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IN VITRO MICROPROPAGATION OF *ERIA LASIOPETALA* (WILLD.) ORMEROD- A NATIVE MEDICINAL ORCHID IN BANGLADESH

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Abstract

The present study focuses on the asymbiotic germination of seeds through *in vitro* culture of *Eria lasiopetala,* a native medicinal orchid in Bangladesh. Out of the four basal media, MS was found to be the most effective medium, where 93.58±0.374% seed germinations were recorded. Different plant growth regulators (PGRs), e.g. BAP, Kin, NAA and were used for secondary protocorm proliferation, plantlet development, and multiplication of shoot buds through *in vitro* conditions. The maximum number of secondary protocorms (12.80±0.115) were developed that were derived from primary protocorms on MS medium fortified with 0.5 mg/l BAP and 1.0 mg/l NAA. Data revealed that 0.5 mg/l BAP + 0.25 mg/l NAA showed the best combination for height elongation of plantlets (3.12±0.012 cm) in MS medium. While for multiple shoot buds (MSBs) 1.0 mg/l BAP + 0.5 mg/l NAA showed better (6.8±0.127) performance than other combinations of PGRs. The single shoots were isolated from the multiple shoots and sub-cultured on ½MS medium supplemented with NAA (0.5 - 2.0 mg/l), IAA (0.5 - 2.0 mg/l) and IBA (0.5 - 2.0 mg/l) for root induction. The maximum number of roots (7.53±0.176) and its growth (4.61±0.01 cm) were found in ½MS medium supplemented with 1.0 mg/l IAA. The well rooted plantlets were hardened and successfully transferred after acclimatization in pots containing the mixture of coconut husk, charcoal and brick pieces in the ratio of 2:1:1 and eventually established under natural condition.

Key words: *Eria lasiopetala*, *In vitro,* Medicinal orchid, Multiple shoot buds, Plant growth regulators, Protocorms.

Introduction

The orchidaceae is a diverse and wide spread family of flowering plant that are of great value in ornamental, medical, conservation, and evolutionary research (Bhattacharjee and Islam 2014a, Zang et al. 2018). It is the largest and most diverse family of flowering plants, consisting of 28,000 accepted species belonging to 763 genera (Christenhusz and Byng 2016). They are estimated to make up 3.7% of all angiosperms (Renner 2006). According to the diversity of genome size, they are currently the most variable angiosperm family (Leitch et al. 2009). Many orchid species are rare or widely dispersed and epiphytic orchids occur in almost every habitat in the world (Jersáková et al. 2006, Waterman and Bidartondo 2008). Many orchid species are threatened; and at present, they are declining worldwide because of habitat loss, over-extraction, and other

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factors like climate change (Joshi et al. 2009, Lim et al. 2017, Bhattacharjee et al. 2022, Kindlmann et al. 2023). Orchids are grown primarily as ornamentals and are valued as cut flowers not only because of their exotic beauty but also because of their long shelf life. At present, orchids are a million-dollar industry in several countries like Thailand, Australia, Singapore, Malaysia and several other countries in the world (Chugh et al. 2009). Though orchids are grown as ornamentals, some of them are employed as herbal medicines and food by many different cultures and tribes (Bhattacharjee et al. 2015). Orchids have been used in many parts of the world in traditional healing systems as well as in the treatment of several diseases since ancient times (Pant 2013). Orchid grows all over Bangladesh but Chittagong, Chittagong hill tracts, Sylhet, Gazipur and Sundarban are enriched with it. In Bangladesh, the orchidaceae family is represented by 72 genera and 188 species (Islam et al. 2015, Rahman et al. 2017, Bhattacharjee et al. 2022). The agro-climatic conditions of Bangladesh are conducive to natural orchid vegetation in different regions, especially in the Chittagong Hills tracks, Rangamati, Sylhet, and Sundarbans. But habitat destruction, deforestation, climate change, and indiscriminate collection by hobbyists and traders are some reasons for the extinction of local orchids. Therefore, it is very important to collect and conserve the local orchid varieties that would be useful for the improvement of orchids in Bangladesh. *Eria lasiopetala* is an epiphytic and local medicinal orchid in Bangladesh (Rahman et al. 2017). It belongs to the genus *Eria,* which is one of the most important medicinal orchid genera and contains 39 compounds of secondary metabolites. It has a lot of economic importance such as medicinal, glycosidic, alkaloidal, etc. Flavanthrin, flavanthrinin, flavanthridin and coelonin 3,7-dihydroxy dimethoxyphenanthrene have been characterized by *Eria lasiopetala as* reported by Nurfadilah (2023).

It has a woody rhizome that has 1.6 to 2" between each fusiform-ellipsoid, laterally compressed, furrowed pseudobulb enveloped basally by membranous sheaths and carrying 2 to 5, lanceolate-oblong, acute to acuminate, grooved petiolate base leaves that bloom in April on a lateral, basal, erect, pubescent, racemose, 4 to 8" [10 to 20 cm] long, sub densely 7 to 12 flowered inflorescences with imbricate, ovate, acute sheaths and lanceolate, persistent, acute floral bracts. *Eria lasiopetala* is a medicinal epiphytic orchid used for the treatment of many human ailments (Hoque et al. 2021). The juice of the pseudobulb of *Eria lasiopetala* is used to treat body swelling and headaches, and its leaf paste is also used to treat inflammation. Indigenous people also used leaf juices to treat constipation and heavy menstruation. The majority of orchid species have relatively undifferentiated seeds with no cotyledons and no endosperm. Orchids produce large quantities of seeds and their germination is limited. Only 0.2%-0.3% of them can germinate. This family produces a large number of minute seeds with only minimal reserves of nutrients (Arditti and Ghani 2000). They cannot utilize their scanty lipid serves, break down starch or photosynthesize and fail to develop in absence of fungal infection (Chugh et al. 2009). Mycorrhizal fungi provide the developing embryo with water, carbohydrates, minerals and vitamins, as reported by Kauth et al. (2008). This necessitates the application of an *in vitro* seed propagation technique for orchids. Thus, *in vitro* cultural techniques are now being adopted for the quick propagation of commercially important orchid species. In this study, we developed an *in vitro* micropropagation technique using plant growth regulators for mass multiplication and germplasm conservation of *Eria lasiopetala*.

Materials and Methods

Plant materials

The immature capsule of *Eria lasiopetala* was used as a seed source and was collected from Sylhet, Bangladesh. The plantlets of *Eria lasiopetala* were grown *in vitro* for further use as the source of explants for experimentation purposes.

Seed sterilization and *in vitro* seed germination

At first, the capsules were washed by running tap water and surface sterilized by detergent. Then capsules were treated with a 0.2% (w/v) $HgCl_2$ solution for 5 minutes and finally dipped in 70% ethanol for 10-12 seconds. Then washed with sterile distilled water, and sterilized capsules were cut longitudinally by a sterile surgical blade. Around 100 mg seeds were cultured per vessel (Fig. 1a). Various media, *viz.* MS (Murashige and Skoog 1962), B5 (Gamborg et al.1968), PM (Phytamax™, Sigma, USA) and MVW (modified after Vacin and Went medium) (Vacin and Went 1949) were used in this study. As basal media, MS and B5 were amended with 3% (w/v) sucrose and the PM and MVW were amended with 2% (w/v) sucrose. The pH for all mediums was adjusted to 5.6 - 5.8 before autoclaving at 121°C for 15 minutes at 15 lbs of pressure. The medium was solidified with 0.7% agar. Inoculated vessels were maintained in the culture room for a period of 16 hrs light and dark, for 8 hrs at 25±2°C. After two weeks of inoculation, some of the seeds were taken out and dispersed in one drop of water on a glass slide and examined under a light microscope. Once the spherules were formed, then protocorm developmental stages were recorded every week. Germination percentages were calculated employing the following formula:

$$\text{Percentage (\%) of seed germination} = \frac{\text{Number of seed swelling of the embryo}}{\text{Total number of seeds}} \times 100$$

Protocorms were taken out aseptically from culture vessels and transferred into fresh culture vessels containing the same germinating medium. Young plantlets with 1-2 leaves and 1-2 weak roots were regenerated from the protocorm. Further sub-culture of the plantlets was done at an interval of 15 days. Before each subculture, the density of plantlets per vessel was reduced.

Development of secondary protocorm

In vitro, grown protocorms were used as explants for the development of secondary protocorm. Agar solidified MS medium supplemented with different concentrations of PGRs, either alone or in combination, was used to assess their efficacy on the growth and development of secondary protocorms. The PGRs used during study were BAP (0.5, 1.0, 0.5, 2.0 mg/l), Kin (0.5, 1.0, 1.5, 2.0 mg/l) and NAA (0.5, 1.0, 2.0 mg/l). MS

medium with no PGRs was considered as control. Data were recorded based on the production of several protocorms from one primary protocorm and their viability to develop plantlets every week of culture initiation.

Plant height development and MSBs formation

Based on the germination and survival rate MS medium was selected for further study of growth and development of explant. For estimating plant height development and multiple shoot bud's formation, young plantlets were taken as explant and transferred into different types of agar solidified media supplemented with BAP (0.5, 1.0, 1.5, 2.0 mg/l), Kin (0.5, 1.0, 1.5, 2.0 mg/l) and NAA (0.25, 0.5, 0.75, 1.0 mg/l). MS media without PGRs were used as control.

Rooting and acclimatization

Newly developed adventitious shoots were transferred into the rooting medium for root development. We used three plant growth regulators, *viz.* IAA (0.5, 1.0, 1.5, 2.0 mg/l), IBA (0.5, 1.0, 1.5, 2.0 mg/l) and NAA (0.5, 1.0, 1.5, 2.0 mg/l) in ½MS medium to assess their efficacy on root development. The well-rooted *in vitro* grown plantlets were successfully hardened in the potting mixture containing coconut husk, charcoal and brick pieces in the ratio of 2:1:1.

Statistical analysis

The experiment was designed by a completely randomized design (CRD). Data were recorded for different parameters and were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple range test (DMRT). The calculations were done at a 5% level of significance ($p<0.05$). All data were represented as mean with standard error (mean ± SE). Three replicates were taken per medium for seed germination The other experiment was carried out three times for each treatment. 10 explants were taken each time. The data analysis was performed using the IBM SPSS software.

Results

***In vitro* seed germination and secondary protocorm development**

Seeds were germinated after four weeks of culture initiation. In this study, four different agar solidified media were investigated. The embryo swelled and emerged from the testa, indicating germination. The first sign of seed germination was swelling, and after four weeks, the undifferentiated embryos developed spherules, which were irregularly shaped cell masses. Within two weeks, the spherules became green and produced circular structures known as protocorms (Fig. 1b). Later, protocorms became visible with a vegetative apex (Fig. 1c-d) followed by the development of 2-3 leaf primordia (Fig. 1e). Young plantlets developed after 1-2 weeks of leaf primordia formation (Fig. 1f-g). The highest percentage of seed germination (93.58±0.374%) was obtained with the MS medium, whereas the lowest (58.49±0.829%) was recorded in the B5 medium (Table 1).

Table 1: Comparative effect of four culture media on germination of seeds in *Eria lasiopetala.*

Culture media	Time required (weeks)		Percentage (%) of seed germination (M±SE)
	Spherule formation	Protocorm formation	
MS	4 -5	6 - 7	93.58±0.374a
Modified VW	7 - 8	9 - 10	74.93±0.907b
PM	9 -10	11 -12	65.97±1.837c
B_5	11 -12	13-14	58.49±0.829d

MS = Murashige and Skoog (1962), PM = Phytamax™, P-1056, Sigma, USA; B5 = Gamborg et al. (1968), MVW (modified after Vacin and Went medium 1949).

It was also revealed that the period of germination on MS medium was comparatively shorter (average germination time 31.5 days) than other mediums used in this study. The maximum time required for seed germination was recorded on B5 medium (Table 1).

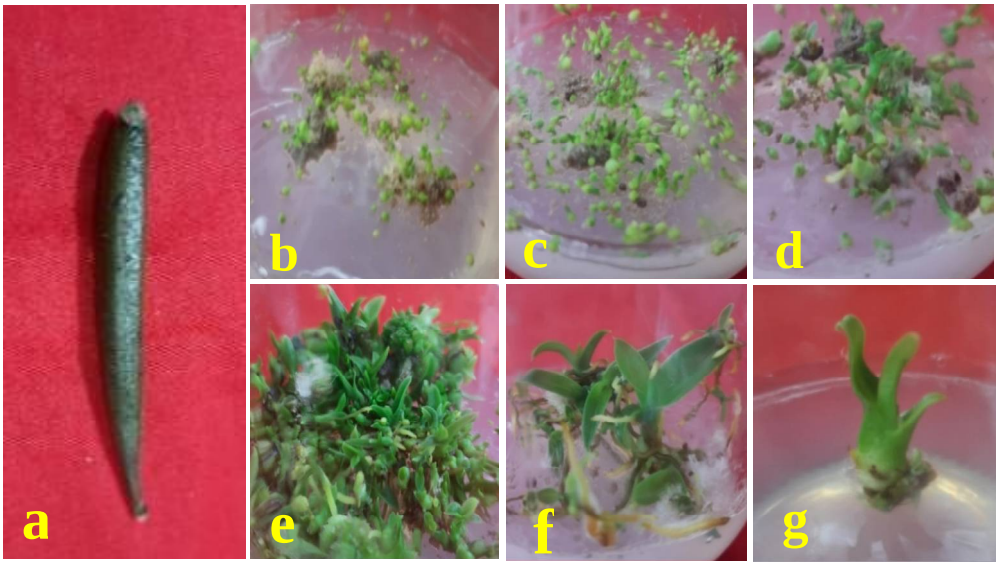

Fig. 1 (a-g): *In vitro* seed germination stages of *Eria lasiopetala.* a) seed pod, b) germinated seeds after 4-5 weeks, c-d) round primary protocorms with shoot apex, e) seedlings with leaf primordia, f) young plantlets, and g) a single plantlet.

Production of secondary protocorms

Primary protocorms were transferred to the culture medium enriched with PGRs to observe their efficacy in multiplication. Sixty days old primary protocorms was used as explants. Different concentrations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l), Kin (0.5, 1.0, 1.5, 2.0 mg/l) and NAA (0.5, 1.0, 2.0 mg/l) were used alone or in combination. Secondary protocorms generated from primary protocorm instead of shoot formation directly (Fig. 2a). MS medium fortified with BAP and NAA proved more effective for secondary protocorm development. The maximum number of secondary protocorms (12.80±0.115) developed on the medium enriched with 0.5 mg/l BAP and 0.1 mg/l NAA. The minimum result was obtained from the medium supplemented with 2 mg/l Kin, while the control medium did not develop any secondary protocorm but directly formed shoots. Almost all the secondary protocorms were converted into plantlets in the following 3-4 weeks (Fig. 2b-c). The shortest time for secondary protocorms formation was taken by the medium supplemented with BAP and NAA (Table 2).

Table 2: Effect of PGRs on production of secondary protocorm of *Eria lasiopetala*.

PGRs (mg/l)			No. of secondary protocorms (Mean±SE)	Time (weeks)
BAP	Kin	NAA		
0.5	-	-	4.00±0.115^{b}	
1.0	-	-	4.67±0.176^{a}	5-7
1.5	-	-	4.27±0.176^{ab}	
2.0	-	-	4.07±0.176^{b}	
-	0.5	-	3.60±0.115^{ab}	
-	1.0	-	3.80±0.115^{a}	6-8
-	1.5	-	3.20±0.306^{bc}	
-	2.0	-	2.73±0.067^{c}	
0.5	-	0.5	10.00±0.115^{d}	
1.0	-	0.5	11.13±0.176^{c}	
0.5	-	1.0	12.80±0.115^{a}	3-4
2.0	-	1.0	9.87±0.176^{d}	
1.0	-	2.0	11.60±0.115^{b}	
1.0	-	1.0	12.00±0.115^{b}	
2.0	-	2.0	9.33±0.176^{e}	

Contd. Table 2

-	0.5	0.5	6.13±0.176[d]	
-	1.0	0.5	9.00±0.115[a]	
-	0.5	1.0	6.40±0.115[d]	
-	2.0	1.0	8.40±0.115[b]	5-6
-	1.0	2.0	5.93±0.176[d]	
-	1.0	1.0	7.53±0.176[c]	
-	2.0	2.0	6.27±0.176[d]	
Control (MS0)			-	

PGRs = Plant growth regulators, values represent mean ± S.E. Each treatment was repeated three times. Means in a column with different letters (superscript) are significantly different according to the least significant difference at *p<0.05* levels.

Plantlets development and MSBs induction

Data showed that the MS medium supplemented with BAP and NAA proved to be best for elongation of plant height, followed by Kin and IAA. The maximum plant height (3.12±0.012 cm) was recorded in medium fortified with 0.5 mg/l BAP + 0.25 mg/l NAA. The minimum elongation of the plant among PGRs fortified medium was found when 1.0 mg/l BAP and 1.0 mg/l Kin was supplemented (Fig. 2c). The lowest elongation of the plant was obtained in control where no PGR was added in MS medium (Table 3).

Table 3: Effect of MS medium fortified with different PGRs on elongation of plantlets of *E. lasiopetala* after 30 days of culture initiation

PGRs (mg/l)			Plant height elongation (cm)		
BAP	Kin.	NAA	Initial length Mean	Final length Mean	Increased length Mean±SE
0.5	-	-	2.47	3.97	1.50±0.012[b]
1.0	-	-	2.39	4.12	1.73±0.018[a]
1.5	-	-	2.39	3.73	1.34±0.023[c]
2.0	-	-	2.43	3.65	1.22±0.012[d]
-	0.5	-	2.42	3.81	1.39±0.012[b]
-	1.0	-	2.41	3.96	1.55±0.013[a]
-	1.5	-	2.45	3.71	1.26±0.012[c]
-	2.0	-	2.43	3.53	1.10±0.023[d]

Contd. Table 3

0.5	-	0.25	2.43	5.55	3.12±0.012[a]
1.0	-	0.5	2.42	5.42	3.00±0.050[b]
1.5	-	0.75	2.45	5.15	2.70±0.012[c]
2.0	-	1.0	2.43	4.67	2.24±0.012[d]
-	0.5	0.25	2.45	4.72	2.27±0.037[b]
-	1.0	0.5	2.49	5.31	2.82±0.012[a]
-	1.5	0.75	2.53	4.62	2.09±0.018[c]
-	2.0	1.0	2.50	4.30	1.80±0.035[d]
0.5	0.5	-	2.46	4.86	2.40±0.042[a]
1.0	0.5	-	2.48	4.48	2.00±0.058[b]
0.5	1.0	-	2.43	4.19	1.76±0.031[c]
1.0	1.0	-	2.50	3.55	1.05±0.018[d]
Control (MS0)			2.43	3.25	0.82±0.012

PGRs = Plant growth regulators, values represent mean ± SE. Each treatment was repeated three times. Means in a column with different letters (superscript) are significantly different according to the least significant difference at $p<0.05$ levels.

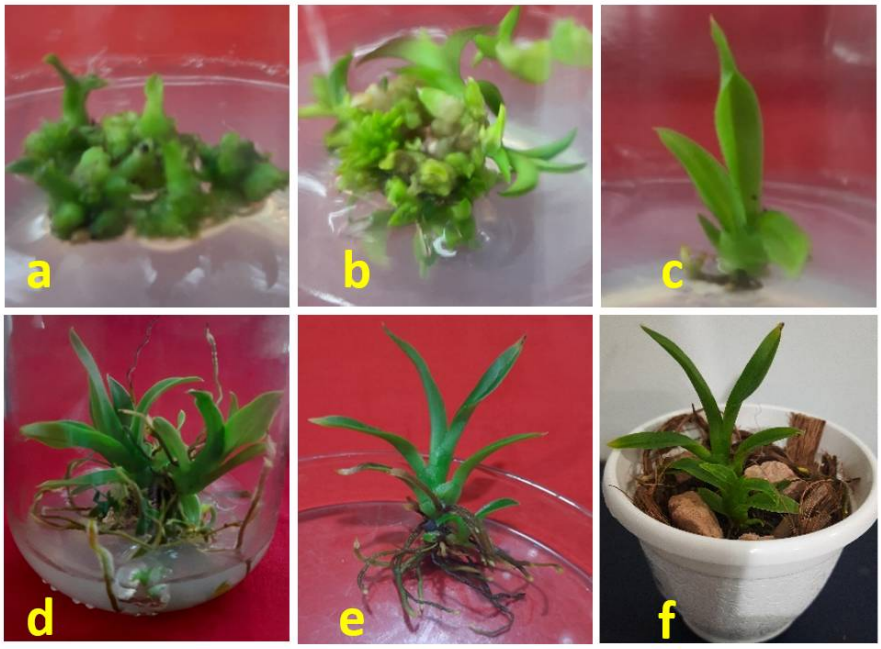

Fig. 2(a-f): Regenerated plants of *E. lasiopetala* derived from seed culture. (a) Secondary protocorm with shoot apex, (b) Plantlets after 30 days of culture initiation, (c) A single elongated plant, (d) Multiple shoot induction from single shoots, (e) A mature plantlet with increased height and well-developed roots, (f) Acclimatized plants transferred to pots.

Single plantlets were used for multiple shoot bud induction and were transferred into fresh culture media enriched with PGRs to assess their efficacy on MSBs formation. MS medium supplemented with BAP and NAA proved beneficial for MSBs induction. The highest induction (6.8 ±0.127) of MSBs was recorded on the medium enriched with 1.0 mg/l BAP and 0.5 mg/l NAA. The minimum induction of MSBs was observed on 2.0 mg/l Kin supplemented medium (Fig. 2d). The lowest number of MSBs inductions was recorded on the control medium (Table 4). Results indicated that for height elongation and MSBs formation, the combined effect of cytokinin + auxin showed better performance than single uses.

Table 4: Effect of MS medium fortified with PGRs on multiple shoot Buds (MSBs) development from single shoot segments after 30 days of culture.

PGRs (mg/l)			No. of shoots per single shoot
BAP	Kin	NAA	Mean ±SE
0.5	-	-	3.53 ± 0.174[b]
1.0	-	-	4.67 ± 0.148[a]
1.5	-	-	3.20 ± 0.069[b]
2.0	-	-	2.53 ± 0.123[c]
-	0.5	-	2.87 ± 0.086[b]
-	1.0	-	3.80 ± 0.092[a]
-	1.5	-	3.00 ± 0.088[b]
-	2.0	-	2.33 ± 0.080[c]
0.5	-	0.25	6.20 ± 0.141[b]
1.0	-	0.5	6.80 ± 0.127[a]
1.5	-	0.75	5.00 ± 0.124[c]
2.0	-	1.0	4.20 ± 0.127[d]
-	0.5	0.25	4.00 ± 0.124[c]
-	1.0	0.5	5.40 ± 0.104[a]
-	1.5	0.75	4.60 ± 0.121[b]
-	2.0	1.0	3.27 ± 0.099[d]
0.5	0.5	-	3.47 ± 0.123[bc]
1.0	0.5	-	4.07 ± 0.116a[b]
0.5	1.0	-	4.13 ± 0.108[a]
1.0	1.0	-	3.07 ± 0.116[c]
Control (MS0)	-	-	2.13 ± 0.067

Values represent mean ±SE Each treatment was repeated three times. Means in a column with different letters (superscript) are significantly different according to the least significant difference (LSD test, $p<0.05$).

Root induction and acclimatization

All elongated shoots were transferred into the rooting medium for root development and results indicate that the average length of induced roots reached the highest (4.61 cm) when the shoots were cultured on half-strength MS medium supplemented with 1 mg/l IAA (Fig. 3b). In addition, this combination of medium was also effective for developing adventitious roots. In this medium, the average number of roots per shoot was 7.53 (Fig. 3a). Data were recorded after 30 days of culture initiation. The well-rooted plantlets were successfully hardened in the potting mixture containing coconut husk, charcoal, and brick pieces in the ratio of 2:1:1 and eventually established under natural conditions. It was observed that 80% of plantlets survived the acclimatization process and grew to a normal flowering plant under field conditions (Fig. 2f).

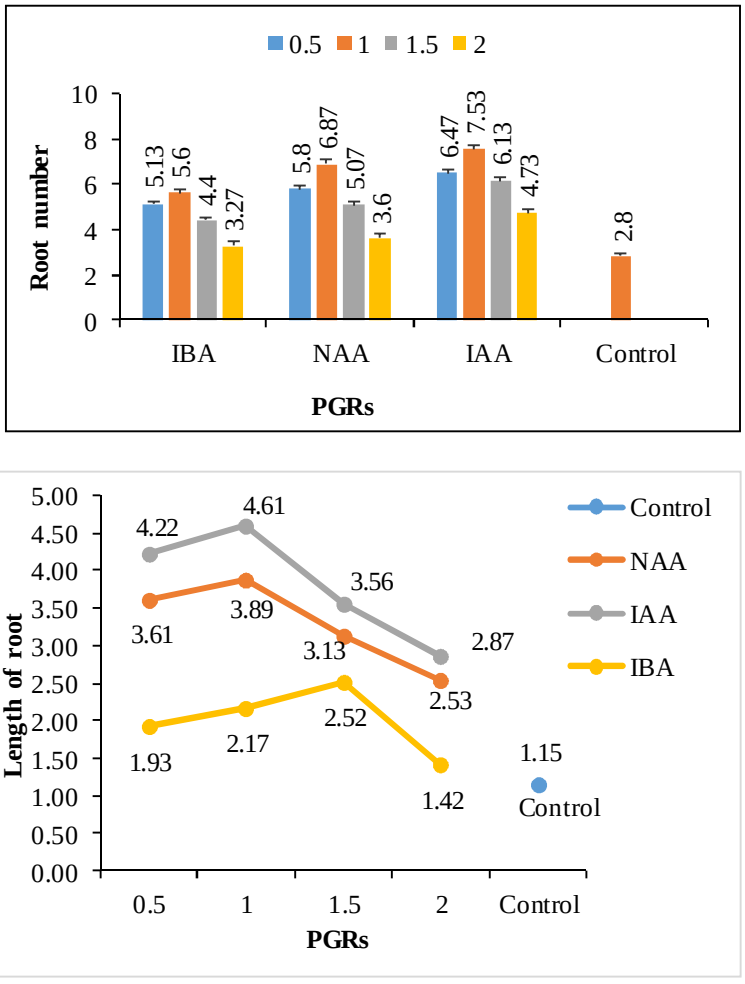

Fig. 3 (a & b): Effect of ½MS medium enriched with auxins on root development of *E. lasiopetala*. a) numbers of roots, and b) length of roots.

Discussion

The tissue culture process very successfully established the *in vitro* micropropagation techniques of *Eria lasiopetala*. Among the four investigated basal media MS medium was the most suitable one for seed germination (93.58%) (Table 1). Considering these two features, rate and time of germination the present findings suggest that MS medium was effective for *in vitro* seed germination of *Eria lasiopetala*. A similar type of finding was also reported on several orchid species such as *Cymbidium mastersii, Vanda tessellata, Rhynchostylis resuta, Dendrobium jenkinsii,* and *Bulbophyllum auricomum* (Mohanty et al. 2012, Bhattacharjee and Islam 2014b, Bhattacharjee and Islam 2015b, Barman and Banu 2020, Aung et al. 2022). On the contrary PM medium was reported to be best for seed germination of *Micropera obtuse* and *Dendrobium transparens* (Bhowmik and Rahman 2020 a,b). According to this investigation, the highest rate of secondary protocorm production was recorded in MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l NAA. The positive effect of MS medium supplemented with BAP and NAA was also reported on protocorm multiplication of *Cymbidium mastersii* and *Rhynchostylis retusa* (Mohanty et al. 2012, Bhattacharjee and Islam 2015b). In the case of *Cremastra appendiculata,* BAP and Kin supplemented medium provide the highest number of secondary protocorm development (Faisal et al. 2022). The height elongation of plantlets is very important for the better hardening of *in vitro*-grown plantlets. That can also be used as the source for regenerating multiple shoots. That is why it is one of the key steps of *in vitro* propagation. To investigate this feature, we have exposed the young orchid plantlets to various elongation media containing different plant growth regulators in different combinations and concentrations. We found that the rate of elongation reached its highest when the young plantlets were cultured on MS medium containing low concentrations of BAP and NAA (0.5 mg/l BAP and 0.25 mg/l NAA). In this medium, the average length of germinated seedlings was about 3.12 cm (Table 2). The beneficial effect of BAP and NAA on the height increase of plantlets of different orchid species was reported by many other researchers (Hossain 2014, Islam SMS et al. 2015, Bhowmik and Rahman 2020c,d). A good effect of BAP and Kin was recorded on shoot elongation of *Bulbophyllum leopardinum* (Thapa et al. 2024) Analyzing the results obtained in the multiplication of the induced shoot, we found that the media containing BAP and NAA developed the highest number of adventitious shoots (in average, 6.8 shoots per explant). The present findings are in agreement with the researchers who reported about the positive effect of BAP and NAA on multiple shoot buds development in *Vanda tesselata Cymbidium aloifolium*, *Vanda tesselata*, *, Aerides multiflora,* and *Dendrobium palpebrae* (Rahman et al. 2009, Pant and Shresta 2011, Bhattacharjee and Islam 2014b, Bhattacharjee and Islam 2017, Bhowmik and Rahman 2020c,e) while Castillo-Pérez et al. (2022) reported BAP and IAA was most effective for multiple shoot induction of *Catasetum integerrimum*. On the contrary, MS medium supplemented with BAP was found to be the most effective for multiple shoot induction of *Cymbidium aloifolium* (Kumar et al. 2022). It was observed that the addition of BAP and NAA in the MS medium leads to optimum micropropagation of *Eria lasiopetala* species and this combination can be successfully employed by orchid breeders. Well-rooted plantlets are important for acclimatization, but often *in vitro*-grown plantlets fail to develop good quality roots. To investigate this problem, we tested three potential plant growth regulators of IBA, IAA, and NAA in ½MS medium. The highest number of adventitious roots (7.53) developed in ½MS medium fortified with 1mg/l IAA (Fig. 3a). The length of these roots was also the

longest (4.61 cm) in the same medium (Fig. 3b). Several authors also reported the positive effect of IAA on root development of other orchid species (Decruse et al. 2003, Malabadi et al. 2004, Bhattacharjee and Islam 2015b, Bhowmik and Rahman 2020f, Castillo-Pérez et al. 2022). MS medium supplemented with NAA and coconut water proved to be best for the root development of *Dendrobium transparens* (Joshi et al. 2023). Finally, the seedlings were taken out of the culture vessels and rinsed with running tap water for the removal of agar attached to the roots. Then the seedlings were transferred to plastic pots containing a potting mixture of sterilized coconut husk, small brick pieces, and charcoal at a ratio of 2:1:1 in according by the methods of (Rahman et al. 2009, Bhattacharjee and Islam 2015a, b) and kept in the orchid shed house, IBSc, RU.

Conclusion

The results suggest that MS medium was best for seed germination and the combined effect of PGRs with low concentrations was more effective for secondary protocorm development, plantlet development, and multiple shoot induction of *Eria lasiopetala*. On the other hand, single effect of IAA with ½MS medium was proved the best for root induction from the individual elongated shoot. In this study, an efficient regeneration protocol for *in vitro* micropropagation in *Eria lasiopetala* through seed culture has been established. For advanced levels of biotechnological research and orchid conservation, those experimental findings can be helpful to plant breeders and the scientific community.

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Author contributions

SMSI conceived the idea, designed the experiments and improvement of the manuscript. AMN conducted experiments, sub-culturing, transferring plants to pots, data recording, maintaining plants up to maturity, and statistical analysis and draft preparation. SRS providing *Eria lasiopetala* Wild. pods/capsules, contribute for writing the manuscript partially and its improvement.

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