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In vitro propagation and PLBs multiplication of *Eulophia graminea* Lindl. on MS medium supplemented with different additives or PGRs

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Abstract
Eulophia graminea Lindl. a terrestrial medicinal orchid occasinally found in Chittagong Hill Tracts and the seeds were cultured on 0.8% (w/v) agar solidified MS medium supplemented with eight different types of additives *viz.* activated charcoal, banana powder, peptone, vanilla powder, amaranth juice, coconut water, pineapple juice and tomato juice. The required minimum time for initiation of seed germination (9.36 ± 0.34 wks) and induction of protocorms (15.21 ± 0.57 wks) was recorded on MS medium fortified with 2 g/l peptone. For the development of seedlings, the lowest required time was 31.50 ± 0.56 wks on MS medium supplemented with 15% CW. The highest percentage (93.34%) of seed germination was achieved on MS medium supplemented with 1 g/l AC, 2 g/l P, 20% CW. The highest increase of PLBs multiplication was observed on MS medium fortified with 2.0 mg/l 2,4-D + 0.8 mg/l BAP after 30d (0.78 ± 0.04 g, 37.2 ± 0.86 no) and 60d (1.94 ± 0.04 g, 87.4 ± 0.51 no) of culture respectively. The maximum increase in length (3.52 ± 0.03 cm) and number of roots (4.50 ± 0.22 no) were induced in seed originated plantlets on MS medium with 1.0 mg/l IBA + 1.0 mg/l NAA. The hardened seedlings were successfully transplanted in outside green house condition.

Keywords: additives, *Eulophia graminea,* PLBs, 2,4-D

Introduction

Orchids are the most appealing and diversified category among monocots in angiosperms. Many diverse civilizations and tribes use various orchids as food and herbal medicine (the tubers of *Cynorchis* and *Eulophia*), while being predominantly cultivated as ornamentals [1]. The diversity of orchid species offers a wonderful opportunity for investigation of the distinctive and intriguing traits that developed during adaptation to varied habitats. In addition to being present in a variety of habitats, orchids constitute up about two-thirds of the world's epiphytic flora and are primarily found (70%) growing epiphytically on tree trunks in forests; the other 25% of orchids are terrestrial, and the rest 5% are found on various substrates [2-3].

In Bangladesh, 178 different orchid species have been found so far; 29 of these have medicinal properties and are frequently utilized as traditional and folk medicines to cure 45 different ailments in ethnic groups [4]. *Eulophia graminea* Lindl., a terrestrial orchid that is indigenous to tropical and subtropical Asia [5]. Although *E. graminea* is a self-compatible, out-crossing species that depends on pollinators [6], less fruit was produced in self-pollinated flowers than in out-crossed flowers [5]. *E. graminea* tuber extracts have been applied as ear drops for treating ear pain [7] and it is also regarded as a great diet for kids and anyone recovering from illness [8]. The juice extract of *E. graminea* is used as tonic, and effective in curing cough and paralysis and the active chemical constituents are linalool, ferulic acid [9].

Due to their need for certain michorrhizal fungi for growth and inclination for strata with low nutrient levels, orchids are frequently constrained in particular habitats [10]. The embryo in undifferentiated orchid seeds lacks the enzymes necessary to metabolize polysaccharides and is very tiny [11-12]. Due to year-round availability of plant materials and an exponential growth rate, regeneration from tissue culture grown explants is preferable to seed culture for mass multiplication [13]. Plant tissue culture methods have been established as a useful technique for orchid micropropagation as well as the preservation of many rare or endangered orchid species [14-15]. Different additives, such as fruit juices, fruit water, and other organic supplements, are applied as an alternative approach [16].

The goal of the current study was to evaluate the asymbiotic germination potential of seeds *in vitro* after protocorm development, differentiation of the first leaf and root primordia, seedlings development, strong and stout root system and PLBs multiplication.

Material and methods

Sterilization of capsules

Green capsules collected from Naikhongchhari, Bandarban, Bangladesh and were scrubbed with Teepol (0.01%), rinsed under running water for ten to fifteen minutes, and then washed twice with sterile distilled water. Following a 30 second dip in 70% ethyl alcohol, the capsules were three times rinsed with double-distilled water. The capsules were then surface sterilized for 10 min with 0.1% (w/v) $HgCl_2$, and washed three times with double distilled water. In a laminar airflow cabinet, under aseptic conditions, the sterilized capsules were split open lengthwise with a sterilized blade to scoop out the seeds.

Culture medium and culture conditions

E. graminea was aseptically cultured on a 0.8% (w/v) agar solidified MS [17] medium that was 3% (w/v) sucrose-fortified and contained eight different types of additives *viz*

activated charcoal (AC), banana powder (BP), peptone (P), vanilla powder (VP), amaranth juice (AJ), coconut water (CW), pineapple juice (PJ), and tomato juice (TJ). For the MS medium, pH (Fisons, UK) was adjusted to 5.8 using 0.1N NaOH or HCl. Agar (Himedia, India) was dissolved in the mixture by boiling it in a water bath. Then, roughly 100 ml of medium was distributed into each 250 ml culture vessel (Duran, Germany), and the containers were autoclaved (Hisense, South Korea) at 121 °C for 30 min at 15 psi pressure. The cultures received 14 hours of 3500 lux illumination while being kept at a constant temperature of 25±2 °C [18].

Hardening and transplantation
Through a series of acclimation phases, healthy seedlings with 3-4 leaves and roots were gradually hardened. To remove the agar, the hardened seedlings were completely rinsed in sterile distilled water, and then the seedlings were moved to a pot with a mixture of sterilized small bricks: coconut husk, sawdust, and activated charcoal were combined in a 1:1:1:1 ratio.

Multiplication of PLBs
To increase the weight and quantity of PLBs, *in vitro* produced protocorms were grown on 2,4-D alone and in addition with BAP or Kn enriched PGRs (Merck, Germany) fortified MS media.

Computation and presentation of data
Following the necessary days of culture, the data on various parameters from several tests were recorded. The parameters were:

1. Percent (%) of culture vessels showing seed germination

The percentage of culture vessels that germinated in MS medium under various strength and condition settings was calculated using the formula below.
% of culture vessel germinate

$$= \frac{Total\ number\ of\ cultured\ vessels}{Number\ of\ cultured\ vessels\ aerminated} \times 100$$

2. Increased weight of PLBs

The following formula was used to determine the increase in PLBs weight at 30 and 60 days after inoculation:
Increased weight of PLBs (30/60 days) = Weight of PLBs after inoculation (30/60 days) - Initial weight of PLBs
Increased weight of PLBs (g/vessel)

$$= \frac{Total\ increased\ weight\ of\ PLBs\ (30/60\ days)}{Number\ of\ cultured\ vessels}$$

3. Increased number of PLBs (number/vessel)

The following formula was used to determine the increase number of PLBs at 30 and 60 days after inoculation:
Increased number of PLBs (30/60 days) = Number of PLBs after inoculation (30/60 days) - Initial number of PLBs
Increased number of PLBs (number/vessel)

$$= \frac{Total\ increased\ number\ of\ PLBs\ (30/60\ days)}{Number\ of\ cultured\ vessels}$$

4. Per cent of seedlings survived

The percent of seedlings that survived was calculated using the following formula:
% of seedlings survived

$$= \frac{Number\ of\ seedling\ survived}{Total\ number\ of\ transplanted\ seedlings} \times 100$$

Statistical analysis
The tests were carried out three times using a Completely Randomized Design (CRD) with varying numbers of replicates for each treatment. The results were provided as means ± standard errors (Mean ± SE) in Tables 1-2 and Figs 1-2. Also, using Microsoft Excel 2013 software, the standard deviation (SD) was computed. The significant differences were identified using Duncan's Multiple Range Test [19] at a 5% level of significance (P<0.05) after the data was subjected to an analysis of variance (ANOVA). The statistical software application IBM SPSS (Statistical Product and Service Solutions) was used to analyze the data.

Results
Seed germination in additives supplemented MS media
The required minimum time for initiation of seed germination (9.36 ± 0.34 wks; Fig. 1) and induction of protocorms (15.21 ± 0.57 wks; Fig. 2) was recorded on MS medium fortified with 2 g/l peptone followed by MS medium supplemented with 1 g/l AC (9.71 ± 0.26, 15.57 ± 0.52; wks) accordingly. For the development of seedlings, the lowest needed time was 31.50 ± 0.56 wks (Fig. 3-4) on MS medium supplemented with 15% CW. The highest percent (93.34%) of seed germination of *E. graminea* was achieved on MS medium supplemented with 1 g/l AC, 2 g/l P, 20% CW followed by 86.67% responses in MS with 1.5 g/l AC, 1 g/l P, 8 g/l VP, 15% CW and 15 ml/l PJ, respectively (Table 1). Peptone additive showed significant variation (P<0.05) with its concentrations (2 g/l and 3 g/l) for initiation of seed germination, induction of protocorms and development of seedling. 20% CW and 15% CW supplemented MS medium showed significant differences (P<0.05) in the initiation of seed germination and induction of protocorms. Higher concentrations of AC, BP, P, PJ and TJ illustrated significant variation (P<0.05) than medium concentration for initiation of seed germination. The medium concentration of BP and P showed significant variation (P<0.05) to higher concentrations for the development of seedlings.

Rooting of seed originated plantlet
The overall comparative results of rooting are illustrated in Graph 1 and 2. The maximum increase in length, as well as the number of roots of *E. graminea* induced in seed originated plantlets (3.52 ± 0.03 cm; 4.50 ± 0.22 no) were achieved in MS medium supplemented with 1.0 mg/l IBA + 1.0 mg/l NAA (Fig. 5). MS medium fortified with 0.5 mg/l IBA + 0.5 mg/l NAA gave almost same results in seed originated (3.37 ± 0.08 cm; 4.33 ± 0.33 no) plantlets accordingly. The lowest findings of increased length and number of roots were recorded on agar solidified MS medium supplemented with 1.5 mg/l IBA in seed originated (1.82 ± 0.08 cm; 2.50 ± 0.34 no) plantlets.
Increase in length and number of roots in both seed originated plantlets gave the insignificant variation (P<0.05) was recorded in 1.0 mg/l IBA + 1.0 mg/l NAA and 0.5 mg/l

IBA + 0.5 mg/l IAA treatments. In seed derived plantlets, individual and combined treatment of different PGRs *i. e.* IAA, IBA, NAA with different concentrations proved that moderate concentration is significantly lower (P<0.05) in higher or lower concentrations of PGRs treatment. After 30 days of culture in rooting media both seed originated plantlets illustrate the insignificant differences (P<0.05) in different concentrations and combinations of PGRs treatments. The insignificant variation (P<0.05) for increase in length and number of roots in 1.0 mg/l IBA + 1.0 mg/l NAA and 1.0 mg/l IAA + 1.0 mg/l NAA treatments.

Hardening and transplantation

For hardening, a gradual system was taken place in order to grow healthy plantlets. In this process, cultured vessels were kept open in the culture room for several hours, and then it was exposed to natural light for a day. Further, plantlets were washed by double distilled water to remove the adhering agar. Plants were treated with auxins to induce *ex vitro* rooting and roots were treated with fungicide. Then the seedlings of *E. graminea* were transferred to plastic pots containing a potting mixture of sterilized small bricks: coconut husk, sawdust, and activated charcoal were combined in a 1:1:1:1 ratio and kept in the green house (at 25-30 ^{0}C and RH 60-70%). Transplanted seedlings were watered regularly for about 2-3 months where the seedlings established and grew well.

Multiplication of PLBs (Protocorm Like Bodies)

The highest increase of PLBs multiplication after 30d and 60d of culture respectively (0.78 ± 0.04 g, 37.2 ± 0.86 no; 1.94 ± 0.04 g, 87.4 ± 0.51 no) of *E. graminea* was observed on MS medium fortified with 2.0 mg/l 2,4-D + 0.8 mg/l BAP (Fig. 6). The induced PLBs was greenish hairy in colour and friable in texture. Similar types of result were found in the medium having MS + 2.5 mg/l 2,4-D (0.73 ± 0.04 g, 34.8 ± 0.73 no after 30d; 1.87 ± 0.04 g, 81.4 ± 0.81 no after 60d). The minimum PLBs multiplication after 30d and 60d of culture respectively (0.59 ± 0.04 g, 21.8 ± 0.86 no; 1.08 ± 0.04 g, 37.6 ± 0.81 no) was obtained in MS medium containing 1.0 mg/l 2,4-D + 0.4 mg/l Kn. The overall results of PLBs multiplication are presented in the Table - 2.

The increased weight of PLBs after 30 and 60 DAI showed insignificant variation (P<0.05) between 2.0 mg/l 2,4-D + 0.8 mg/l BAP and 2.5 mg/l 2,4-D combinations, while the effect of increased number of PLBs is significant (P<0.05) in the same combinations. PLBs number increased for 60 DAI showed significant variation (P<0.05) among different concentration of all treatments, whereas in case of 30 DAI the effects is insignificant (P<0.05). In few cases, MS medium 30 and 60 days after inoculation, the PLBs weight and number showed significant variation (P<0.05) among different concentrations of PGRs treatments combinations.

Discussion

Different inorganic and oranic additives have been widely used in orchid propagation through tissue culture [20-21]. The seeds cultured on media fortified with pineapple juice (PJ) was shown the highest germination rate in *Orchis simia* [22]. Coconut water (CW) has been shown to improve seed germination in *Rhynchostylis retusa* [23], *Paphiopedilum wardii* [24], *Acampe papillosa* [25], *Cypripedium macranthos* [26] and *Smithsonia maculate* [27]. Peptone has been reported to improve seed germination in *Dendrobium lasianther* [28], *Epidendrum ibaguense* [29], *Spathoglottis plicata* [30], *Dendrobium parishii* [31] and *Aerides ringens* [32]. The amino acids, amides and vitamin contents of peptone are thought to be important for the improvement of germination of seeds [33]. Activated charcoal was also showed effective seed germination of *Epidendrum ibaguense* [29]. One of the potential benefits of charcoal in culture media is the adsorption of unknown morphogenetically active or toxic substances [34], while the other is the adsorption of inhibitory phenolics and carboxylic compounds produced by tissues [35]. Banana powder at low concentrations in MS medium was found to be effective in improving seedling growth and development [36]. Banana powder is high in minerals, vitamins, and amino acids, all of which aid in the growth of protocorm-like bodies [37].

Auxin-supplemented medium was more effective for inducing strong and stout root system [38-39]. IAA was reported to be the most effective in inducing strong and stout roots in *Acampe praemorsa* [40], *Ipsea malabarica* [41] and *Cymbidium* [42]. NAA was found to be the most efficient for rooting in *Cymbidium* in another investigation [43]. On the other hand, IBA was the most beneficial at inducing rooting in *Cymbidium iridioides* [44]. In *Dendrobium bensoniae*, a combination of BAP (0.5 mg/l) and IBA (1.0 mg/l) was more efficient for induced rooting [45].

The highest increase of PLBs multiplication of hybrid orchid (*Dendrobium alba* × *Ascanda dongtarm*) were observed on MS medium supplemented with 2.0 mg/l IAA + 0.5 mg/l Kinetin and 0.5 mg/l IAA + 0.5 mg/l Kinetin respectively [46]. PLBs multiplication of *Cypripedium formosum* was better on MS medium fortified with 2,4-D and BAP [47].

Conclusion

According to the results of the current study, MS media enriched with various additives AC, P and CW were preferred over MS0 medium for *in vitro* seed germination, protocorm development, differentiation of first leaf primordia, differentiation of first root primordia and development of seedlings in *E. graminea*. Furthermore, from seedling germination to growth, every basal media that had PGRs added to it performed at its peak. Increased length and quantity of roots were attained in seed-originating plantlets when different PGRs IBA and NAA were treated in combination at moderate concentrations. The combination of 2, 4-D and BAP in MS medium was the most effective for increasing the number and weight of PLBs.

Full meaning of abbreviated words

MS (Murashige and Skoog medium), MS0 (Hormone free Murashige and Skoog medium), activated charcoal (AC), banana powder (BP), peptone (P), vanilla powder (VP), amaranth juice (AJ), coconut water (CW), pineapple juice (PJ) and tomato juice (TJ), PGRs (Plant Growth Regulators), 2,4-dichloro phenoxy acetic acid (2,4-D), BAP (6-benzyl Amino Purine), IAA (Indole Acetic Acid), IBA (Indole Butaric Acid), Kinetin (Kn), NAA (Napthelene Acetic Acid), Protocorm Like Bodies (PLBs), Day After Innoculation (DAI).

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Author contributions

Tapash Kumar Bhowmik and Md. Mahbubur Rahman were involved in conception and design of the experiments and finally approved for publication. Tapash Kumar Bhowmik contributed to perform the experiments, analyzed the data and drafting the article. Md. Mahbubur Rahman contributed critically revising for important intellectual content.

Conflicts of interest

Authors declared that they have no conflict of interest.

Table 1: Effect of different additives in MS medium on asymbiotic seed germination, protocorms induction and seedlings development of *E. graminea.*

Sl. No.	Additives	Concen-tration	% of culture vessel germinated	Time taken in weeks (Mean ± SE)		
				Initiation of seed germination	Induction of protocorms	Development of seedlings
1.	AC (g/l)	0.5	80.00	10.93 ± 0.25^{bcdefg}	17.14 ± 0.52^{bcdef}	35.71 ± 0.64^{cdef}
2.		1.0	93.34	9.71 ± 0.26^{ab}	15.57 ± 0.52^{ab}	33.14 ± 0.75^{ab}
3.		1.5	86.67	11.57 ± 0.41^{efgh}	16.07 ± 0.48^{abcd}	34.64 ± 0.58^{bcd}
4.	BP (g/l)	2.0	73.34	10.14 ± 0.34^{abcd}	16.86 ± 0.57^{abcdef}	35.71 ± 0.59^{cdef}
5.		4.0	80.00	9.86 ± 0.45^{ab}	16.07 ± 0.48^{abcd}	34.21 ± 0.52^{bc}
6.		6.0	73.34	11.93 ± 0.41^{fgh}	17.57 ± 0.60^{defg}	36.64 ± 0.51^{defg}
7.	P (g/l)	1.0	86.67	11.64 ± 0.42^{efgh}	16.71 ± 0.58^{abcde}	32.93 ± 0.66^{ab}
8.		2.0	93.34	9.36 ± 0.34^{a}	15.21 ± 0.57^{a}	31.57 ± 0.57^{a}
9.		3.0	80.00	10.86 ± 0.45^{bcdefg}	17.21 ± 0.57^{bcdef}	34.21 ± 0.52^{bc}
10.	VP (g/l)	4.0	73.34	11.36 ± 0.42^{cdefgh}	18.14 ± 0.57^{efg}	36.86 ± 0.60^{efg}
11.		8.0	86.67	10.07 ± 0.38^{abc}	16.58 ± 0.47^{abcde}	34.86 ± 0.71^{bcde}
12.		12.0	80.00	10.79 ± 0.42^{bcdefg}	17.36 ± 0.52^{cdef}	34.79 ± 0.75^{bcd}
13.	AJ (ml/l)	5.0	73.34	11.57 ± 0.40^{efgh}	18.36 ± 0.51^{efg}	37.79 ± 0.57^{g}
14.		10.0	80.00	10.14 ± 0.39^{abcd}	16.86 ± 0.50^{abcdef}	35.57 ± 0.69^{cdef}
15.		15.0	73.34	10.93 ± 0.37^{bcdefg}	17.64 ± 0.56^{defg}	36.36 ± 0.71^{defg}
16.	CW (%)	10.0	80.00	11.29 ± 0.29^{cdefgh}	17.79 ± 0.58^{defg}	32.93 ± 0.66^{ab}
17.		15.0	86.67	10.79 ± 0.42^{bcdefg}	16.29 ± 0.50^{abcd}	31.50 ± 0.56^{a}
18.		20.0	93.34	9.86 ± 0.50^{ab}	15.79 ± 0.54^{abc}	34.29 ± 0.52^{bc}
19.	PJ (ml/l)	10.0	73.34	11.21 ± 0.42^{cdefgh}	17.71 ± 0.57^{defg}	36.57 ± 0.54^{defg}
20.		15.0	86.67	10.50 ± 0.41^{abcde}	16.93 ± 0.48^{abcdef}	35.79 ± 0.60^{cdef}
21.		20.0	80.00	12.07 ± 0.41^{gh}	18.57 ± 0.49^{fg}	37.50 ± 0.53^{fg}
22.	TJ (ml/l)	5.0	73.34	11.43 ± 0.44^{defgh}	17.57 ± 0.47^{defg}	36.50 ± 0.57^{defg}
23.		10.0	80.00	10.64 ± 0.37^{abcdef}	17.71 ± 0.50^{defg}	35.64 ± 0.64^{cdef}
24.		15.0	73.34	12.29 ± 0.42^{h}	18.36 ± 0.51^{efg}	37.43 ± 0.49^{fg}
25.	MS0 (Control)		66.67	13.50 ± 0.33^{i}	19.29 ± 0.43^{g}	38.21 ± 0.42^{g}

Activated Charcoal (AC); Banana Powder (BP); Peptone (P); Vanilla Powder (VP); Amaranth Juice (AJ); Coconut Water (CW); Pineapple Juice (PJ) and Tomato Juice (TJ). Values represent mean ± SE of each experiment consist of fifteen replicates. Mean values followed by different superscript letters within a column are significantly different at p = 0.05 according to DMRT.

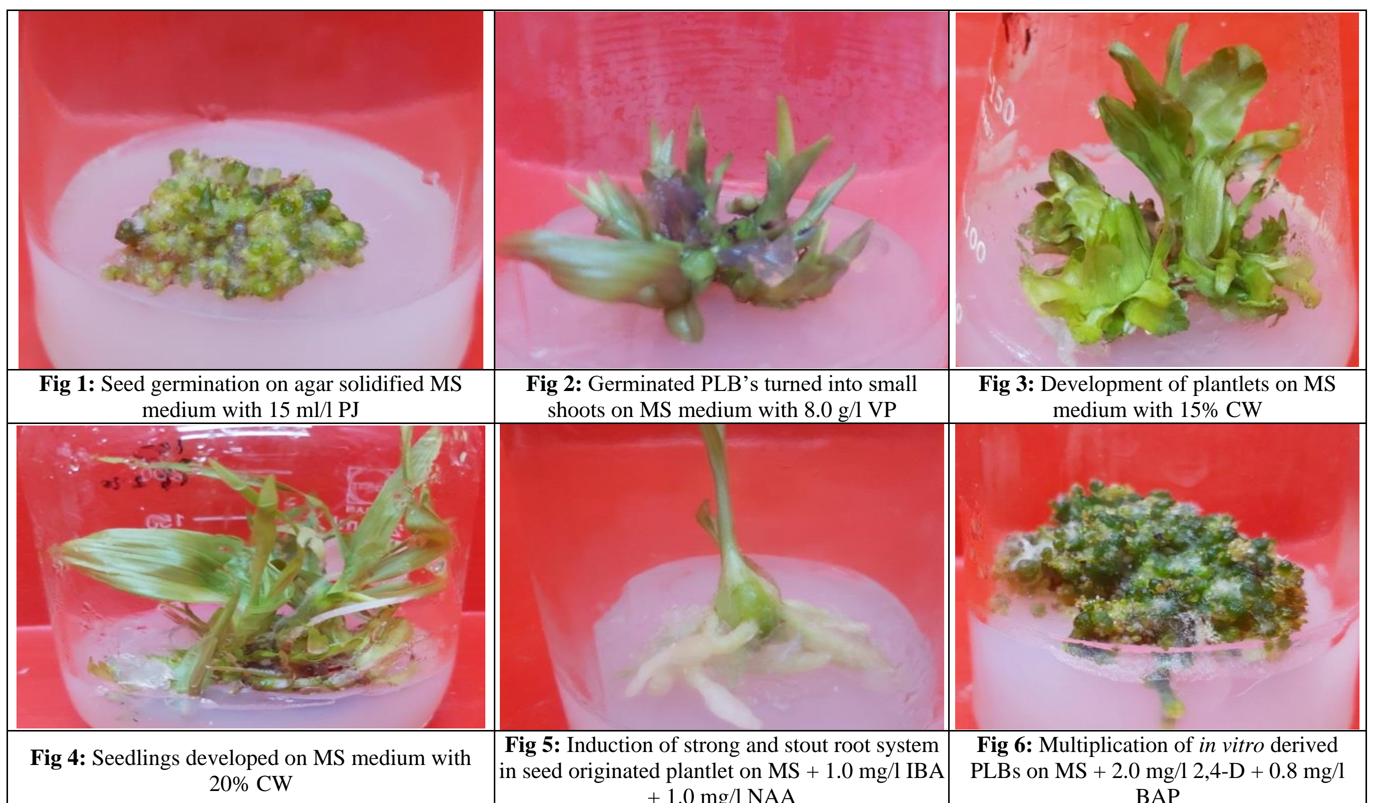


Fig 1: Seed germination on agar solidified MS medium with 15 ml/l PJ

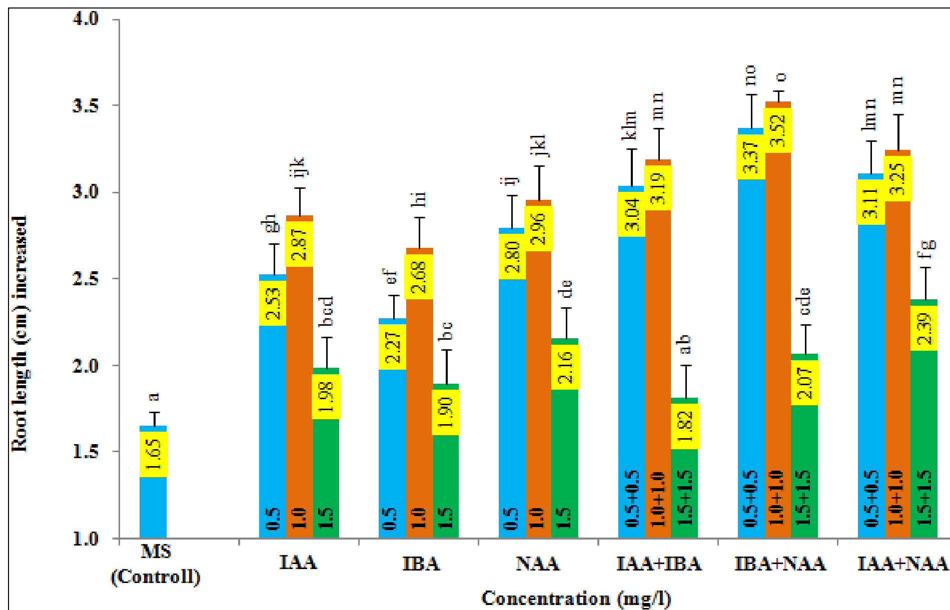
Fig 2: Germinated PLB's turned into small shoots on MS medium with 8.0 g/l VP

Fig 3: Development of plantlets on MS medium with 15% CW

Fig 4: Seedlings developed on MS medium with 20% CW

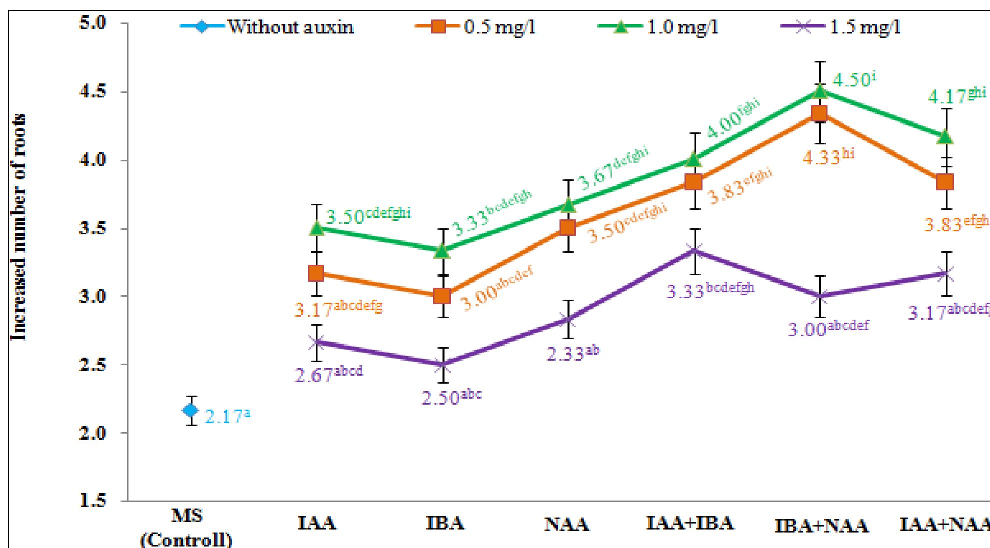
Fig 5: Induction of strong and stout root system in seed originated plantlet on MS + 1.0 mg/l IBA + 1.0 mg/l NAA

Fig 6: Multiplication of *in vitro* derived PLBs on MS + 2.0 mg/l 2,4-D + 0.8 mg/l BAP



Graph 1: Increased root length (cm) of seed originated plantlets of *E. graminea* in auxin supplemented MS medium after 30d of culture.

Values represent mean ± SE of each experiment consist of six replicates. Mean values of each bar of a graph followed by different letters at the upper position of SE bar are significantly different at p ≤ 0.05 according to Duncan's Multiple Range Test.



Graph 2: Increased root number of seed originated plantlet of *E. graminea* in auxin supplemented MS medium after 30d of culture.

Values represent mean ±SE of each experiment consist of six replicates. Mean values of each point of a graph followed by different superscript letters are significantly different at p≤0.05 according to Duncan's Multiple Range Tes

Table 2: Effect of 2,4-D individually and in combination with BAP or Kn in MS medium on PLBs multiplication of *E. graminea*.

Sl. No.	PGRs Concentration (mg/l)			Increased weight of PLBs (g/vessel) (Mean ± SE)		Increased number of PLBs (No/vessel) (Mean ± SE)		Quantity of PLBs	Colour and texture of induced PLBs
	2,4-D	BAP	Kn	30 DAI	60 DAI	30 DAI	60 DAI		
1.	0.5	-	-	$0.60±0.04^{abc}$	$1.12±0.05^{b}$	$22.8±0.58^{b}$	$40.8±0.58^{c}$	Few	YGC
2.	1.0	-	-	$0.63±0.03^{abc}$	$1.31±0.04^{de}$	$24.2±0.58^{bc}$	$50.2±0.86^{e}$	Moderate	GHC
3.	1.5	-	-	$0.65±0.03^{abc}$	$1.44±0.04^{efg}$	$27.4±0.93^{de}$	$56.0±0.71^{g}$	Moderate	GYHC
4.	2.0	-	-	$0.70±0.03^{bcd}$	$1.75±0.04^{ijk}$	$32.4±0.81^{hi}$	$73.4±0.93^{l}$	Many	YGC
5.	2.5	-	-	$0.73±0.04^{cd}$	$1.87±0.04^{kl}$	$34.8±0.73^{j}$	$81.4±0.81^{n}$	Many	GYHC
6.	0.5	0.2	-	$0.64±0.02^{abc}$	$1.38±0.04^{def}$	$26.0±0.71^{cd}$	$53.2±0.80^{f}$	Moderate	YGF
7.	1.0	0.4	-	$0.69±0.04^{bcd}$	$1.69±0.05^{hij}$	$31.4±0.93^{ghi}$	$70.6±0.93^{k}$	Many	WGC
8.	1.5	0.6	-	$0.71±0.05^{bcd}$	$1.81±0.03^{jkl}$	$33.6±0.81^{ij}$	$76.8±0.86^{m}$	Many	GYHF
9.	2.0	0.8	-	$0.78±0.04^{d}$	$1.94±0.04^{l}$	$37.2±0.86^{k}$	$87.4±0.51^{o}$	Many	GHF
10.	2.5	1.0	-	$0.66±0.03^{abcd}$	$1.51±0.05^{fg}$	$28.4±0.68^{ef}$	$59.6±0.81^{h}$	Moderate	GYHC

11.	0.5	-	0.2	0.61±0.04[abc]	1.18±0.04[bc]	23.0±0.71[b]	43.0±0.71[c]	Few	YGC
12.	1.0	-	0.4	0.59±0.04[ab]	1.08±0.04[ab]	21.8±0.86[b]	37.6±0.81[b]	Few	YGF
13.	1.5	-	0.6	0.67±0.04[bcd]	1.57±0.05[gh]	29.2±0.80[efg]	62.2±0.86[i]	Moderate	WGC
14.	2.0	-	0.8	0.69±0.04[bcd]	1.64±0.05[hi]	30.6±0.93[fgh]	66.0±0.71[j]	Moderate	GYHC
15.	2.5	-	1.0	0.63±0.05[abc]	1.25±0.04[cd]	24.2±0.80[bc]	46.4±0.93[d]	Few	WGC
16.	MS0 (Control)			0.54±0.03[a]	0.98±0.04[a]	15.0±0.71[a]	28.0±0.71[a]	Few	WGF

DAI = Days After Inoculation; Few (0 ≤ Few ≤ 49 no), Moderate (50 ≤ Moderate ≤ 69 no), Many (70 ≤ Many ≤ Above 70 no); Greenish Yellow Hairy Compact (GYHC), Greenish Yellow Hairy Friable (GYHF), Yellowish Green Compact (YGC), Yellowish Green Friable (YGF), Greenish Yellow Hairy (GYH), Whitish Green Compact (WGC), Whitish Green Friable (WGF), Whitish Green Hairy (WGH), Greenish Hairy Compact (GHC), Greenish Hairy Friable (GHF); Values represent mean ± SE of each experiment consist of five replicates. Mean values followed by different superscript letters within a column are significantly different at p = 0.05 according to DMRT

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