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Study on rapid mass multiplication of orchid by using immature seeds

Vitthal N. Bhosale*, **Krishan P. Singh, A.K. Singh** and R. Jyothi**
Division of Floriculture and Landscaping, Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110 012

ABSTRACT

Orchid species for present research work used was Bamboo orchid (*Arundina graminifolia*). The seeds were used as source of *ex-plant* obtained from immature capsule (120 day old). *In vitro* culture was initiated on Murashige and Skoog's medium supplemented with BAP (1.0 mg/l) + NAA (1 mg/l) provided with 16/8 h photoperiod light condition were found to be most suitable for *in vitro* seed germination and Protocorm Like Bodies (PLB's) development. The maximum multiplication of the PLB's was obtained on the MS medium supplemented with, BAP (0.5 mg/l) + NAA (0.5 mg/l) and BAP (1 mg/l) + NAA (1 mg/l) forms plb's in less period of time i.e. 12.00 and 13 days after inoculation. The MS + BAP (1.5 mg/l) + NAA (1 mg/l) gives highest vegetative growth of plantlets. A well-developed cluster of plantlets were selected and transferred to second subculture for root induction. The half strength MS medium provided with NAA (0.5 mg/l) gives good rooting. Rooted plantlets were transferred in earthen pot with polythene cover filled with peat + soilrite (1:1 v/v) for temporary *ex-vitro* hardening gives high survival of the seedlings. The plantlets were then successfully acclimatized by additional application of Ohio W. P. solution on same medium using earthen pots.

Key words: *Arundina graminifolia*, immature seeds, mass multiplication, orchid.

INTRODUCTION

Orchids enjoy the place of royalty in the world of ornamental horticulture and one of the most important groups of flowering plants is known for their lovely blooms. They exhibit a great diversity in colour, shape, size and shelf life. Today, creation of newer orchid hybrids for production of quality cut flowers has become a competitive business with potential to earn multi-million dollars for the orchid producing nation in the international market. There is approximately more than one lack hybrids have been registered till date. Despite orchids have non-endospermic seeds, which don't have any source of food as embryos are virtually devoid of food therefore orchids are not able to germinate in nature. The seeds of orchids took help of mycorrhizal to germinate in nature, this act referred as 'symbiotic germination.' Seed germination is main hindrance in orchid breeding. This is only when Prof. Knudson (3) discover the chemically defined medium for orchid germination. After this discovery two big bangs which are described as 'Revolution in orchid industry' and 'Rush of orchid hybrids.' Since then tissue culture became standard technique for mass propagation of orchids.

*Corresponding author's E-mail: vitthalbhosale@gmail.com
** Division of Fruits and Horticultural Technology, IARI, New Delhi

Till date great success has been reported in the epiphytic orchids compared to terrestrial orchids as the terrestrial orchids have very complex nutrient requirement and grater dependence on the mycorrhizal association (Pathak *et al.,* 7), orchid taken for the present study i.e. *Arundina graminifolia* which is a terrestrial orchid. The first objective of study was set as standardization of culture medium and culture conditions for *in vitro* propagation of orchid using immature seeds. Use of seeds for *in vitro* propagation is beneficial to get variation.

MATERIALS AND METHODS

The present investigation was carried out at the Central Tissue Culture Laboratory of National Research Centre on Plant Biotechnology and Division of Floriculture and Landscaping, Indian Agricultural Research Institute, New Delhi, during 2007 to 2008. The explants of *Arundina graminifolia* (Immature seed pods) were collected from National Research Centre for Orchids, Pakyong, Sikkim (India). The explants were subjected to pretreatment, using fungicides namely, Carbendazim (0.1%) and Endofil (0.1%) alone and in combination for 1h, followed by three times distilled water wash. The pre treated explants were taken to the laminar air-flow chamber and surface sterilized using mercuric chloride for 4-6 min., followed by 3 times distilled water wash. The

decontaminated explants were cultured on Murashige and Skoog media supplemented with growth regulators BAP (0.5-4.0 mg/l) and NAA (0.5-2.5 mg/l). The inoculated cultures were exposed to different photoperiodic conditions *viz.,* 1. In complete darkness; 2. In diffused light; 3. 7 days in dark followed by 16/8 h photoperiod; 4. 16/7 h photoperiod followed by continuous light. The observations *viz.,* per cent microbial contamination and per cent survival were recorded after 21 day of culture period along with this other observations like days to complete greenness and days to Protocorm Like Bodies (PLBs) formation were also recorded accordingly.

The initiated plb's from immature seeds were sub-cultured on shoot elongation media containing BAP (0.5-3.0 mg/l) in combination with NAA (0.1- 2.5 mg/l) and the observations on number of leaves per explant, length of shoots (cm) and width of shoots (cm) were recorded. The regenerated shoots were separated and placed in different rooting media comprising half-strength MS medium supplemented with different auxins *viz.,* IBA and NAA (1-2 mg/l) alone and the observations on rooting per cent, days to root initiation, average number of roots and mean length of root (cm) were recorded. Acclimatization of *in vitro* raised orchid plantlets was done by washing the roots with sterile distilled water to dislodge the adhering agar and then rooted plantlets were transferred to sterilized potting mixture comprising of peat + soilrite (1:1 v/v) in glass jar with polypropylene cap and earthen pot with polythelene cover. The plants were moistened regularly and supplemented with foliar spray of NPK mixture and Ohio W.P. fertilizer solution. After 30 days plants were transferred to glasshouse condition.

RESULTS AND DISCUSSION

For establishment of aseptic culture from immature capsules, seed capsules were pre-treated with carbendazim (0.1%) for 1 h followed by surface sterilization with $HgCl_2$ (0.1%) for 6 min proved to be the best to get contamination free culture. This treatment gave 83 % of survival rate; we got huge success in establishment of aseptic culture by using above stated method despite most of the scientists use alcohol dip method of sterilization as a standardized method of sterilization as given by Roy *et al.* (8), use of calcium chloride in combination of Teepol (Pathak *et al.,* 7), sterilization with calcium hypochlorite and sodium hypochlorite.

Light plays very important role in advancement of germination in most of the crops. keeping in view this fact the present study was also included the study of effect of light on the orchid seed germination. In our study 16/8 h photoperiod followed by continuous light found to be the best over other treatments which gave plb's in 13.6 day after inoculation (Table 1). However, there is no data available to support the findings except Pedroza-Manrique (6) who had use four light regimes (white light, darkness, red light and far red light) on asymbiotic germination of *Odontoglossum gloriosum*, the best germination percentage and germination time were obtained NAA under red light with a 16 h photoperiod.

Table 1. Effect of light conditions on *in vitro* seed germination in *Arundina graminifolia.*

Treatment	Days required for plb's formation
T_1 - In complete darkness	00.0
T_2- In diffuse light	16.0
T_3- 7 days in dark followed by 16/8 h photoperiod	14.3
T_4- 16/8 h photoperiod followed by continuous light	13.6
CD at 5 %	1.21*

*Indicate significant difference

With reference to the different media, MS medium concluded to be the best. MS medium supplemented with MS + BAP (0.5 mg/l) + NAA (0.5 mg/l) treatment found to be the best for early getting greening of seeds and production of plb's in less period of time i.e. in 5.67 and 12.67 days after inoculation, respectively. Relative ranking of plb's development among various combinations was $T_6 > T_7 > T_8 > T_3 = T_5 > T_4 = T_2 > T_1$ (Table 2). Aseptic germination of seeds and subsequent growth and development of plantlets of some Indian orchids was shown to be poor in Knudson C and Vacin and Went media. This was mainly attributed to presence of $FeSO_4.7H_2O$ instead of Fe- EDTA in both Knudson C and Morel medium. Better seedling growth was attributed to Fe- EDTA present in Murashige and Skoog medium (Lee *et al.,* 4). In literature there are several reports which indicate BAP alone or in combination with NAA contributes to efficient plb's production. Inclusion of BAP (0.05 – 0.5 mg/l) into the MS medium induced vigorous production of plb's in clonal propagation of *Dendrobium* 'Madampompadour' (Mujib and Jana, 5).

This was found that treatment MS + BAP (1 mg/l) + NAA (0.5 mg/l) gives highest shoot length i.e. 4. 96 cm. For the same treatment negative correlation was observed for number of leaves per plantlet. In relation to our findings, successful mass multiplication method has been established in orchid by using 2 mg/l BAP + 1.5

Table 2. Effect of media on seed germination and Protocorm Like Bodies (PLBs) formation in *Arundina graminifolia.*

Treatment	Days to greening of seeds	Days to PLBs formation	Germination (%)	Contamination (%)	Survival (%)
T_1	12.67	20.00	75.0	36.67	63.33
T_2	7.00	14.33	86.7	33.33	66.67
T_3	7.00	14.00	90.0	36.67	63.33
T_4	7.67	14.33	91.7	30.00	70.00
T_5	8.00	14.00	93.3	26.67	73.33
T_6	5.67	12.00	88.3	40.00	60.00
T_7	6.00	12.33	86.7	36.67	63.33
T_8	7.33	13.00	73.3	26.67	73.33
CD at 5 %	0.93*	1.05*	6.11*	NS	NS

T_1- MS + No hormone (control), T_2- MS + BAP (0.5 mg/l) T_3- MS + BAP (1.0 mg/l), T_4- MS + NAA (0.5 mg/l) T_5- MS + NAA (1.0 mg/l), T_6- MS + BAP (0.5 mg/l) + NAA (0.5 mg/l) T_7- MS + BAP (1 mg/l) + NAA (1 mg/l), T_8- MS + BAP (1.5 mg/l) + NAA (1.5 mg/l)

mg/l NAA by Arditi (1). Micro-propagation of *Cymbidium* (24 week old) on Vacin and Went medium containing BAP 1.5 mg/l and NAA 1.0 mg/l proved to be the best for accelerated development of plb's and proliferations (Vij *et al.* 12). Better plantlets were produced and low cytokinin to auxin ratio resulted in the formation of callus in *Bletella striata* on modified MS Medium containing 1 mg/l NAA + 0.2 mg/l BA (Yam and Weatherhead, 13). Significant differences were recorded for all root parameters i.e. number of roots, root length, days required for root initiation and per cent rooting. ½ MS + NAA (0.5 mg/l) has given best result for number of roots/ plantlet and length of roots (cm) with almost 100 % rooting (99.17 %) (Table 3).

Table 3. Effect of growth regulators supplemented to MS médium on rooting in *Arundina graminifolia.*

Treatment	Days required for root initiation	Rooting per cent
T1	7.67	61.67
T2	6.33	91.67
T3	5.67	100.00
T4	6.00	99.17
T5	6.33	83.33
CD at 5%	0.93	10.43

T1 = MS + No hormone (Control), T2 = ½ MS + IBA (0.5 mg/l)
T3 = ½ MS + IBA (1 mg/l), T4 = ½ MS + NAA (0.5 mg/l)
T5 = ½ MS + NAA (1 mg/l)

This result was correlated to the root number and root length of micro shoots of *Dendrobium* hybrid Sonia-17 were the highest in KC medium supplemented with IBA - 5.0 mg/l (Sharma *et al.,* 9). Maximum rooting of *in vitro* cultured *Dendrobium oesterholt* was observed in half strength MS medium with IBA 1 mg/l + NAA 1 mg / l (Vejsadova, 11). Among two different treatment Earthen pots with polythelene cover filled with peat + soilrite (1:1) gave good, healthy and vigorous plants over Glass jar with polypropylene cap filled with peat + soilrite (1:1). The pots were provided with brick pieces to drain the excess nutrient liquid solution. The growing media and the container into which *in vitro* rooted plantlets are transferred are important for their survival (Jones, 2). The potting mixture comprising leaf mould, perlite, vermiculite, and dry sphagnum in 1:1:1:2 ratios were used by Sharma and Chauhan (10) in *Phalaenopsis* reveals high success.

The established protocol is efficient for mass multiplication of the orchid *Arundina graminifolia* a

Fig. Established *in vitro* mass multiplication protocol for orchid *(Arundina graminifolia).*

terrestrial orchid (Fig.1), which can be exploited for further orchid development and can be used as a source of purple colour in orchid breeding programme. The mass multiplication of bamboo orchid using seeds from immature green pod through tissue culture has made availability of the disease free seedlings in large number with in short duration of time than mature seed culture.

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REFERENCES

1. Arditi, J. 1967. Factors affecting the germination of orchid seeds. *Botanical Review* **33**: 1-97.
2. Jones, J.B. 1982. How we can get micro-cuttings out of the lab? *Combined Proceedings of International Plant Propagation Society,* **33**: 618-22.
3. Kundson, L. 1946. A new nutrient solution for germinating of orchid seed. *American Orchid Society Bull.* **15**: 214-17.
4. Lee, J.K., Paek, K.Y. and Lee, H.S. 1983. Studies on the non-symbiotic germination of seeds of *Laelia briegeri.* I. Morphological characters of germinating seeds, and effects of pH, sucrose and growth regulators on seed germination. *Journal of the Korean Soc.Hort.Sci.* **24**: 169-74.
5. Mujib, A. and Jana, B.K. 1994. Clonal propagation of *Dendrobium* 'Madame Pompadour' through apical meristem culture. *Adv. Plant Sci.* **7**: 340-46.
6. Pedroza-Manrique, J. 2006. *In vitro* cellular and developmental biology of plant. *Sceintia Hort.* **42**: 543-47.
7. Pathak, P., Mahant, K.C. and Vij, S.P. 2001. *In vitro* propagation as an aid to conservation of Indian orchids: Seed culture. In: *Orchids, Science and Commerce.* (Eds.). Pathak, P., Mahant K.C. and Vij, S.P., Dehradun, India: Bishen Singh and Mahendra Pal Singh, Dehradun, Uttarakhand, India pp. 319-62.
8. Roy, A.R., Patel, R.S., Patel, V.V. and Kumar, R. 2007. *In vitro* propagation of *Thunia marshalliana* Rchb.f. an epiphytic-cum-terrestrial orchid. *J. Orn. Hort.***10**: 15-19.
9. Sharma, A., Tandon, P. and Kumar A. 1992. Regeneration of plantlets from encapsulated *Dendrobium* protocoms. *Indian J. Exp. Biol.* **30**: 747.
10. Sharma, J. and Chauhan, Y.S. 1995. Establishment of *in vitro* raised seedlings of *Dendrobium* and *Phapiopedium*. *J.Orchid Soc.India,* **9**: 37-41.
11. Vejsadova, H. 2006. Factors affecting seed germination and seedling growth of terrestrial orchids cultured *in vitro*. *Acta Biologia Cracoviensia Series Botanica,* **48**: 109-13.
12. Vij, S.P., Aggarwal, S. and Pathak, P. 2004. Regeneration competence of Cymbidium great Waltz X Valley flower roots: A study *in vitro*. *J. Orchid Soc.India*, **18**: 109-15.
13. Yam, T.W. and Weatherhead, M.A. 1990. Nodal culture of native orchids of Hong Kong. *Lindleyana,* **5**: 218-23.

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