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IN VITRO REGENERATION OF *ARUNDINA GRAMINIFOLIA* (D. DON) HOCHR

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ABSTRACT

We can see Orchids come in a wide variety of shapes, sizes, colours, and textures far beyond the human mind's imagination. They emerge from seeds in nature, but in the absence of suitable hosts, they do not germinate in sufficient numbers. This problem was solved by using the tissue culture technique for its germination. One of the successful method used for mass propogation of orchid plantlets is *in vitro* techniques. Therefore, an initial analysis was conducted in order to establish an appropriate procedure for mass multiplication of *Arundina graminifolia*. MS (Murashige and Skoog) medium was found to be suitable for the asymbiotic seed germination of *Arundina graminifolia*. Direct protocorm like bodies were induced by using combinations and individual supplement of MS medium with IAA (Indole-3-acetic acid), IBA (Indole-3- butyric acid), BAP (6-Benzylaminopurine) and KIN (Kinetin). Hormone-free MS basal medium was found suitable for the conversion of PLBs (protocorm-like bodies) into complete plantlets

Keywords: Contaminate, spoilage, fungi, bacteria and surface sterilized

INTRODUCTION

Among angiosperms orchidaceae is one of the largest families. Orchidaceae family includes 800 genera and 25000 species. Orchids are well known for their economic importance and ornamental beauty. *Arundina* comes from Latin word arundo which means reed and graminifolia means grass like leaves. *Arundina graminifolia* is commonly known as bamboo orchid. Bamboo orchid is a terrestrial perennial orchid with erect stem, forming into large clumps growing to a height between 70cm to 2m (Supriya das *et al*; 2013).

Orchids are the most fascinatingly beautiful flowers and unique group of plants of nature. With their exotic shapes, hues and the added advantage of longevity, these flowers of rare beauty have become increasingly popular in the 21st century. They belong to the Orchidaceae family and consisting 600-800 genera and 25,000-35,000 species. Today growing orchid is more than just a hobby; it is an international business covering around 10% of the world floriculture trade. Especially, some of the exquisitely rare hybrids of orchid are among the top ten cut flowers. It has become possible by adopting *in vitro* tissue culture techniques for their rapid multiplication. Since, orchids are strictly out breeders, seed propagation results in unwanted heterozygous types. So, vegetative propagation techniques require accurate regeneration protocol for obtaining *in vitro* cultured true to type plants. In fact, the technical aspects of micro propagated orchids have improvised significantly in past few years. But the loop holes in micro propagation stems from the somaclonal variation, phenolic compound exudation explants, hardening and so on (Dipika Sarmah; 2017)

A. graminifolia a reedly terrestrial tropical orchid species generally grows in clumps. It is available in newly developed habitats of anthropogenic origin, such as road cuts and abandoned farm fields [1, 2] and mostly occur in limited areas, its natural habitat being steep, rocky sites or open grassy areas [1]. The rhizome of the plant is used as antibacterial agent and its root decoction is commonly used for the ailments of diabetes, tumour, hyperliposis and hepatitis. The phenolic compound of this orchid has antihepatitic and antiHIV activity (Bimal Debnath *et al*., 2016)

High frequency micropropagation of *Arundina graminifolia* (D. Don.) Hochr. through protocorm-like bodies (PLBs) using node explants was established. Node explants derived from three-year-old field grown plants were cultured on half-strength MS medium with either 6.97 µM kinetin (Kn), or 15% coconut water (CW) or 13.3 µM BA favoured sprouting of the axillary bud. Subsequent culture of these emerged buds on medium having 44.4 µM BA facilitated formation of PLBs (a mean of 5.4) from the base. Transfer of PLBs to medium with the same level of BA (44.4 µM) PLBs favoured enhanced proliferation. The 6th subculture of PLBs on medium with 44.4 µM BA yielded > 100 PLBs. The PLB proliferation did not exhibit a decline up to 10th subculture. Conversion of PLBs to shoots or plantlets occurred at high rate (89%) upon transfer to half-strength MS medium containing 6.97 µM Kn. Half-strength MS medium with 1 g l-1 activated charcoal was effective for rooting, and the rooted plants exhibited 91% survival in field condition (Kottackal Poulose Martin; 2007)

MATERIALS AND METHODS

Plant material

The un-dehisced capsules of *Arundina graminifolia*, were collected during July-August from Western Ghats, Tamil Nadu. The seeds from the capsules were used for asymbiotic seed germination studies. The freshly collected capsules were washed with the detergent Teepol (0.1%), rinsed in distilled water, surface sterilized with mercuric chloride solution (0.1%) for 3 min and rinsed thrice in distilled water. The capsules were dipped in 80% ethyl alcohol for 1 min, flamed, cut longitudinally and the seeds were inoculated on five different basal media under aseptic condition. The 80-day-old protocorms, (before the emergence of first leaves) obtained from the symbiotically germinated seeds was used as explants for further *in vitro* regeneration studies. For each culture, 5 replicates were maintained and all the experiments were repeated at least thrice to confirm the results.

Preparation of Media

Stock solutions were prepared separately for macronutrients, micronutrients, iron EDTA, potassium iodide and vitamins and stored at 4°C. Specific quantity of stock solutions of Murashige and Skoog (1962), Linsmaier and Skoog (1965), Lindemann orchid medium (1970), Schenk and Hildebrandt medium (1972) and Knudson C medium (1946) were used along with the required sucrose. The final volume was made up with distilled water and the pH was adjusted to 5.5-5.8 either with 1NNaOH or 1N HCl.

Asymbiotic seed germination

Agar (0.8% to 0.9%; Hi Media) was added to the above media and melted in water bath. The medium (10 mL to 15 mL) was dispensed into 250 mm × 150 mm culture tubes, covered, autoclaved (1.06 kg pressure for 20 min at 120°C), cooled, allowed to solidify as slants and the inoculations were carried out. Bursting of the seed coat and emergence of the enlarged embryo, i.e., the protocorm was considered as germination. The germination of seeds was recorded and percentage of seed germination was calculated.(fig 1)

% of germination = Total No. of seeds germinated Total No. of seeds inoculated × 100

Multiple protocorm-like body (PLB) induction from seed-derived protocorms

The MS (1962) medium, in which the germination percentage was highest was selected for PLB induction and supplemented with growth regulators such as 2,4-D (2,4-Dichlorophenoxyacetic acid)(2.26, 4.52 and 9.03 µM), IAA (Indole-3-acetic acid) (2.9, 5.7 and 11.4 µM) both individually and in combinations with BA (6-benzyladenine) (2.22, 4.44 and 8.88 µM) and Kinetin (2.32, 4.64 and 9.29 µM). The percentage frequency of explants producing PLBs and average number of PLBs per explant were observed periodically and the results were tabulated.

Regeneration of plantlets from PLBs

The PLBs were transferred to hormone - free MS medium after 40 days and the percentage frequency of conversion of PLBs into complete plantlets was calculated.

Rooting

The regenerated plantlets developed roots on the hormone-free MS medium after 10 days.

Hardening

Well-rooted healthy plantlets were washed with distilled water and transferred to potting mixture containing sand and perlite in the ratio 1:1 for hardening. The containers with the plantlets were covered with polythene bags to ensure humidity, after 4 weeks the plants were transferred to earthen pots.

RESULTS AND DISCUSSION

Asymbiotic seed germination the immature pods of *A. graminifolia* showed differential response (Table 1) when cultured in five different basal media. Swelling and greening of the seeds was found to be the first significant change after 14 days of culture. The greening and germination of the seed commenced first in MS basal medium within 14 days (Fig 1) followed by KC basal medium (16 days). Seed germination frequency of 100% was observed on MS basal medium followed by LS and LOM medium (80%). However, only 60% of the seeds germinated on KC basal medium (Figure 2). The poor results obtained on KC medium can be attributed to the nutrient ion imbalance, a primary defect in this medium (Ichihashi, 1979). Studies on micropropagation showed that the presence of sucrose in the culture medium to be important for root development, shoot multiplication as well as for increase in height of the plant (Kozai *et al*., 1991). Sucrose concentrations of 0.59M and 0.88M have been most commonly used in orchid tissue culture studies.

Multiple protocorm-like body (PLB) induction from seed-derived protocorms
Effect of auxins

Direct PLB inductions have been observed from intact protocorms of *in vitro* germinated seeds. Seed derived protocorms and longitudinal section of PLBs were found to be efficient in inducing somatic embryogenesis (Huan

Table 1. Effect of various basal media on asymbiotic seed germination of *A. graminifolia*

Basal Media	MS	LS	LOM	SH	KC
No: of days taken for seed germination	14	20	25	25	16
Frequency of germination (%)	100	80	80	75	60

MS- Murashige and skoog (1962); LS- Linsmaier and skoog (1965); LOM- Lindemann orchid medium (1970); SH- Schenk and Hildenbrandt medium (1972); KC -Knudson C medium (1946)

Table 2. Effect of auxins on multiple protocorm induction in *A. graminifolia*

MS + Growth Regulators (µM)		Time taken for multiple protocorm initiation (days)	Frequency of multiple protocorm formation (%)	Average number of multiple protocorms initiated (after 40 days)	Frequency of protocorm survival (%)
MS Basal		0	0	0	100
2,4 D	2.26	0	0	0	10
	4.52	0	0	0	10
	9.03	0	0	0	20
IAA	2.9	0	0	0	100
	5.7	60	20	3.5	100
	11.4	0	0	0	90

MS- Murashige and Skoog (1962); 2,4-D - 2,4-Dichlorophenoxyacetic acid; IAA- Indole-3-acetic acid

Table 3. Effect of auxins on multiple protocorm induction in *A. graminifolia*

MS + Growth Regulators (µM)		Time taken for multiple protocorm initiation (days)	Frequency of multiple protocorm formation (%)	Multiple protocorms initiated (after 40 days)	Frequency of protocorm survival (%)
MS basal		0	0	0	100
BAP	2.22	60	60	5.5	100
	4.44	0	0	0	90
	8.88	0	0	0	70
KIN	2.32	0	0	0	100
	4.64	0	0	0	100
	9.29	0	0	0	100

MS - Murashige and Skoog (1962); BAP - 6-Benzylaminopurine; KIN – Kinetin

Table 4. Effect of combination of BAP with auxins on multiple protocorm induction in *A. graminifolia*

MS +Growth regulators (µM)		Time taken for multiple protocorm initiation (days)	Frequency of multiple protocorm formation (%)	Multiple protocorms initiated (after 40 days)	Frequency of protocorm survival (%)
BAP+2,4 D	2.22+4.52	0	0	0	20
	4.44+4.52	0	0	0	10
	8.88+4.52	0	0	0	10
BAP+ IAA	2.22+2.90	0	0	0	70
	4.44+2.90	57	30	3	100
	2.22+5.70	0	0	0	0
	8.88+5.70	50	90	10	100

MS - Murashige and Skoog (1962); BAP-6- Benzyl aminopurine; 2, 4 - D - 2, 4 - dichlorophenoxyacetic acid; IAA- Indole - 3 - acetic acid

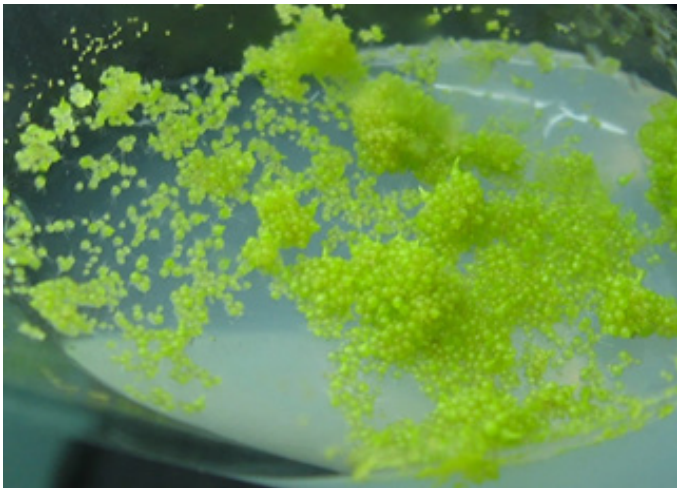

Figure 1. seed germination of *Arundina graminifolia*

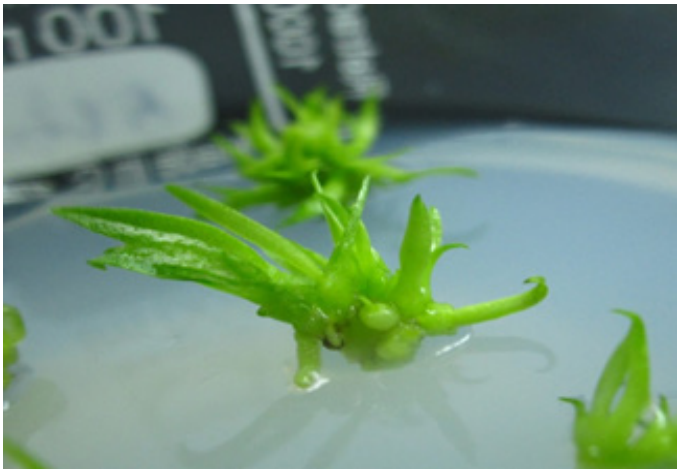

Figure 2. *In vitro* regeneration of *Arundina graminifolia*

et al., 2004). Plantlet regeneration via callus mediated PLB formation from seed-derived protocorms has been well-documented in a number of orchids (Diaz and Alvarez, 2009; Chen *et al.,* 2000). Micropropagation through PLBs is preferred over other methods because of their regeneration potential and efficient conversion into plantlets (Ng and Saleh, 2011). In the present study, single protocorms when inoculated on MS medium supplemented with IAA at 5.7 µM produced a maximum average of 3.5 protocorms .All other concentrations of IAA failed to induce any observable change. 2,4-D at all concentrations (2.26, 4.52 and 9.03 µM) induced protocorm death. MS medium when used without growth regulators showed 100% protocorm survival rate. Auxins were efficient in inducing PLBs in *Aerides maculosum*, however, the regeneration frequency was lower when compared to cytokinins. In Dendrobium draconis, the addition of auxin alone did not have any effect on PLB formation (Rangsayatorn, 2009)

Effect of cytokinins

BAP at 2.22 µM induced maximum number of protocorms within 60 days of inoculation (Table 3). At higher concentrations of BAP (4.44 µM and 8.88 µM), protocorm death was observed. KIN at all concentrations did not induce protocorms. However, 100% survival of protocorms was observed at all concentrations of KIN. The presence of BAP in the culture medium enhances direct PLB induction and proliferation when compared to Kin (Martin and Madassery, 2006; Rangsayatron, 2009).

Effect of combination of BAPwith auxins

BAP in combination with 2,4-D induced protocorms death at all concentrations (Table 4). However, best results were observed when BAP was combined with IAA. Higher frequency and early induction (50 days) of multiple protocorms (90%) and a maximum average of 10 protocorms per explant was observed when BAP (8.88 µM) was combined with IAA(5.7 µM).

Regeneration of plantlets from PLBs

The PLBs developed normal shoots with well-developed leaves when transferred to hormone-free MS basal medium. A total of around 50 shoots were obtained in all the treatments. The effect of hormone free MS basal medium in promoting maturation and subsequent conversion of the somatic embryos and PLBs into complete plantlets has been well documented (Chen and Chang, 2000) and has been reported in many orchids (Ng and Saleh, 2010; Rangsayatorn, 2009).

Rooting and hardening

The PLBs germinated into complete plantlets with shoots and roots on hormone-free MS basal medium. The regenerated plantlets were successfully transferred to earthen pots with a survival rate of 50%.

CONCLUSION

MS medium was found to be suitable for the asymbiotic seed germination of *Arundina graminifolia*. MS medium supplemented with IAA, IBA, BAP and KIN individually and in combinations induced direct PLBs. Hormone-free MS basal medium was suitable for the conversion of PLBs into complete plantlets. The results of this preliminary study may help in contributing to the development of protocols for the medium- and long-term conservation of the plant.

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REFERENCES

Chen, J.T. and Chang, W.C. 2000. Efficient plant regeneration through somatic embryogenesis from callus cultures of Oncidium (Orchidaceae). *Plant Sci*.160, 87 - 93.

Diaz, M.D.S.S. and Alvarez, C.C. 2009. Plant regeneration through direct shoot formation from leaf culture and from protocorm-like bodies derived from callus of Encycliamariae (Orchidaceae), a threatened Mexican orchid. 2009. *In Vitro* Cell. Dev. *Biol. Plant* 45, 162 - 170.

Dressler, R.L. 1981. The Orchids: Natural History and Classification. Harvard University Press, Cambridge, Massachusetts.

Hoque, M.M., Khaleda, L. and Al-Forkan, M. 2016. Evaluation of pharmaceutical properties on microbial activities of some important medicinal orchids of Bangladesh. *J Pharmacogn. Phytochem.* 5, 265 - 269.

Huan, L.V.T., Takamura, T. and Tanaka, M. 2004. Callus formation and plant regeneration from callus through somatic embryo structures in Cymbidium orchid. *Plant Sci*. 166, 1443 - 1449.

Ichihashi, S. 1979. Studies on the media for orchid seed germination. J. Jpn. Soc. Hortic. Sci. 48, 345 - 352. Kasulo, V., Mwabumba, L. and Cry, M. 2009. A review of edible orchids in Malawi. J. Hortic. For. 1, 133 - 139.

Knudson, L. 1946. A new nutrient solution for the germination of orchid seeds. Amer. Orchid Soc. Bull.15, 214 - 217.

Kozai, T., Iwabuchi, K., Watanabe, K. and Watanabe, I. 1991. Photoautotrophic and photo mixotrophic growth of strawberry plantlets *in vitro* and changes in nutrient composition of the medium. *Plant Cell Tiss. Org. Cult.*

25, 107 - 115.

Lindemann, E.G.P., Gunckel, J.E. and Davidson, O.W. 1970. Meristem culture of Cattleya. American Orchid Soc. Bull. 39,1002 - 1004. Linsmaier, E.M. and Skoog, F. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant*.18, 100 - 127.

Martin, K.P. and Madassery, J. 2006. Rapid *in vitro* propagation of Dendrobium hybrids through direct shoot formation from foliar explants and protocorm like bodies. *Sci. Hort.* 108, 95 - 99.

Mohanty, J.P., Pal, P. and Barma, A.D. 2015. An overview on orchids. Univers. J. Pharm. Sci. Res. 1, 45 - 50. Murashige, T. and Skoog, F. 1962. Arevised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473 - 497.

Nagananda, G.S., Patil, A., Kambli, J, V. and Rajath, S. 2014. Phytochemical evaluation and *in vitro* free radical scavenging activity of cold and hot successive pseudo bulb extracts of medicinally important orchid Pholidota pallid Lindl. *Adv. Biores*. 5, 100 - 105.

Prasathkumar, M. and Ramesh, T. 2016. Conservation of Pholidota pallida Lindl. Medicinal orchid – *in vitro. Int. J. Res. Ins.* 3, 678 - 688.

Quattrocchi, U. 2012. CRC world dictionary of medicinal and poisonous plants: Common Names, scientific names, eponyms, synonyms, and etymology. CRC Press, Taylor and Francis group, USA, p. 2888.

Rangasayatorn, N. 2009. Micropropagation of Dendrobium draconis Rchb.f. from thin cross- section culture. *Sci. Hort.*122, 662 - 665.

Rasmussen, H.N. 1992. Seed dormancy patterns in Epipactis palustris (Orchidaceae): Requirements for germination and establishment of mycorrhiza. *Physiol. Plant.* 86, 161 - 167.

Rasmussen, H.N., Anderson, T.F. and Johansen, B. 1990. Temperature sensitivity of *in vitro* germination and seedling development of Dactylorhiza majalis (Orchidaceae) with and without a mycorrhizal fungus. *Plant Cell and Environ.*13, 171-177.

Schenk, R.V. and Hildebrandt, A.C. 1972. Medium techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot.* 50, 199 - 204.

Teoh, E.S. 2016. Medicinal orchids of Asia. Springer International Publishing, Switzerland, p. 594.

The Plant List 2013. Version 1.1. Published on the Internet; http://www.theplantlist.org/.