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COMBINED EFFECT OF NAA AND BAP ON PROCOCORM LIKE BODIES (PLBs) DERIVED PLANTLETS PRODUCTION FOR COMMERCIAL PROPAGATION OF *DENDROBIUM SONIA* ORCHID

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Abstract

The experiment was conducted to find out the optimum concentration and combination of plant growth regulators (PGRs) and their effects on *in vitro* growth and development of *Dendrobium Sonia* orchid. The experiment was caried out at the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during the period from February 2009 to May 2010. The experiment was laid out in Completely Randomized Design (CRD) with five replications. Protocorm-like bodies (PLBs) were inoculated as explants on half MS medium supplemented with four levels (0.0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}) of Naphthalene acetic acid (NAA) and 6-Benzylaminopurine (BAP) independently to proliferate and multiply the PLBs. Then, these multiplied PLBs were cultured on different concentrations and combinations of NAA (0.0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}) and BAP (0.0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}) for the growth and development of *Dendrobium Sonia* plantlets. The maximum fresh weight of PLBs (3.5 g), the highest percentage of regenerated plantlets (95%) was obtained from the combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP. At the same time, maximum number of shoots (6.40) as well as length of shoot (4.82 cm) and fresh weight of single shoot (0.414 g) was also achieved at this combination. Similarly, the highest number of leaves (6.00), longest leaf (2.36 cm) and width of leaf (0.78 cm) was observed from 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP. Moreover, the treatment combination, 0.5 mgL^{-1} each of NAA + BAP was found to be the best for producing maximum number of roots (6.40), length (2.82 cm) and fresh weight of roots (0.141 g). In contrast, higher concentration of 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP combinations showed poor results for all parameters.

Keywords: *Dendrobium Sonia*, Plantlets, NAA, BAP, Orchid.

Introduction

Orchids constitute one of the most economically important groups of ornamental plants worldwide and are extensively cultivated as both cut flowers and potted plants, contributing approximately 8% of the global floriculture trade. Their exceptional commercial value is attributed to remarkable floral diversity in terms of color, size, shape, and form, as well as their long-lasting blooms, which make

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them highly desirable in domestic and international markets (Tokuhara and Mii, 2001). In addition to their ornamental appeal, orchids possess multifaceted uses, including applications in perfumery, traditional medicine, adhesives, and food and beverage flavoring, further enhancing their economic significance (Goh *et al.,* 1992). Bangladesh possesses favorable climatic conditions for orchid growth throughout the year, offering substantial potential for orchid cultivation and commercialization. However, despite suitable environmental conditions, the orchid industry in Bangladesh remains at an early developmental stage compared to other orchid-producing countries. Globally, commercial orchid cultivation for both plant sale and cut flower production has expanded into a major industry, generating millions of dollars annually (Singh, 1998). Among orchids, *Dendrobium* is the most popular orchid, with amazing variants like Emma white that are praised for their purity of blossoms and long shelf life (Vendrame, 2008). In Bangladesh as well, *Dendrobium* is one of the most popular orchid genera due to its rapid growth, aesthetic appeal, and long-lasting flower spikes (Talukder *et al.,* 2002). In the Asian floriculture market, *Dendrobium* hybrids dominate the cut flower trade. For instance, Thailand exports *Dendrobium* flowers worth over 12 million US dollars annually to European markets (Rao, 1977). *Dendrobium*, though widely cultivated, includes several species that are considered rare or endangered in their native environments (Chen *et al.,* 2014; Ye *et al.,* 2016). Conventional methods of orchid propagation, particularly vegetative propagation, are extremely slow and inefficient, producing only 2-4 plants per year (Nasiruddin *et al.,* 2003), which is inadequate to meet commercial demand or conservation needs. Micropropagation through tissue culture has emerged as a highly effective alternative for the mass propagation of orchids, especially those with slow sexual reproduction and high genetic heterozygosity (Kanjilal *et al.,* 1999). Successful *in vitro* regeneration of *Dendrobium* orchids largely depends on the appropriate combination of plant growth regulators (PGRs) in the culture medium (Aktar *et al.,* 2008). Previous studies have demonstrated that cytokinins such as BAP play a crucial role in protocorm-like body (PLB) induction and shoot regeneration, while auxins like NAA enhance PLB growth and root formation (Fujii *et al.,* 1999; Martin and Madassery, 2006). However, the response to PGRs varies significantly among species and hybrids, making it essential to optimize hormone concentrations empirically for each genotype (Tokuhara and Mii, 1993; Teixeira da Silva, 2013). Recent findings have shown that an optimized combination of BAP and NAA significantly enhances shoot regeneration, while higher auxin concentrations favor effective root development in *Dendrobium* orchids (Hossen *et al*., 2021). Considering the aforesaid factors, the present study was conducted to establish an efficient *in vitro* propagation protocol by standardizing the optimal concentrations of BAP and NAA for enhanced shoot regeneration and high-frequency plantlet production through PLB multiplication in *Dendrobium Sonia* orchid.

Materials and Methods

The experiment was carried out at the USDA Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh durin February 2009 to May 2010, to investigate the effect of NAA and BAP on Protocorm Like Bodies (PLBs), shoot regeneration and multiplication and rooting of *Dendrobium sonia* orchid. In this experiment, *in vitro* multiplied shoots were cultured on ½ MS medium (Murasihge and Skoog, 1962) supplemented with different combinations of NAA (0.0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}) and BAP (0.0, 0.5, 1.0, 2.0 and 4.0 mgL^{-1}). The medium was supplemented with 3% sucrose as carbon source and solidified with 0.3% gelrite. The pH of the medium was adjusted to 5.8 before autoclaving. PLBs of *Dendrobium Sonia* orchids were used as explants. These PLBs were initiated earlier and maintained in the Tissue Culture Laboratory of the Department of Biotechnology. One-month-old *in vitro* grown PLBs were used as explants. PLBs were cultured on ½ MS medium supplemented with different concentrations of BAP and NAA individually for 60 days to investigate their effects on the proliferation and multiplication of PLBs. In each vial, four uniform PLBs were cultured. Gelrite was dissolved by boiling the mixture and about 20 ml medium was dispensed into each vial. The culture vials containing 20 ml media were autoclaved at $121^{O}C$ for 20 min at 1.16 Kg cm^{-2} pressure. The culture vials were maintained in a growth chamber at $25\pm1^{O}C$ under 16 hours photoperiod, illuminated with fluorescent tubes of 50 µmol $m^{-2}s^{-2}$. Data were collected at 60 days after culture for the following parameters: fresh weight of PLBs, percentage of PLBs, number of multiple shoots per explants, length of shoot, weight of shoot, number of leaves per plantlet, length and width of leaves, number and fresh weight of roots, length of roots. The experiment was laid out in Completely Randomized Design (CRD) with five replications. The analysis of variance was performed, and means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984). Well rooted shoots were removed from culture vials, thoroughly washed with running tap water to remove residual medium and transferred to earthen pots containing a mixture of coconut husk. The pots were then kept in a shade house and irrigated with mist.

Results and Discussion

In vitro regeneration of plantlets offers a feasible propagation method for orchid and can be utilized for year-round and rapid propagation of orchid plants. The subsequent growth and effective shoot and root development on suitable medium,

acclimatization and plantlet establishment in pot play a vital role in orchid production. The results obtained from the experiments are presented and discussed in the following Table 1 and Fig.1 & 2.

In the present study, early *in vitro* grown Protocorm Like Bodies (PLBs) and PLBs derived plantlets of *Dendrobium Sonia* were cultured on ½ MS medium supplemented with different combinations of NAA and BAP as well as data were collected after 60 days of culture. The highest fresh weight of PLBs (3.5 g) was obtained for the combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP followed by 0.0 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and whereas the lowest fresh weight of PLBs (0.1 g) found on 4.0 mgL^{-1} NAA + 0.0 mgL^{-1} BAP (Fig. 1 & Plate. a). So, it is assumed that plant growth regulators play a vital role in PLBs growth. A low concentration of auxin and cytokinin had an influence on the PLBs in this study. The effects of auxin and cytokinin on *in vitro* induction, proliferation, and regeneration of PLBs of orchids are reported by several researchers (Niknejad *et al.,* 2011; Mondal *et al.,* 2013; Chew *et al.,* 2018; Chookoh *et al.,* 2019). The finding of the present study was agreed with the findings of Khatun *et al.,* (2010). They found the highest PLBs proliferation in the medium combination of 1.0 mgL^{-1} NAA and 1.0 mgL^{-1} BAP for *Dendrobium* hybrid. Similarly, the maximum percentage of plantlets (95.00%) was observed at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP, while the least percentage (4%) was found at 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP (Fig. 2 & Plate. b). It is observed that the growth of PLBs was severely reduced when the concentration increased further. The efficacy of multiple shoot formation with the combinations of BAP and NAA (Table 1 & Plate. a-e). After 60 days of culture PLBs regenerated plantlets produced multiple shoots which varied from 1.00 to 6.4 shoots per plant (Table 1& Plate. b). The highest number of multiple shoots (6.4) was observed for the combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Table 1& Plate. c), whereas the lowest number of multiple shoots found from control treatment after 60 days of culture. This result was supported by Polonca *et al.,* (2004) who showed the same was amount of BAP and NAA containing medium performed best for shoot multiplication. Moreover, the longest shoot length (4.82 cm) was also observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP whereas the shortest (1.67 cm) was observed at 4.0 mgL^{-1} NAA + 0.0 mgL^{-1} BAP (Table 1 & Plate. d, e). Results are in agreement with the findings of Khatun *et al.* (2008) who reported that lower concentration of auxin and cytokinin favoured the enhancement of plantlet growth. Similarly, Prasad *et al.,* (2001) and Khatun *et al.* (2010) found that the highest mean shoot height was obtained from 1.0 mgL^{-1} each of BAP + NAA on MS medium.

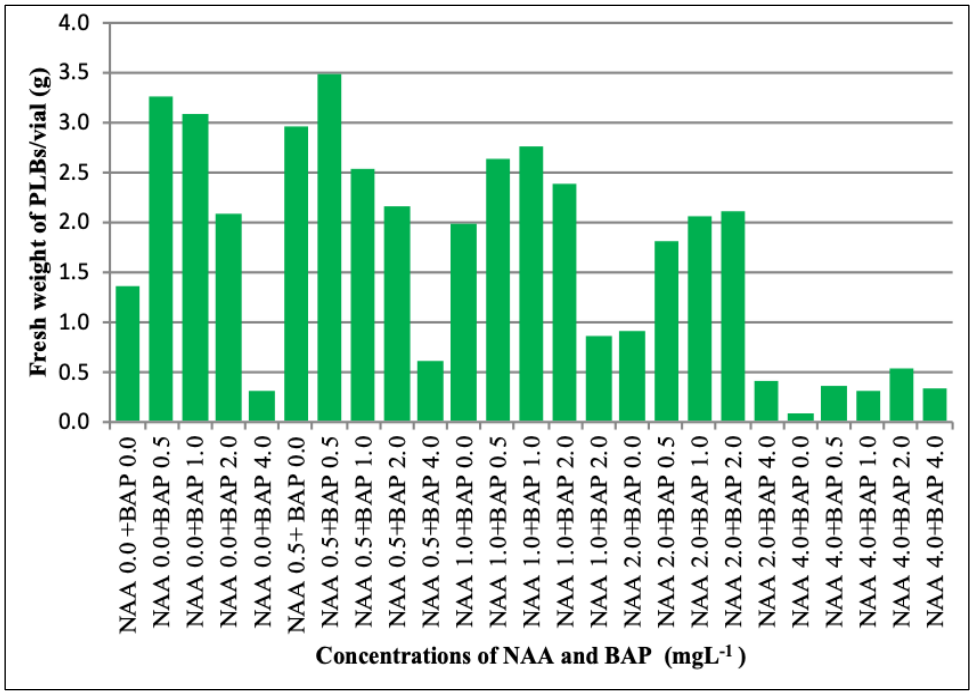

Fig. 1. Combined effect of different levels of NAA and BAP on *in vitro* fresh weight of PLBs per vial after 60 days of culture.

The number, length and width of leaves per plantlet were significantly influenced by the combined effect of NAA and BAP. The highest number of leaves (6.0) was obtained from 0.5 mgL^{-1} each of NAA + BAP (Table 1 & Plate. d, e), while, the minimum number (1.0) was recorded at 4.0 mgL^{-1} each of NAA + BAP. These findings are in agreement with the findings of Prasad *et al.* (2001). Furthermore, the largest leaf (2.36 cm) was found in the medium containing 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP whereas shortest (0.74 cm) leaf was observed from the medium containing 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP (Table 1 & Plate. d, e). According to Khatun *et al.* (2010) the highest leaf length was obtained from 1.0 mgL^{-1} of each NAA + BAP. In addition, the highest width (0.78 cm) of leaf was found from the combined use of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Table 1 & Plate. 3. d, e).

The lowest width (0.28 cm) of leaf was recorded from 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP after 60 days of culture. The combined application of NAA and BAP significantly influenced the number, length, and weight of roots. After 60 days of culture, the maximum number of roots (6.40) was obtained from the medium containing 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP whereas the minimum number of roots (2.00) were found from the each 4.0 mgL^{-1} NAA + BAP (Table 1 & Plate. e). These results were supported by Nayak *et al.,* (1998) who observed that NAA and

BAP combination induced rooting in regenerated shoots thereby producing complete plantlets. Similarly, Vij and Kaur (1998) found a positive effect of NAA and BAP to promote rooting in multiplied shoots. After 60 days of culture, the longest root (2.82 cm) was obtained from the medium containing a combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP, while the shortest root (0.88 cm) was obtained from 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP (able 1 & Fig. 3. e). The present result is supported by Khatun *et al.* (2010) who reported that the maximum root length was obtained from 1.0 mgL^{-1} NAA + 1.0 mgL^{-1} BAP.

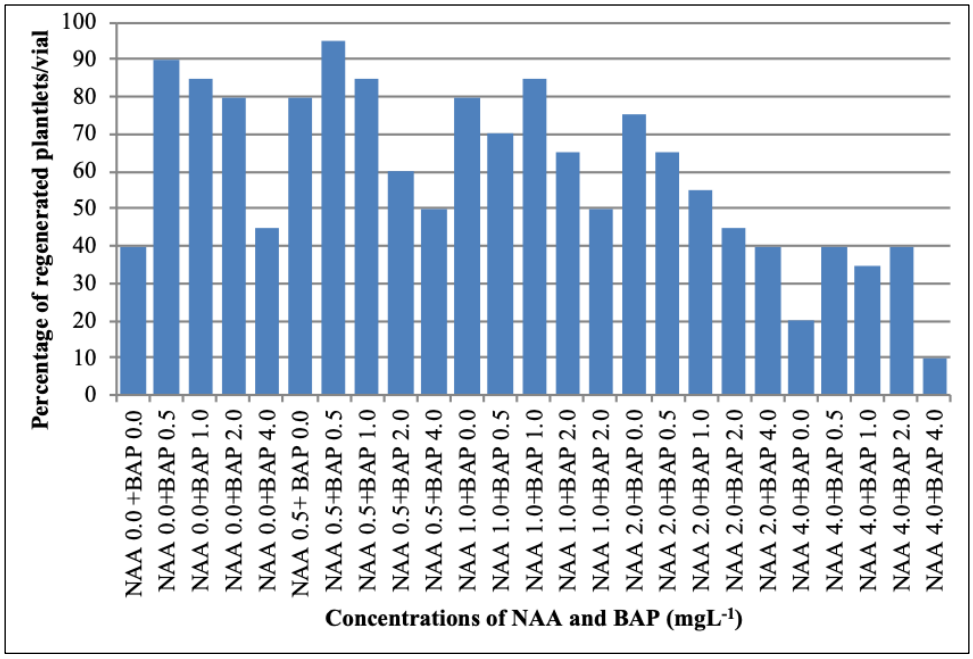

Fig. 2. Combined effect of different levels of NAA and BAP on *in vitro* percentage of PLBs regenerated plantlets after 60 days of culture.

The highest fresh weight of roots (0.141 g) was recorded at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP while the lowest (0.067 g) was from 4.0 mgL^{-1} NAA + 4.0 mgL^{-1} BAP after 60 days of culture (Table 1). This result supported by Khatun *et al.,* (2005) who showed that 0.5 mgL^{-1} each of BAP and NAA performed better growth and development of *Dendrobium Sonia* orchid root.

The result of this study supports the establishment of a protocol for *in vitro* mass propagation of *Dendrobium Sonia*. In the combined effect of NAA and BAP into ½ MS medium, the highest number of shoot and their length and weight of shoot were obtained at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP. Similarly, the maximum number of leaves, width and length of leaf was also found at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP after 60 days of culture. Moreover, half the strength of MS medium containing a

Table 1. Combined effect of different levels of NAA and BAP on growth and development of plantlets after 60 days after of culture

Treatments		No. of shoots	Length of shoot (cm)	Fresh wt. of single shoot (g)	No. of Leaves	Length of leaf (cm)	Width of leaf (cm)	No. of roots	Length of root (cm)	Fresh wt. of roots (g)
NAA (mgL^{-1})	BAP (mgL^{-1})									
0.0	0.0	3.20 efg	2.48 b-f	0.064 b	4.00 a-e	1.36 c-i	0.48 g-k	3.00 fgh	1.10 c-f	0.115 a-e
	0.5	5.20 ab	4.04 ab	0.296 a	4.90 a	2.10 ab	0.66 a-f	5.00 a-d	1.52 a-d	0.14 ab
	1.0	4.60 abc	3.99 ab	0.200 ab	4.80 ab	1.88 a-d	0.72 a-d	5.20 a-d	1.62 ab	0.12 a-d
	2.0	4.20 bce	3.39 a-e	0.164 ab	4.20 a-d	1.66 bg	0.58 c-i	4.20 b-f	1.44 a-e	0.111 a-e
	4.0	2.80 fgh	1.76 ef	0.056 b	2.60 def	0.84 hi	0.42 i-k	3.20 e-h	1.00 ef	0.091 b-e
0.5	0.0	3.40 d-g	2.66 b-f	0.138 ab	3.60 a-f	1.42 b-i	0.56 d-j	5.00 a-d	1.86 a-d	0.115 a-e
	0.5	6.40 a	4.82 a	0.414 a	6.00 a	2.36 a	0.78 a	6.40 a	2.82 a	0.141 a
	1.0	5.00 abc	3.60 abc	0.391 a	4.40 abc	1.94 abc	0.64 a-g	5.40 a-d	2.62 ab	0.128 abc
	2.0	4.60 abc	3.30 a-f	0.345 a	4.40 abc	1.68 b-g	0.58 c-i	4.80 a-e	1.96 abc	0.11 a-e
	4.0	2.80 fgh	2.08 c-f	0.115 ab	3.60 a-f	1.16 e-i	0.46 h-	3.20 f-h	1.40 a-e	0.08 cde
1.0	0.0	1.80 hij	3.10 b-f	0.081 b	4.80 ab	1.52 b-h	0.70 a-d	5.80 ab	2.42 ab	0.125 abc
	0.5	5.30 ab	3.58 a-d	0.395 a	4.20 a-d	1.74 a-f	0.74 abc	5.80 ab	2.88 a	0.136 ab
	1.0	4.40 a-d	4.11 ab	0.405 a	4.20 a-d	1.80 a-e	0.76 ab	5.40 a-d	2.76 a	0.125 abc
	2.0	4.00 cde	3.31 af	0.126 ab	3.80 a-e	1.42 c-i	0.68 a-e	5.60 abc	2.60 ab	0.111 a-e
	4.0	2.40 ghi	2.01 c-f	0.079 b	4.00 a-e	0.96 hi	0.68 a-e	3.20 e-h	1.73 a-f	0.083 cde
2.0	0.0	1.10 jk	2.50 b-f	0.12 b	3.80 a-e	1.06 f-i	0.52 f-k	3.20 e-h	2.00 abc	0.121 abc
	0.5	3.80 c-f	3.03 b-f	0.085 b	3.60 a-f	1.24 di	0.60 b-h	4.00 c-g	2.56 ab	0.135 ab
	1.0	3.40 d-g	2.70 b-f	0.082 b	3.20 b-f	1.18 e-i	0.56 d-j	4.60 b-f	2.32 ab	0.12 a-d
	2.0	3.20 efg	2.52 b-f	0.082 b	4.00 a-e	1.04 ghi	0.50 f-k	3.20 e-h	1.98 abc	0.10 a-e
	4.0	2.00 hij	1.91 def	0.071 b	3.00 c-f	0.94 hi	0.40 jkl	3.00 fgh	1.64 a-c	0.071 de
4.0	0.0	1.00 k	1.67 f	0.046 b	2.80 c-f	0.88 hi	0.40 jkl	2.40 gh	1.00 ef	0.10 a-e
	0.5	2.60 ghi	2.71 b-f	0.056 b	2.40 efg	1.08 f-i	0.42 i-l	3.20 e-h	1.26 b-f	0.125 abc
	1.0	2.40 ghi	2.21 c-f	0.051 b	2.80 c-f	0.92 hi	0.42 i-l	3.80 d-g	1.36 a-f	0.121 abc
	2.0	1.60 ijk	2.08 c-f	0.05 b	2.00 fg	0.90 hi	0.38 kl	3.80 d-g	1.24 b-f	0.113 a-e
	4.0	1.20 jk	2.47 b-f	0.049 b	1.00 g	0.74 i	0.28 l	2.00 h	0.88 f	0.067 e
CV (%)		23.21	3.74	8.40	24.88	9.14	13.54	20.82	7.29	1.94

In a column, mean values followed by a common letter are not significantly different at the 5 % level as per DMRT.

combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP showed also maximum number of roots, the longest root and fresh weight of root. Similarly, the highest weight of PLBs was found at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP. The maximum percentage of plantlets production was observed at 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP. Considering the above results, ½ MS medium supplemented with the combination of 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP could be recommended for commercially large scale of PLBs production, percentage of plantlet regeneration, growth and development of plantlets and root formation of *Dendrobium Sonia* orchid which may be applicable for other *Dendrobium* species. In contrast, the highest concentration of NAA and BAP did not show any advantage.

Finally, the plantlets were taken out from the culture vials carefully and washed under running tap water to remove medium from the basal part of plantlets (Plate. f). Then the basal part of plantlets was wrapped with coconut fiber and set into small earthen pots (Plate. g). The plantlets were kept in the hardening room under shade and supplied water twice daily as mist and gradually exposed to natural light (Plate. g).

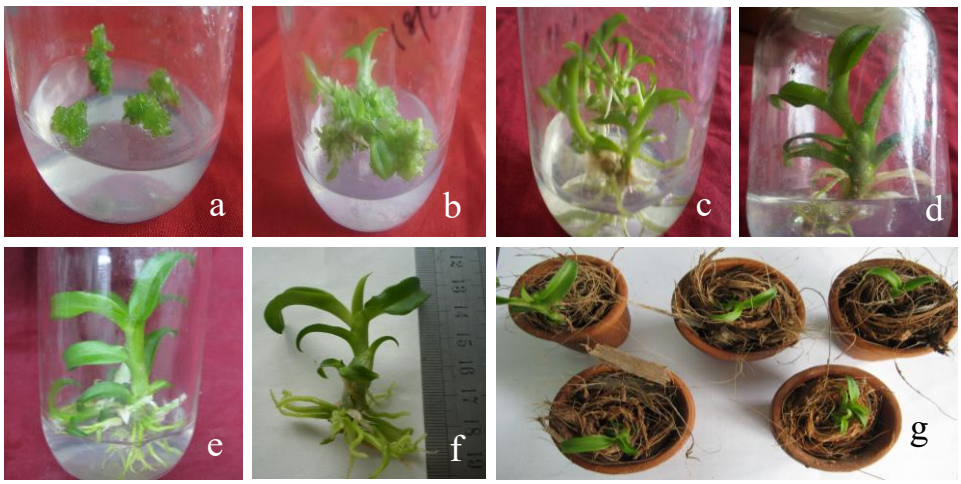

Plate (a-g): Combined effect of NAA and BAP on *Dendrobium Sonia in vitro.*

a. Multiplication of PLBs and development of PLBs. b. Shoot initiation from PLBs. c. Maximum number of shoots proliferation. d. Maximum length of shoot and number of leaf development. e. Maximum number of roots initiation and development. f. Well-rooted plantlet was removed from the culture vial prepared for acclimatization. g. Hardening of *in vitro* raised well-rooted healthy plantlets were transplanted to earthen pots filled with small pieces of coconut husks.

Conclusion

The study demonstrated that the combined application of 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP in ½ MS medium was the most effective for PLBs proliferation, shoot regeneration, leaf development, and root formation in *Dendrobium Sonia*. This optimized protocol ensures high plantlet regeneration (up to 95%) and superior growth performance, making it suitable for large-scale commercial propagation of this orchid. Conversely, higher concentrations of NAA and BAP negatively affected plant growth. Therefore, the standardized medium with 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP may serve as a reliable protocol for efficient orchid micropropagation.

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