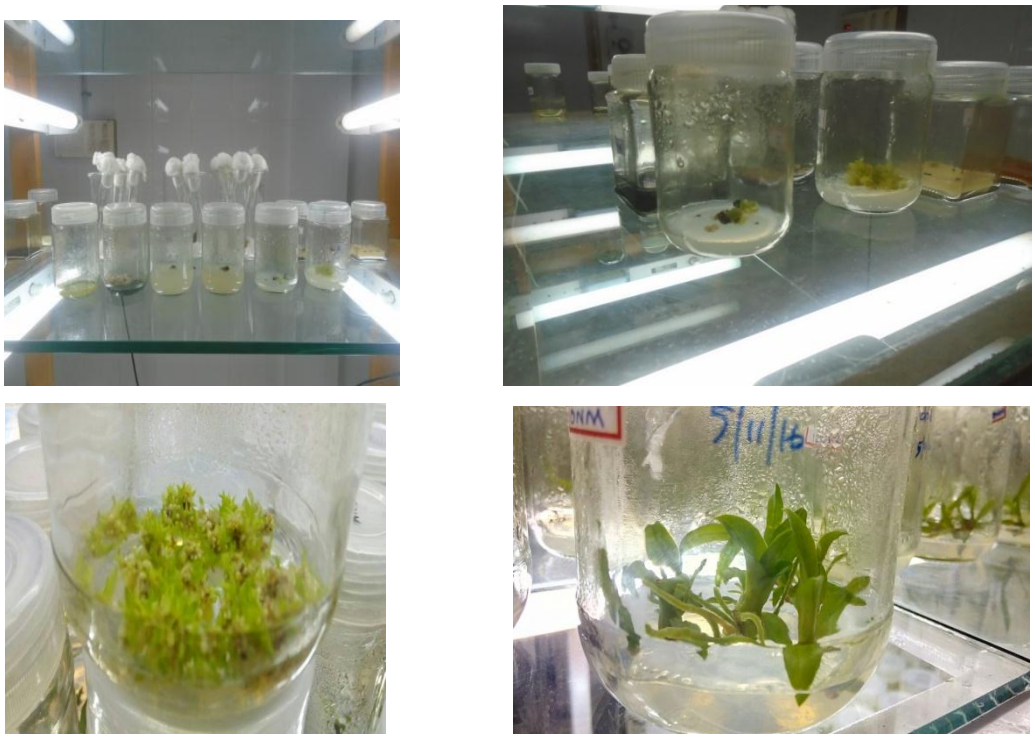

Plate: 2 Micropropagation of *Geodorum densiflorum* (Lam.) Schltr.

The well-developed seedlings with 2 to 3 leaves and 1 to 2 roots were gradually sub-cultured on hormone free and subsequently on one half and one fourth strength nutrient medium, respectively for 3 months as a part of hardening procedure. Root intact plantlets were taken out of the culture vessel, transferred to orchid pots filled with garden soil and forest leaves (1:1). During the part of transfer 4.5% of plants died and 50% of the field-transferred regenerates were successfully acclimatized to soil. Acclimatization of the rooted shoots was greatly influenced by the potting mixtures used for plant transfer. The combined mixture of

autoclaved garden soil and forest leaves (1:1) act as the most effective substrate for the acclimatization of *in-vitro* regenerated plantlets of *Geodorum densiflorum* (Lam.) Schltr. (Plate: 3). Transfer of plants in the pots and field was accomplished after hardening and acclimatization.

The earlier studies showed that the rhizome sections cultured on Murashige and Skoog (MS) and Knudson C (KC) media supplemented with various growth regulators and 0.1% activated charcoal of *Geodorum densiflorum* (Lam.) Schltr. rhizome sections responded on MS medium. Naphthaleneacetic acid (NAA) at 2.0 M stimulated rhizome growth, benzyladenine (BA) at 5.0 M 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 M. Well-developed plantlets were transferred to community pots and then to a greenhouse where the plants have been acclimatized.[17]

Plate: 3 Hardening of *Geodorum densiflorum* (Lam.) Schltr.

In-vitro phytochemical analysis of *Geodorum densiflorum* (Lam.) Schltr. leaf extract showed the presence of flavonoid, tannin, terpenoid, alkaloid, saponin and steroid; benzene extract of *Geodorum densiflorum* (Lam.) Schltr. leaf showed the presence of terpenoid, alkaloid, saponin and steroid; ethanol extract showed the presence of terpenoid, flavonoid, tannin and steroid; acetone extract showed the presence of terpenoid, alkaloid, steroid and chloroform extract showed the presence of flavonoid, alkaloid and steroid.

CONCLUSION

The regenerated plantlets were phenotypically similar when compared with wild plants. Further standardization of medium or selection of other explants is under progress to develop an efficient *in vitro* clonal propagation.

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