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In vitro propagation in *Malaxis acuminata* D.Don using pseudobulb explants

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INTRODUCTION

Orchids, besides their ornamental values are therapeutically important in curing many diseases. The propagation of orchids is hampered because of their low viability of seeds and mandatory micorrhizal association for seed germination.

In India the medicinal properties of orchids have been used since Vedic period. "Ashtawarga" is a group of 8 drugs in Ayurvedic system which is used for preparation of tonics, such as 'Chyavanparas', which consists of 4 orchid species, viz *Malaxis muscifera* (Lindl.) Kuntze, *Malaxis acuminata* D.Don, *Habenaria intermedia* D.Don and *Habenaria edgeworthi* Hook. *f*. Around 40 other orchid species are being used in indigenous medicine systems[10]. The state Uttarakhand, having 237 species of orchids[3], ranks fifth among the Indian states in terms of orchid species richness. Orchids are distributed throughout the state ranging from foot hills to the alpine region but their diversity and abundance is comparatively higher in. the riverine area and moist pockets of the forest[4].

The genus *Malaxis* includes 300 species and are distributed from temperate to tropical regions. The dried pseudobulbs are used in making of ayurvedic tonic "Chyvanprash". Plant pacifies vitiated pitta, vata, oligospermia, burning sensation, emaciation, general debility, arthritis and blood vomiting. Vij and Kaur[12] have successfully micropropagted this orchid.

The present study deals with the *in vitro* propagation of *M.acuminata* using pseudobulbs.

MATERIALS AND METHODS

The pseudobulbs were collected from one year old plants from Nagdeva forest divison (Figure 1A). These were washed thoroughly with tap water followed by a wash with 1 % (v/v) Labolene detergent for 15 minutes and then in running tap water for 30 minutes and were surface sterilized with 70 - 90% ethyl alcohol for 30 seconds, followed by 0.1 % (w/v) $HgCl_2$ with two drops of Tween 80 per 100 ml. of solution for two minutes. The explants were then rinsed 6 times with sterile double distilled water to remove all traces of $HgCl_2$ and blotted on sterile filter paper discs. The pseudobulbs were cut transversally and placed on the medium. All the cultures were incubated in an Environmental Chamber at $24^0 \pm 2^o$ C with 16: 8 hrs light: dark photoperiod controlled by clock timer and 60 % relative humidity. All the experiments were repeated thrice to optimize the results.

RESULTS

Shoot regeneration

In vitro regeneration was achieved by using pseudobulb segments as explants. The explants were

incubated in MS media fortified with BA (1.0-5.0 mgl^{-1}), 0.5 mgl^{-1} IAA / IBA/NAA with or without GA_3 (0.5 mgl^{-1}).

Shoot proliferation was observed after two weeks of incubation. Observations reveal that 5.0 mgl^{-1} BA with 0.5 mgl^{-1} IAA and GA_3 induced maximum number of shoots. 2.30 ±1.08 number of shoots with 3.80±0.12 cm shoot length was recorded on the final day of study period (Figure 1B). 1.0 mgl^{-1} of BA reflected very poor response (1.66± 1.24 number of shoots) in this study (TABLE 1).

In another experiment, IAA was replaced with IBA and best results were obtained in 2.0:0.5 mgl^{-1} concentration of BA:IBA combination. Shoot number was recorded as 2.65 ± 0.68 with 3.40 ± 0.44 cm shoot length (Figure 1C).

TABLE 1 : Effect of different concentrations of BA and auxins (IAA/IBA/NAA) on shoot regeneration in *M.acuminata*.

MS + Cytokinins	Auxins			GA_3	Shoot number	Shoot length
	IAA	IBA	NAA			
BA						
1.0	0.5			0.5	1.66±1.24	3.80±0..56
2.0	0.5			0.5	1.80±0.88	3.82±0.60
3.0	0.5			0.5	2.22±1.42	3.90±0.40
4.0	0.5			0.5	2.16±0.10	3.44±0.10
5.0	0.5			0.5	2.30±1.08	3.80±0.12
1.0		0.5			2.14±1.20	2.10±0.10
2.0		0.5			2.65±0.68	3.40±0.44
3.0		0.5			1.90±1.12	1.78±0.10
1.0			0.5		1.12±0.68	0.80±0.12
2.0			0.5		2.20±0.45	1.70±0.40
3.0			0.5		2.40±0.95	2.60±1.00
1.0	1.0				1.10±0.80	0.68±0.10
2.0	1.0				0.68±0.44	0.40±0.10
3.0	1.0				1.08±0.44	1.10±0.12
1.0		1.0			2.10±0.88	2.40±0.60
2.0		1.0			2.22±1.02	2.68±1.00
3.0		1.0			2.12±0.66	2.38±0.10
1.0			1.0		2.30±1.12	2.80±1.00
2.0			1.0		2.32±1.00	2.78±1.10
3.0			1.0		2.04±0.68	2.08±1.00

Values are mean of three replicates ± SD, study period=120 days

Addition of 0.5 mgl^{-1} NAA in MS media with BA (1.0-3.0 mgl^{-1}) reflected that increasing concentration of BA improved shoot induction frequency as well as shoot length. Maximum number of shoots (2.40± 0.95) were recorded in 3.0 mgl^{-1} of BA and NAA combination.

1.0 mgl^{-1} concentration of auxin did not induce good results, as the maximum number of shoots recorded was 1.10 ± 0.80 in BA and IAA and 2.22±1 in BA and IBA whereas 2.32±1.00 in BA and NAA combination (TABLE 1).

The results depicted in the TABLE 1 reflect that 2.0: 0.5 mgl^{-1} of BA:IBA combination proved to be the best in terms of shoot regeneration whereas 3.0:0.5:0.5 mgl^{-1} concentration of BA:IBA:GA_3 combination reflected maximum shoot length in the present study.

Rooting of *in vitro* raised shoots

The in *vitro* regenerated shoots were again inoculated in MS media fortified with IBA/NAA (1.0-2.0 mgl^{-1}) for root induction. Rooting was observed in almost all the shoots after 15 days of incubation. The rooting frequency was however, poor in 1.0 mgl^{-1} of IBA, where 1.88±0. roots were recorded. Best rooting was observed in 2.0 mgl^{-1} concentration of IBA with 2.20± 0.10 roots and 1.88± 0.10 cm root length (TABLE 2; Figure 1D & E).

TABLE 2 : Effect of different concentrations of auxins (IBA/NAA) on rooting in *M. acuminata* in MS media.

MS + Auxins (mgl^{-1})	Root number	Root length (cm)
IBA		
1.0	1.88±0.10	0.80±0.12
1.5	2.00±0.40	1.60±0.24
2.0	2.20±0.10	1.88±0.10
NAA		
1.0	2.12±0.20	2.60±0.12
1.5	2.80±0.10	3.22±0.10
2.0	3.60±0.40	3.10±0.08

Values are mean of three replicates ± SD

NAA proved to be better than IBA in the present study and maximum number of roots was observed by supplementing this auxin in the MS media. 1.0 mgl^{-1} of NAA produced 2.12±0.20 roots whereas best rooting was observed in 2.0 mgl^{-1} of NAA where root number was 3.60±0.40 with 3.10±0.08 cm root length (TABLE 2; Figure 1F).

The rooted plantlets were removed from the medium and washed thoroughly with water and planted in thermocol cups for acclimatization. Almost 70% of rooted shoots of *M. acuminata* were successfully ac-

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Figure 1 : Plant of *M. acuminata* (A); multiple shooting 5.0 mgl^{-1} BA with 0.5 mgl^{-1} IAA and GA$_3$ (B); *in vitro* shoot in 2.0:0.5 mgl^{-1} BA:IBA (C); rooting in 2.0 mgl^{-1} IBA (D & E); fully rooted plant in 2.0 mgl^{-1} NAA (F); Hardened plant of *M. acuminata* (G).

climatized and established ex vitro (Figure 1G).

DISCUSSION

Pseudobulb segment culture is an efficient system for the production of a large number of plantlets in short time. Regeneration potential of pseudoblulb explants has been successfully tested in several orchids including *Cattleya, Miltonia, Cymbidium*[7], *Arundina*[6], *Dendrobium* etc.

The regeneration competence of pseudobulbs seems to markedly influenced by physiological age of the mother plant, position of donor and growth stimulus in nutrient pool[11].

The results reflected in the present study are in agreement with the earlier observations in *Ascofinetia*[2], *Dendrobium crepidatum* and *D. pierardianum*[12]. In the present study, the combination of BA and IBA considerably increased the multiple shoot production. 2.0 mgl^{-1} of BA was effective in production of multiple shoot further. The results also agree with findings of Roy and Banerjee[9]. Combined treatment of BA and NAA also proved to be better for multiple shoot induction in the present study which is in agreement with the results in *Cattleya*[5].

1.0-3.0 mgl^{-1} of BA resulted in better shoot induction, these results are similar to the results in *Coleogyne stricta*[1].

Root induction studies reflected maximum root induction in NAA as compared to IBA.

On the basis of present study 2.0:0.5 mgl^{-1} of BA:IBA and 2.0 mgl^{-1} NAA concentration in MS media can be recommended for maximum shoot and root induction respectively using pseudobulb as an explants of *M.acuminata*.

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