APPENDICES

APPENDIX A

PUBLISHED PROCEEDING

The Effect of Sodium Hypochlorite and Culture Medium on Seed Viability and Germination of *Eulophia bicallosa* (D. Don) Hunt & Summerh (Orchidaceae)

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Abstract: *Eulophia bicallosa* (D. Don) Hunt & Summerh., a terrestrial orchid having light green or cream color flowers with purplish markings, is considered a near-threatened species in Australia. In Thailand, it is recognized as a rare species since just two populations have been reported so far. The information on its propagation via seed is required. The objective of this study was to investigate seed characteristics and the effect of sterilant and culture medium on seed viability and germination of *E. bicallosa*. Seeds were sterilized in sodium hypochlorite (NaClO) at three concentrations (0, 0.5, and 1%) and three durations (1, 5, and 10 min), followed by 1%TTC for viability test. Sterilized seeds were sown on Vacin and Went (VW) media for one month for germination assessment. In addition, the effect of six medium types: MM, ½ MM, MS, ½ MS, VW, and ½ VW were assessed for seed germination for 3 months. The result showed that the seeds of *E. bicallosa* weighed only 4.50±0.29 µg per seed, with a length of 628.32 ± 9.15 µm, and a width of 195.68 ± 4.15 µm in size. At one month after sowing, seed treatments with 0.5% NaClO for 5 min or 10 min resulted in high seed viability of 62.06% and 59.10% with seed germination of 58.89% and 58.42%, respectively, without contamination. Among six media tested, the fastest and highest seed germination percentage was observed on MS and ½ MS medium in which protocorm development reached to stage 3 at 3 months after culture. Suitable sterilization and medium are required for this orchid species for optimum germination and growth.

Keywords: *asymbiotic germination, in vitro, Orchidaceae, survival rate, rare species*

1. Introduction

Orchidaceae (orchids) is a family of herbaceous plants that can be found on trees, rocks, and soil. They have a unique and beautiful flower that possesses a highly modified dorsal petal (lip) [1], making them have high market needs and causing them to be removed from natural habitats. Moreover, some orchid seeds are hard to germinate and have a high mortality rate [2]. As a result of these, many species are at risk of extinction.

E. bicallosa is a terrestrial orchid that possesses beautiful white purplish flowers. The flowering period occurs during April and December. It can be found in southeast Asia (India, Indonesia, Malaysia, Myanmar, Nepal, New Guinea, Thailand, and Australia) [3]. It has a high potential to become a commercial, becoming more popular in local markets due to its beautiful flowers, therefore it raises a concern that this orchid species may become a threatened species soon.

Orchid seeds are hard to germinate in a natural environment [2]. The *in-vitro* seed germination protocol is a powerful tool for orchid germination, that is reported to be successful in many orchid species, such as *Calanthe* hybrids

("Hyesung" x "Jeongmong", "Hwagung" x "Heysung") [4], *Habenaria macroceratitis* [5], *Spathoglottis plicata* [6], *Eulophia nuda* [7], *Liparis fujisanensis*, *L. koreojaponica* and *L. kumokiri* [8].

The objective of this study was to investigate seed characteristics and identify suitable sterilizing and culture media for seed germination of *E. bicallosa* through asymbiotic germination.

2. Experiment

2.1 Seed source and sterilization

For seed source, *E. bicallosa* capsules and seeds were collected from Rayong Botanical Garden, Rayong Province with a permit. Then, seed capsules were stored in paper bags and dried on silica gel until the dehiscence. The brown seeds were then stored in a sterile Eppendorf tube at 4 °C (12% Humidity) until use.

To calculate seed weight and maturity, 1.2 mg of
E. bicallosa seeds were weighed and counted. The seeds with fully developed embryos were counted as mature seeds. The length and width of randomized 30 seeds were measured.

For seed sterilization treatment, three concentrations (0, 0.5, 1%) and three durations (1, 5, 10 min) of commercial bleach (Hyter, 6% NaClO) were employed. After sterilization treatment, sterilized seeds were rinsed with deionized water for 5 min, 3 times before sawing. Seed viability using triphenyl tetrazolium chloride (TTC) staining (1%) was evaluated, using a microscope (Fig. 2A). After that approximately 150-200 seeds were sown onto each plate. Four replicate plates were used for each treatment. Seed germination percentage and contamination were recorded at one month, using stereoscope Fig. 2B-D and Fig. 3.

2.2 Effect of different media on seed germination

Seed germination and protocorm development were assessed by spreading seeds on six different types of culture media: (1) Malmgren Modified Terrestrial Orchid Medium (MM) [9], (2) half-strength MM (½MM), (3) Murashige and Skoog (MS) [10], (4) half-strength MS (½MS), (5) Vacin and Went (VW) [11], and (6) half-strength VW (½VW). The pH of all media was adjusted before sterilization and autoclaving at 121°C for 20 min. Sterilized seeds with the best sterilizing condition, 0.5% NaClO for 5 min, were sown on these media for 4 weeks under 0/24 h L/D and for 8 weeks under 16/8 h L/D photoperiod at 25 ± 2C. Approximately 150-200 seeds were sown onto each plate. Five replicate plates were used for each germination medium treatment. Percentages of seeds were recorded every month for three months. Protocorm development was scored on a scale of 0–5 [5].

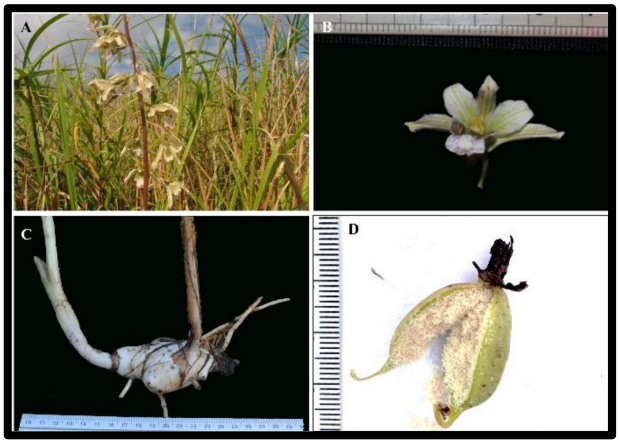

Fig. 1. Habit and inflorescence of *E. bicallosa.* A: habit; B: flower, C: rhizome, D: capsule and seeds.

3. Analysis

3.1 Statistic analysis

For the experiments, seed germination and protocorm development were observed every month for 3 months under a stereomicroscope. According to Stewart and Kane (2006), the germination and developmental stages were graded on a 0-5 scale of incremental growth. Percentages of seed germination and protocorms for each developmental stage were calculated from the total number of viable (maturation) seeds. All the experiments were conducted in a completely randomized design (CRD). Before data processing, the arcsine square root transformation was used to normalize variability. With the SPSS V26.0 statistical software (SPSS Inc., Chicago, USA), an analysis of variance (ANOVA) was performed, and the means were compared using the least significant difference (LSD) test (P=0.05).

4. Results and discussion

4.1 Seed characteristics and effect of NaClO on seed viability and germination

Like many other orchids, *E. bicallosa* seed is very tiny, dust-like, without endosperm, weighing about 4.50±0.29 µg. The seed length and width are about 628.32±9.15 µm and 195.69±4.15 µm respectively, while the embryo length and width are approximately 206.18±0.70 µm, and 117.16±3.76 µm, respectively (Table 1).

Embryos of the viable seeds of *E. bicallosa* stained bright red with 1% TTC, showing the high quality of the seed batches (66.90%) (Fig. 2A). After sterilization, seed viability with TTC showed variable among treatments (Table 1). The concentrations of 0.5% NaClO for 5 min (0.5x5) and 0.5% NaClO for 10 min (0.5x10) treatments gave moderate seed viability (62.06%, 59.10% respectively), whereas 0.5x1 and 1x1 treatments provided much higher percent seed viability (91.95%, and 87.31% respectively). NaClO for 1x 5 and 1x10 treatments had the lowest seed viability (13.82%, and 4.7% respectively). It is indicated that a shorter soaking time with NaClO gave more effectiveness on seed viability. After one month of sawing, some treatments got contaminated. Seed germination results are similar to seed maturity percentages, with 0.5x5 and 0.5x10 treatments providing a moderate percentage of seed germination (58.89%, and 58.42% respectively) without contamination. The 0.5x1 and 1x1 treatments gave the significantly highest percentage of seed germination (91.70%, and 83.52% respectively), but some got contaminated. The 1x5 and 1x10 treatments had the lowest seed germination percentages (21.14% and 1.49% respectively).

Without sterilant (0x10 treatment), all the samples got contaminated within the first week of sawing. Sterilization solution NaClO not only eliminated microbe from the tissue but also caused damage [12] and created stress to the tissue, stimulated metabolism that involved cell damage repair, and resulted in increased respiration. As a result of this, NaClO-treated seeds (0.5x1 and 1x1 treatments) were easier to be detected by TTC staining than untreated seeds (0x10). However, if NaClO is used with high concentration or long-duration treatment, it can cause decreasing in seed viability and germination due to damaged tissue which was noticeable, when compared to 0.5x5 and 0.5x10 treatments. With too low concentration or too short duration of NaClO, some microbe remained in the tissue, resulting in contamination during tissue culturing (0x10, 0.5x1, and 1x1 treatments).

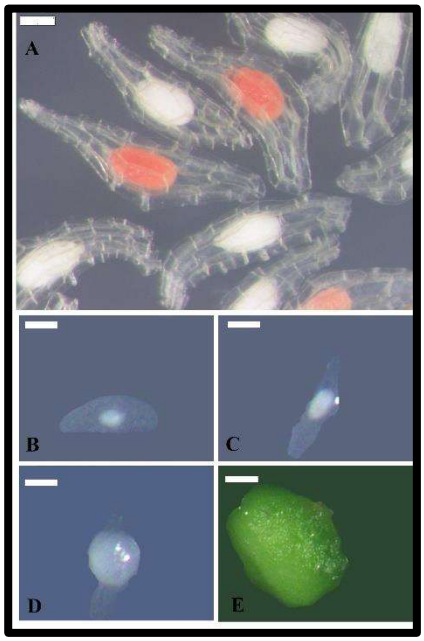

Fig. 2. TTC viability, and seed development A; treated seeds with 1% TTC (red= viability), B: stage 0, C: stage 1, D: stage 2; E: Stage 3 (scale bar= 100 µm).

Table 1. Seed characteristics of *E. bicallosa*.

Characteristics	Average ±SE
Seed weight	4.50 ±0.29 µg
Seed length (SL)	628.32±9.15 µm
Seed width (SW)	195.68±4.15 µm
SL/SW ratio	3.27
Embryo length (EL)	206.18±0.70 µm
Embryo width (EW)	117.16±3.76 µm
EL/EW ratio	1.79
Seed viability	66.90%

Table 2. Effect of NaClO concentration and duration on seed viability and germination.

Concentration (%) x Time (min)	Viability (%)	Germination (%)	Contami-nation
0 x 10	66.90 [a]	nd	++++
0.5 x 1	91.95 [b]	91.70 [a]	+
0.5 x 5	62.06 [a]	58.89 [b]	-
0.5 x10	59.10 [a]	58.42 [b]	-
1 x 1	87.37 [b]	83.52 [a]	+
1 x 5	13.82 [c]	21.14 [c]	+
1 x 10	4.70 [c]	1.49 [c]	-

Note: Different letters indicate significant differences between each treatment tested according to LSD test ($p < 0.05$). nd=not determine.

4.2 Effect of different media on seed germination

From three months of observation, development stages can be separated into 3 stages. At stage 1, seeds had not yet germinated, the testa intact with embryo length and width around 206.16±0.70 µm and 117.16±3.76 µm. Stage 2 seeds germinated with testa rupture due to an increase in the size of the embryo, in which some embryos are free from testa, embryos length and width around 378.33± 47.01 µm, and 303.78±40.95 µm. In stage 3, embryos are turning green and increase in size, embryo length and width are around 705±109.1 µm (twice larger) and 687.34±11.46 µm (6 times larger) (Fig. 2). This stage was noticeable in the second and third month of culture.

According to our findings, the kind and concentration of the basal media altered protocorm growth response. During three months of growth, all tested conditions sustained protocorm development (Fig. 3) to stage 3 protocorm. The rates of seed germination and protocorm development, however, differed between treatments (Table 3 and Fig. 3). MS (92.88 7.02%) and ½ MS (89.21 2.41%) had the highest seed germination rate and the highest proportion of stage 3 protocorms at 3 months after seed sawing compared to MM, ½ MM, VW, and 1/2 VW (Table 3). Protocorms grown in MM and ½ MM medium had well root development that could be due to the effect of activated charcoal in the medium.

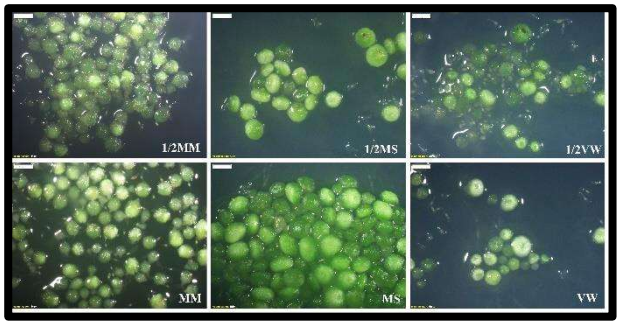

Fig. 3. Seed germination on various media at 3 months after sawing (scale bar= 1 mm).

Table 3. Seed germination stages of *E. bicallosa* on various media for 3 months. Different letters indicate significant differences in each media tested according to LSD test ($p < 0.05$).

Medium	Stage 0	Stage 1	Stage 2	Stage 3	Total germination
MM	29.32± 9.3[a]	0.57± 0.52[a]	9.22± 1.00[a]	60.88± 10.08[b]	70.67 ± 9.38[b]
½ MM*	19.88± 0.10	0.00	2.21 ± 1.10	77.91 ± 0.10	80.13 ± 1.00
MS	8.90± 6.68[b]	0.00	1.52 ± 0.43[b]	91.40 ± 8.60[a]	92.88 ± 7.02[a]
½ MS	10.79± 2.41[b]	0.00	0.00	89.21 ± 2.41[a]	89.21 ± 2.41[a]
VW	43.88± 9.68[a]	0.82 ± 0.42[a]	3.63 ± 3.31[ab]	49.70 ± 4.81[b]	56.12 ± 9.68[b]
½ VW	34.49± 4.24[a]	2.65 ± 2.18a	3.20 ± 2.97[ab]	65.51 ± 4.24[b]	65.51 ± 4.24[b]

**½ MM *had only two replicate plates, unable to perform statistical analysis on this treatment*

The culture medium employed had a significant impact on germination due to the composition variation of organic and inorganic nutrients. The high germination and robust subsequent growth in MS and ½ MS media might be related to the fact that these media are particularly rich in both macro- and micronutrients when compared to MM and VW. It has previously been demonstrated that a high nitrogen supply affects the germination of many orchid species [5], [13], and [14]. Furthermore, orchids have been observed to use a species-specific medium for seed germination. Even within the same genus, medium variations have been found, such as BM1 for *Eulophia nuda* [6] and P723 medium for *Eulophia alta* [15], for *E. promensis* [16]. Using asymbiotic methods have been successfully reported in many *Eulophia* species, such as *E. cucullata*, *E. petersii*, and *E.*

streptopetala were all cultured on MS [17]. Because no plant growth regulators were added in this study, it suggests that sufficient endogenous hormones are already present.

5. Conclusions

Seed treatments with 0.5% NaClO for 5 min or 10 min resulted in moderate seed viability and germination of 58.89% and 58.42%, respectively, without contamination, thus is suitable for this orchid species. MS and ½ MS medium were observed to increase protocorm development in *E. bicallosa*, resulting in the highest seed germination and the largest proportion of Stage 3 protocorms at three months following seed sowing. This is the first report of *in vitro* germination of *E. bicallosa* seeds, which might be applied to other commercially significant, rare species, and endangered orchids for multiplication and conservation.

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APPENDIX B

MEDIA AND COLCHICINE PREPARATION

1. Media preparation

1.1 MM and 1/2MM media were prepared using M534 Malmgren Modified Terrestrial Orchid Medium Without Sucrose and Agar (PhytoTechnology Laboratories ®, USA) according to product description. To prepare 1 liter MM medium; dissolve 21.84 g medium powder and 10 g sucrose in 1 liter DI water then adjust PH to 5.5-6 using NaOH and HCl, add 6 g agar and autoclave at 121 C, 15 PSI for 20 min. For 1/2MM, the method is the same as above except for medium powder and sucrose which are reduced to 10.92 g and 5 g respectively.

1.2 MS and 1/2MS media were prepared using M5800 Murashige and Skoog (MS) Modified Basal Medium (PhytoTechnology Laboratories ®, USA) according to product description. To prepare 1 liter MS; dissolve 4.406 g medium powder and 30 g sucrose in 1 liter DI water then adjust PH to 5.7-5.8 using NaOH and HCl, add 6 g agar and autoclave at 121 ^{O}C, 15 PSI for 20 min. For 1/2MS, the method is the same as above except for medium powder and sucrose which are reduced to 2.203 g and 15 g respectively.

1.3 VW and 1/2VW were prepared from stock solution A, B, and C. To prepare 1 liter VW; pipette 10 ml stock solution A, B, and C into 500 ml DI water, add 20 g sucrose, adjust volume to 1 liter using DI water, adjust PH to 4.8-5.5 using NaOH and HCl, add 6 g agar and autoclave at 121 C, 15 PSI for 20 min. For 1/2MS, the method is the same as above except for stock solution A, B, and C which are reduced to 5 ml. For stock solution preparation, I will describe below.

1) 1-liter stock A preparation. Put 50 g $(NH_4)_2SO_4$, 25 g $MgSO_4*7H_2O$, 52.5 g KNO_3, 25 g KH_2PO_4, 0.75 g $MnSO_4*4H_2O$ into each 200 ml beaker and dissolve in 180 ml DI water separately. Put dissolved solution together in 1000 ml volumetric flask ordinally start from $(NH_4)_2SO_4$ to $MgSO_4*7H_2O$ to KNO_3 to KH_2PO_4 to $MnSO_4$ $*4H_2O$, adjust the volume to 1 liter. Store at 4 C away from light.

2) 1-liter stock B preparation. Dissolve 20 g $Ca_3(PO_4)_2$ in minimal amount of 10% (v/v) HCl. Put the dissolved $Ca_3(PO_4)_2$ in 1000 ml volumetric flask containing small amount of DI water and adjust the volume to 1 liter using DI water. Store at 4 C away from light.

3) 1-liter stock C preparation. Dissolve 2.78 g $FeSO_4*7H_2O$ and 3.73 g Na_2EDTA in minimal amount of 50 C DI water separately. Put dissolved $FeSO_4*7H_2O$ into 1000 ml beaker containing small amount of DI water followed by dissolved Na_2EDTA, mix together, put into 1000 ml volumetric flask containing, and adjust volume to 1 liter. Store at 4 C away from light (use within 3 months).

2. Colchicine preparation

2.1 Prepare 2 ml of 0.2% (w/v) concentration colchicine stock solution by dissolving 0.004g colchicine in 2 ml DI water. Wrap with aluminum foil store at 4 C, away from light.

2.2 2 ml of 0.025, 0.05, 0.1 (w/v) colchicine were prepared from 0.2% (w/v) concentration colchicine stock solution. For 2 ml of 0.025 colchicine, pipette 0.25 ml colchicine stock solution into 5 ml vial bottle, and adjust the volume to 2 ml. For 2 ml of 0.05 (w/v) colchicine, pipette 0.5 ml colchicine stock solution into 5 ml vial bottle, and adjust the volume to 2 ml. For 2 ml of 0.1 (w/v) colchicine, pipette 0.1 ml colchicine stock solution into 5 ml vial bottle, and adjust the volume to 2 ml.

2.3 All colchicine vial were wrapped with aluminum foil and autoclave at at 121 °C, 15 PSI for 20 min. Leave at room temperature for 24 hours, away from the light and will be use within 48 hours after prepared.

APPENDIX C

RAW DATA

Table 6.1 Effect of Na(OCl) treatments on seed viability.

Na(OCl) Concentration (%)	Duration (min)	Replicate	Living seeds	Dead seeds	Unfertilized seeds	Fertilized seed	Total seeds
0	10	1	146	100	4	246	250
0	10	2	74	12	1	86	87
0	10	3	96	47	0	143	143
0	10	4	49	40	0	89	89
0.5	1	1	180	15	4	195	199
0.5	1	2	105	19	12	124	136
0.5	1	3	184	7	10	191	201
0.5	1	4	103	6	9	109	118
0.5	5	1	87	37	4	124	128
0.5	5	2	74	52	11	126	137
0.5	5	3	103	164	2	267	269
0.5	5	4	105	25	2	130	132

Table 6.1 Effect of Na(OCl) treatments on seed viability (Continued).

Na(OCl) Concentration(%)	Duration (min)	Replicate	Living seeds	Dead seeds	Unfertilized seeds	Fertilized seed	Total seeds
0.5	10	1	110	82	8	192	200
0.5	10	2	141	92	5	233	238
0.5	10	3	148	73	14	221	235
0.5	10	4	110	103	15	213	228
1	1	1	100	3	19	103	122
1	1	2	184	21	17	205	222
1	1	3	129	24	2	153	155
1	1	4	112	31	18	143	161
1	5	1	13	225	10	238	248
1	5	2	7	69	4	76	80
1	5	3	41	141	17	182	199
1	5	4	17	77	7	94	101
1	10	1	2	128	11	130	141
1	10	2	17	191	1	208	209
1	10	3	10	125	0	135	135
1	10	4	3	177	7	180	187

Table 6.2 Effect of Na(OCl) treatments on seed germination.

Na(OCl) Concentration(%)	Duration (min)	Replicate	germinated seeds	Total seeds
0	10	1	na	na
0	10	2	na	na
0	10	3	na	na
0	10	4	na	na
0.5	1	1	78	84
0.5	1	2	41	47
0.5	1	3	38	40
0.5	5	1	50	108
0.5	5	2	53	145
0.5	5	3	108	141
0.5	5	4	102	134

Remark: nd= no data (all sample are contaminated)

Table 6.2 Effect of Na(OCl) treatments on seed germination (Continued).

Na(OCl) Concentration(%)	Duration (min)	Replicate	germinated seeds	Total seeds
0.5	10	1	72	116
0.5	10	2	53	98
0.5	10	3	132	188
0.5	10	4	62	131
1	1	1	102	119
1	1	2	137	169
1	1	3	93	111
1	5	1	24	94
1	5	2	21	95
1	5	3	21	133
1	10	1	2	144
1	10	2	3	129
1	10	3	3	134
1	10	4	0	132

Table 6.3 influence of culture media on seed germination and development on the third month of culture.

Media	Replicate	Stage0	Stage1	Stage2	Stage3	Total
MM	1	73	2	16	101	192
MM	2	77	0	29	146	252
MM	3	57	2	23	212	294
1/2MM	1	32	0	5	130	167
1/2MM	2	43	0	3	163	209
MS	1	44	7	5	194	250
MS	2	0	0	0	155	155
MS	3	4	0	2	136	142
MS	4	11	0	0	93	104
MS	5	8	0	2	163	173
1/2MS	1	38	0	0	268	306
1/2MS	2	23	0	0	273	296
1/2MS	3	37	0	0	230	267
1/2MS	4	23	0	0	196	219
1/2MS	5	26	0	0	250	276

Table 6.3 influence of culture media on seed germination and development on the third month of culture (Continued).

Media	Replicate	Stage0	Stage1	Stage2	Stage3	Total
VW	1	57	30	17	95	199
VW	2	82	0	0	85	167
VW	3	91	1	4	85	181
VW	4	61	2	2	88	153
VW	5	84	1	4	74	163
1/2VW	1	97	0	3	167	267
1/2VW	2	92	16	20	154	282
1/2VW	3	57	1	11	134	203
1/2VW	4	109	6	7	177	299
1/2VW	5	97	6	0	146	249

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment.

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
1	0	1	30	30
1	0	2	29	30
1	0	3	27	30
1	0	4	30	30
1	0	5	29	30
1	0	6	27	30
1	0.025	1	30	30
1	0.025	2	30	30
1	0.025	3	30	30
1	0.025	4	30	30
1	0.025	5	30	30
1	0.025	6	30	30

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
1	0.05	1	29	30
1	0.05	2	29	30
1	0.05	3	30	30
1	0.05	4	29	30
1	0.05	5	29	30
1	0.05	6	30	30
1	0.1	1	26	30
1	0.1	2	26	30
1	0.1	3	30	30
1	0.1	4	26	30
1	0.1	5	26	30
1	0.1	6	30	30

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
2	0	1	28	30
2	0	2	25	30
2	0	3	24	30
2	0	4	27	30
2	0	5	26	30
2	0	6	25	30
2	0.025	1	30	30
2	0.025	2	30	30
2	0.025	3	30	30
2	0.025	4	30	30
2	0.025	5	30	30
2	0.025	6	30	30

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
2	0.05	1	29	30
2	0.05	2	29	30
2	0.05	3	29	30
2	0.05	4	29	30
2	0.05	5	29	30
2	0.05	6	29	30
2	0.1	1	26	30
2	0.1	2	26	30
2	0.1	3	29	30
2	0.1	4	26	30
2	0.1	5	26	30
2	0.1	6	27	30

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
3	0	1	28	30
3	0	2	25	30
3	0	3	24	30
3	0	4	26	30
3	0	5	16	30
3	0	6	20	30
3	0.025	1	30	30
3	0.025	2	30	30
3	0.025	3	30	30
3	0.025	4	30	30
3	0.025	5	30	30
3	0.025	6	30	30

Table 6.4 Survival rate of *E. bicallosa* at 1, 2, and 3 months after colchicine treatment (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	living protocorm	total protocorms
3	0.05	1	29	30
3	0.05	2	29	30
3	0.05	3	29	30
3	0.05	4	29	30
3	0.05	5	29	30
3	0.05	6	29	30
3	0.1	1	26	30
3	0.1	2	25	30
3	0.1	3	29	30
3	0.1	4	26	30
3	0.1	5	26	30
3	0.1	6	27	30

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa.*

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
1	0	1	28	2	0	0
1	0	2	29	0	0	0
1	0	3	27	0	0	0
1	0	4	30	0	0	0
1	0	5	29	0	0	0
1	0	6	27	0	0	0
1	0.025	1	30	0	0	0
1	0.025	2	27	2	1	0
1	0.025	3	22	6	2	0
1	0.025	4	30	0	0	0
1	0.025	5	26	4	0	0

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa* (Continued).

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
1	0.025	6	20	9	1	0
1	0.05	1	20	9	0	0
1	0.05	2	20	9	0	0
1	0.05	3	27	2	1	0
1	0.05	4	29	0	0	0
1	0.05	5	26	3	0	0
1	0.05	6	27	2	1	0
1	0.1	1	23	3	0	0
1	0.1	2	24	2	0	0
1	0.1	3	30	0	0	0
1	0.1	4	26	0	0	0
1	0.1	5	26	0	0	0
1	0.1	6	30	0	0	0

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa* (Continued).

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
2	0	1	0	0	0	28
2	0	2	0	0	0	25
2	0	3	0	0	0	24
2	0	4	0	0	0	27
2	0	5	25	1	0	0
2	0	6	23	2	0	0
2	0.025	1	0	0	0	30
2	0.025	2	0	0	0	30
2	0.025	3	0	0	0	30
2	0.025	4	0	0	0	30
2	0.025	5	0	0	0	30
2	0.025	6	0	0	0	30

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa* (Continued).

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
2	0.05	1	0	0	0	29
2	0.05	2	0	0	0	29
2	0.05	3	0	0	0	29
2	0.05	4	2	0	0	27
2	0.05	5	0	0	0	29
2	0.05	6	0	0	0	29
2	0.1	1	0	0	0	26
2	0.1	2	0	0	0	26
2	0.1	3	0	0	0	29
2	0.1	4	1	0	0	25
2	0.1	5	0	0	0	26
2	0.1	6	2	0	0	25

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa* (Continued).

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
3	0	1	0	0	0	28
3	0	2	0	0	0	25
3	0	3	0	0	0	24
3	0	4	0	0	0	26
3	0	5	0	0	0	16
3	0	6	0	0	0	20
3	0.025	1	0	0	0	30
3	0.025	2	0	0	0	30
3	0.025	3	0	0	0	30
3	0.025	4	0	0	0	30
3	0.025	5	0	0	0	30
3	0.025	6	0	0	0	30

Table 6.5 Effect of colchicine treatment on protocorm developmental stages of *E. bicalossa* (Continued).

month (after colchicine treatment)	colchicine concentration (%)	Replicate	stage 3 protocorm	stage 4 protocorm	stage 5 protocorm	rhizome-like bodies protocorm
3	0.05	1	0	0	0	29
3	0.05	2	0	0	0	29
3	0.05	3	0	0	0	29
3	0.05	4	0	0	0	29
3	0.05	5	0	0	0	29
3	0.05	6	0	0	0	29
3	0.1	1	0	0	0	26
3	0.1	2	0	0	0	25
3	0.1	3	0	0	0	29
3	0.1	4	0	0	0	26
3	0.1	5	0	0	0	26
3	0.1	6	0	0	0	27

Table 6.6 influence of colchicine treatment on shoot induction.

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
1	0	1	0	0	0	0
1	0	2	0	0	0	0
1	0	3	0	0	0	0
1	0	4	0	0	0	0
1	0	5	0	0	0	0
1	0	6	0	0	0	0
1	0.025	1	0	0	0	0
1	0.025	2	4	0	0	0
1	0.025	3	6	0	0	0
1	0.025	4	0	0	0	0
1	0.025	5	4	0	0	0
1	0.025	6	6	0	0	0

Table 6.6 influence of colchicine treatment on shoot induction (Continued).

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
1	0.05	1	0	0	0	0
1	0.05	2	2	1	0	0
1	0.05	3	2	0	0	0
1	0.05	4	0	0	0	0
1	0.05	5	2	1	0	0
1	0.05	6	2	0	0	0
1	0.1	1	0	0	0	0
1	0.1	2	0	0	0	0
1	0.1	3	0	0	0	0
1	0.1	4	0	0	0	0
1	0.1	5	0	0	0	0
1	0.1	6	0	0	0	0

Table 6.6 influence of colchicine treatment on shoot induction (Continued).

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
2	0	1	0	0	0	0
2	0	2	0	0	0	0
2	0	3	0	0	0	0
2	0	4	0	0	0	0
2	0	5	1	0	0	0
2	0	6	2	1	0	1
2	0.025	1	3	1	0	0
2	0.025	2	5	2	0	0
2	0.025	3	1	1	0	0
2	0.025	4	6	0	0	0
2	0.025	5	2	3	0	0
2	0.025	6	7	2	0	0

Table 6.6 influence of colchicine treatment on shoot induction (Continued).

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
2	0.05	1	1	1	0	0
2	0.05	2	0	1	0	0
2	0.05	3	2	0	0	0
2	0.05	4	7	2	0	0
2	0.05	5	3	0	1	0
2	0.05	6	5	0	1	0
2	0.1	1	0	0	0	0
2	0.1	2	0	0	0	0
2	0.1	3	1	0	0	0
2	0.1	4	6	0	0	0
2	0.1	5	0	0	0	0
2	0.1	6	0	0	0	0

Table 6.6 influence of colchicine treatment on shoot induction (Continued).

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
3	0	1	1	0	0	0
3	0	2	0	0	0	0
3	0	3	0	0	0	0
3	0	4	0	0	0	0
3	0	5	0	0	0	0
3	0	6	1	1	0	0
3	0.025	1	6	3	0	0
3	0.025	2	8	1	0	0
3	0.025	3	1	1	0	0
3	0.025	4	11	0	0	0
3	0.025	5	8	2	0	0
3	0.025	6	10	3	0	0

Table 6.6 influence of colchicine treatment on shoot induction (Continued).

month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 shoot	Plant with 2 shoots	Plant with 3 shoots	Plant with 4 shoots
3	0.05	1	3	2	0	0
3	0.05	2	13	0	0	0
3	0.05	3	5	2	1	0
3	0.05	4	17	1	2	0
3	0.05	5	8	0	0	0
3	0.05	6	8	1	1	0
3	0.1	1	2	0	0	0
3	0.1	2	4	1	0	0
3	0.1	3	2	0	0	0
3	0.1	4	6	3	0	0
3	0.1	5	5	1	0	0
3	0.1	6	6	0	0	0

Table 6.7 influence of colchicine treatment on root induction.

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
1	0	1	0	0	0	0
1	0	2	0	0	0	0
1	0	3	0	0	0	0
1	0	4	0	0	0	0
1	0	5	0	0	0	0
1	0	6	0	0	0	0
1	0.025	1	0	0	0	0
1	0.025	2	0	0	0	0
1	0.025	3	1	0	0	0
1	0.025	4	0	0	0	0
1	0.025	5	0	0	0	0
1	0.025	6	1	0	0	0

Table 6.7 influence of colchicine treatment on root induction (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
1	0.05	1	0	0	0	0
1	0.05	2	0	0	0	0
1	0.05	3	1	0	0	0
1	0.05	4	0	0	0	0
1	0.05	5	0	0	0	0
1	0.05	6	0	0	0	0
1	0.1	1	0	0	0	0
1	0.1	2	0	0	0	0
1	0.1	3	0	0	0	0
1	0.1	4	0	0	0	0
1	0.1	5	0	0	0	0
1	0.1	6	0	0	0	0

Table 6.7 influence of colchicine treatment on root induction (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
2	0	1	0	0	0	0
2	0	2	0	0	0	0
2	0	3	0	0	0	0
2	0	4	0	0	0	0
2	0	5	0	0	0	0
2	0	6	0	0	0	0
2	0.025	1	4	1	0	0
2	0.025	2	0	2	0	0
2	0.025	3	0	0	0	0
2	0.025	4	1	5	0	0
2	0.025	5	3	3	1	0
2	0.025	6	3	0	1	1

Table 6.7 influence of colchicine treatment on root induction (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
2	0.05	1	0	2	0	0
2	0.05	2	2	2	0	0
2	0.05	3	3	0	0	0
2	0.05	4	4	1	0	0
2	0.05	5	3	1	0	0
2	0.05	6	4	3	1	0
2	0.1	1	0	0	0	0
2	0.1	2	0	0	0	0
2	0.1	3	1	0	0	0
2	0.1	4	3	1	0	0
2	0.1	5	3	0	0	0
2	0.1	6	3	0	0	0

Table 6.7 influence of colchicine treatment on root induction (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
3	0	1	0	0	0	0
3	0	2	0	0	0	0
3	0	3	0	0	0	0
3	0	4	0	0	0	0
3	0	5	0	0	0	0
3	0	6	1	0	0	0
3	0.025	1	2	2	0	1
3	0.025	2	1	3	1	1
3	0.025	3	2	0	0	0
3	0.025	4	3	2	0	0
3	0.025	5	3	2	0	0
3	0.025	6	6	4	0	0

Table 6.7 influence of colchicine treatment on root induction (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant with 1 root	Plant with 2 roots	Plant with 3 roots	Plant with 4 roots
3	0.05	1	3	2	0	0
3	0.05	2	3	2	0	0
3	0.05	3	3	2	0	0
3	0.05	4	5	2	1	0
3	0.05	5	6	2	0	0
3	0.05	6	4	2	4	0
3	0.1	1	3	0	0	0
3	0.1	2	2	1	0	0
3	0.1	3	1	1	0	0
3	0.1	4	4	5	2	0
3	0.1	5	4	2	0	0
3	0.1	6	3	2	0	0

Table 6.8 Various shoot length due to the effect of colchicine.

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
1	0	1	no shoot	no shoot
1	0	2	no shoot	no shoot
1	0	3	no shoot	no shoot
1	0	4	no shoot	no shoot
1	0	5	no shoot	no shoot
1	0	6	no shoot	no shoot
1	0.025	1	no shoot	no shoot
1	0.025	2	1	0.3
1	0.025	2	2	0.2
1	0.025	2	3	0.2
1	0.025	2	4	0.2
1	0.025	3	1	0.7
1	0.025	3	2	0.2
1	0.025	3	3	0.4
1	0.025	3	4	0.3
1	0.025	3	5	0.2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
1	0.025	3	6	0.2
1	0.025	4	no shoot	no shoot
1	0.025	5	1	0.3
1	0.025	5	2	0.2
1	0.025	5	3	0.2
1	0.025	5	4	0.2
1	0.025	6	1	0.7
1	0.025	6	2	0.2
1	0.025	6	3	0.4
1	0.025	6	4	0.3
1	0.025	6	5	0.2
1	0.025	6	6	0.2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
1	0.05	1	no shoot	no shoot
1	0.05	2	1	0.4
1	0.05	2	2	0.3
1	0.05	2	3	0.3
1	0.05	3	1	0.7
1	0.05	3	2	0.3
1	0.05	4	no shoot	no shoot
1	0.05	5	1	0.4
1	0.05	5	2	0.3
1	0.05	5	3	0.3
1	0.05	6	1	0.7
1	0.05	6	2	0.3

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
1	0.1	1	no shoot	no shoot
1	0.1	2	no shoot	no shoot
1	0.1	3	no shoot	no shoot
1	0.1	4	no shoot	no shoot
1	0.1	5	no shoot	no shoot
1	0.1	6	no shoot	no shoot
2	0	1	no shoot	no shoot
2	0	2	no shoot	no shoot
2	0	3	no shoot	no shoot
2	0	4	no shoot	no shoot
2	0	5	1	0.7
2	0	6	1	1
2	0	6	2	1
2	0	6	3	1
2	0	6	4	9

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
2	0.025	1	1	1.8
2	0.025	1	2	3.46
2	0.025	1	3	1.21
2	0.025	1	4	0.71
2	0.025	2	1	2.4
2	0.025	2	2	2.06
2	0.025	2	3	1.28
2	0.025	2	4	1.14
2	0.025	2	5	0.82
2	0.025	2	6	1.21
2	0.025	2	7	0.51
2	0.025	3	1	0.86
2	0.025	3	2	1.39
2	0.025	4	1	1
2	0.025	4	2	1
2	0.025	4	3	1

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
2	0.025	4	4	0.5
2	0.025	4	5	0.2
2	0.025	4	6	0.2
2	0.025	5	1	0.5
2	0.025	5	2	0.5
2	0.025	5	3	2.5
2	0.025	5	4	2.5
2	0.025	5	5	0.3
2	0.025	6	1	1.5
2	0.025	6	2	4
2	0.025	6	3	1
2	0.025	6	4	2
2	0.025	6	5	5
2	0.025	6	6	5
2	0.025	6	7	1.5
2	0.025	6	8	1.2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
2	0.025	6	9	5
2	0.05	1	1	1.44
2	0.05	1	2	0.76
2	0.05	2	1	2.07
2	0.05	2	2	4.37
2	0.05	2	3	1.58
2	0.05	2	4	2.07
2	0.05	3	1	1.72
2	0.05	3	2	0.64
2	0.05	4	1	1.5
2	0.05	4	2	0.2
2	0.05	4	3	0.2
2	0.05	4	4	1
2	0.05	4	5	1
2	0.05	4	6	0.5
2	0.05	4	7	0.5

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
2	0.05	4	8	0.5
2	0.05	4	9	0.5
2	0.05	5	1	4
2	0.05	5	2	1.5
2	0.05	5	3	1.5
2	0.05	5	4	3
2	0.05	6	1	9
2	0.05	6	2	2
2	0.05	6	3	2
2	0.05	6	4	2
2	0.05	6	5	4
2	0.05	6	6	2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
2	0.1	1	no shoot	no shoot
2	0.1	2	no shoot	no shoot
2	0.1	3	1	1.51
2	0.1	4	1	1
2	0.1	4	2	0.5
2	0.1	4	3	0.5
2	0.1	4	4	0.5
2	0.1	4	5	0.5
2	0.1	4	6	0.3
2	0.1	5	no shoot	no shoot
2	0.1	6	no shoot	no shoot

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0	1	1	1.25
3	0	2	no shoot	no shoot
3	0	3	no shoot	no shoot
3	0	4	no shoot	no shoot
3	0	5	no shoot	no shoot
3	0	6	1	9
3	0	6	2	5
3	0.025	1	1	2.51
3	0.025	1	2	5.82
3	0.025	1	3	2.8
3	0.025	1	4	4.84
3	0.025	1	5	5.66
3	0.025	1	6	5.68
3	0.025	1	7	4.84
3	0.025	1	8	4.86
3	0.025	1	9	1.28

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.025	2	1	4.17
3	0.025	2	2	6.62
3	0.025	2	3	8.09
3	0.025	2	4	5.07
3	0.025	2	5	6.12
3	0.025	2	6	3.48
3	0.025	2	7	1.61
3	0.025	2	8	4.35
3	0.025	2	9	3.94
3	0.025	3	1	5.02
3	0.025	3	2	1.08
3	0.025	4	1	4
3	0.025	4	2	0.5
3	0.025	4	3	2
3	0.025	4	4	2.5
3	0.025	4	5	7

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.025	4	6	1
3	0.025	4	7	4
3	0.025	4	8	1
3	0.025	4	9	1
3	0.025	4	10	0.5
3	0.025	4	11	1
3	0.025	5	1	6
3	0.025	5	2	9
3	0.025	5	3	3
3	0.025	5	4	4
3	0.025	5	5	7
3	0.025	5	6	6
3	0.025	5	7	9
3	0.025	5	8	2
3	0.025	5	9	3
3	0.025	5	10	2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.025	5	11	2
3	0.025	6	1	9
3	0.025	6	2	5
3	0.025	6	3	3
3	0.025	6	4	2
3	0.025	6	5	5
3	0.025	6	6	7
3	0.025	6	7	3
3	0.025	6	8	4
3	0.025	6	9	9
3	0.025	6	10	3
3	0.025	6	11	3
3	0.025	6	12	6
3	0.025	6	13	2

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.05	1	1	4.62
3	0.05	1	2	3.98
3	0.05	1	3	2.48
3	0.05	1	4	1.74
3	0.05	1	5	1.46
3	0.05	2	1	5.98
3	0.05	2	2	2.46
3	0.05	2	3	0.94
3	0.05	2	4	4.38
3	0.05	2	5	1.77
3	0.05	2	6	7.42
3	0.05	2	7	4.56
3	0.05	2	8	8.14
3	0.05	2	9	8.14
3	0.05	2	10	3.48
3	0.05	2	11	2.46

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.05	2	12	1.78
3	0.05	2	13	1.63
3	0.05	3	1	5.33
3	0.05	3	2	10.05
3	0.05	3	3	5.07
3	0.05	3	4	3.15
3	0.05	3	5	4.46
3	0.05	3	6	7.65
3	0.05	3	7	3.64
3	0.05	3	8	1.85
3	0.05	4	1	5
3	0.05	4	2	1
3	0.05	4	3	5
3	0.05	4	4	3
3	0.05	4	5	1
3	0.05	4	6	5

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.05	4	7	2
3	0.05	4	8	2
3	0.05	4	9	0
3	0.05	4	10	5
3	0.05	4	11	8
3	0.05	4	12	1
3	0.05	4	13	2
3	0.05	4	14	5
3	0.05	4	15	3
3	0.05	4	16	5
3	0.05	4	17	9
3	0.05	4	18	8
3	0.05	4	19	3
3	0.05	4	20	3

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.05	5	1	3
3	0.05	5	2	3
3	0.05	5	3	5
3	0.05	5	4	4
3	0.05	5	5	3
3	0.05	5	6	4
3	0.05	5	7	3
3	0.05	5	8	2
3	0.05	6	1	9
3	0.05	6	2	5
3	0.05	6	3	9
3	0.05	6	4	3
3	0.05	6	5	5
3	0.05	6	6	5
3	0.05	6	7	9
3	0.05	6	8	3

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.05	6	9	6
3	0.05	6	10	2
3	0.1	1	1	2.21
3	0.1	1	2	7.12
3	0.1	2	1	1.21
3	0.1	2	2	1.84
3	0.1	2	3	2
3	0.1	2	4	0.61
3	0.1	2	5	1.74
3	0.1	3	1	0.93
3	0.1	3	2	0.43
3	0.1	4	1	7
3	0.1	4	2	2
3	0.1	4	3	4
3	0.1	4	4	4
3	0.1	4	5	4

Table 6.8 Various shoot length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine concentration (%)	Replicate	Plant number	Length of the longest shoot
3	0.1	4	6	6
3	0.1	4	7	3
3	0.1	4	8	3
3	0.1	4	9	2
3	0.1	5	1	2
3	0.1	5	2	1
3	0.1	5	3	1
3	0.1	5	4	1
3	0.1	5	5	0.5
3	0.1	5	6	0.5
3	0.1	6	1	1.5
3	0.1	6	2	1.5
3	0.1	6	3	6
3	0.1	6	4	3
3	0.1	6	5	1
3	0.1	6	6	4

Table 6.9 Various root length due to the effect of colchicine.

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
1	0	1	no root	no root
1	0	2	no root	no root
1	0	3	no root	no root
1	0	4	no root	no root
1	0	5	no root	no root
1	0	6	no root	no root
1	0.025	1	no root	no root
1	0.025	2	no root	no root
1	0.025	3	1	1.1
1	0.025	4	no root	no root
1	0.025	5	no root	no root
1	0.025	6	1	1.1

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
1	0.05	1	no root	no root
1	0.05	2	no root	no root
1	0.05	3	1	0.2
1	0.05	4	no root	no root
1	0.05	5	no root	no root
1	0.05	6	1	0.2
1	0.1	1	no root	no root
1	0.1	2	no root	no root
1	0.1	3	no root	no root
1	0.1	4	no root	no root
1	0.1	5	no root	no root
1	0.1	6	no root	no root

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
2	0	1	no root	no root
2	0	2	no root	no root
2	0	3	no root	no root
2	0	4	no root	no root
2	0	5	no root	no root
2	0	6	no root	no root
2	0.025	1	1	0.73
2	0.025	1	2	0.76
2	0.025	1	3	0.68
2	0.025	1	4	0.57
2	0.025	1	5	0.49
2	0.025	2	1	1.54
2	0.025	2	2	1.13
2	0.025	3	no root	no root

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
2	0.025	4	1	1
2	0.025	4	2	1
2	0.025	4	3	1
2	0.025	4	4	0.5
2	0.025	4	5	0.5
2	0.025	4	6	0.5
2	0.025	5	1	0.5
2	0.025	5	2	0.5
2	0.025	5	3	1
2	0.025	5	4	0.5
2	0.025	5	5	0.5
2	0.025	5	6	0.5
2	0.025	5	7	1

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
2	0.025	6	1	1.5
2	0.025	6	2	1
2	0.025	6	3	1
2	0.025	6	4	1
2	0.025	6	5	1
2	0.05	1	1	0.65
2	0.05	2	1	0.82
2	0.05	2	2	0.81
2	0.05	2	3	1.03
2	0.05	2	4	0.93
2	0.05	3	1	1.16
2	0.05	3	2	0.73
2	0.05	3	3	0.53

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
2	0.05	4	1	1
2	0.05	4	2	2
2	0.05	4	3	1
2	0.05	4	4	1
2	0.05	4	5	1
2	0.05	5	1	0.4
2	0.05	5	2	0.2
2	0.05	5	3	0.2
2	0.05	5	4	0.3
2	0.05	6	1	1.2
2	0.05	6	2	0.5
2	0.05	6	3	1
2	0.05	6	4	1
2	0.05	6	5	1
2	0.05	6	6	1
2	0.05	6	7	1

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
2	0.05	6	8	1
2	0.1	1	no shoot	no shoot
2	0.1	2	no shoot	no shoot
2	0.1	3	1	0.47
2	0.1	4	1	1
2	0.1	4	1	0.3
2	0.1	4	2	1
2	0.1	4	3	0.5
2	0.1	5	1	0.3
2	0.1	5	2	0.3
2	0.1	5	3	0.3
2	0.1	6	1	0.3
2	0.1	6	2	0.3
2	0.1	6	3	0.3

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0	1	no root	no root
3	0	2	no root	no root
3	0	3	no root	no root
3	0	4	no root	no root
3	0	5	no root	no root
3	0	6	1	0.5
3	0	6	2	1
3	0.025	1	1	1.34
3	0.025	1	2	1.19
3	0.025	1	3	1.38
3	0.025	1	4	0.56
3	0.025	1	5	0.68

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.025	2	1	1.29
3	0.025	2	2	1.08
3	0.025	2	3	1.11
3	0.025	2	4	1.25
3	0.025	2	5	2.05
3	0.025	2	6	1.2
3	0.025	3	1	0.79
3	0.025	3	2	1.19
3	0.025	4	1	1.5
3	0.025	4	2	1.2
3	0.025	4	3	1.5
3	0.025	4	4	2
3	0.025	4	5	0.5

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.025	5	1	1
3	0.025	5	2	1
3	0.025	5	3	1
3	0.025	5	4	1.5
3	0.025	5	5	1
3	0.025	6	1	1
3	0.025	6	2	1
3	0.025	6	3	1
3	0.025	6	4	1
3	0.025	6	5	2
3	0.025	6	6	1.5
3	0.025	6	7	2
3	0.025	6	8	1.5
3	0.025	6	9	1
3	0.025	6	10	1

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.05	1	1	1.1
3	0.05	1	2	0.88
3	0.05	1	3	0.92
3	0.05	1	4	0.69
3	0.05	1	5	0.9
3	0.05	2	1	1.45
3	0.05	2	2	1
3	0.05	2	3	1.24
3	0.05	2	4	0.94
3	0.05	2	5	0.87
3	0.05	3	1	0.859
3	0.05	3	2	1.328
3	0.05	3	3	1.902
3	0.05	3	4	0.779
3	0.05	3	5	0.746

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.05	4	1	5
3	0.05	4	2	1.5
3	0.05	4	3	3
3	0.05	4	4	1.5
3	0.05	4	5	2
3	0.05	4	6	2
3	0.05	4	7	0.5
3	0.05	4	8	8
3	0.05	5	1	3
3	0.05	5	2	3
3	0.05	5	3	5
3	0.05	5	4	4
3	0.05	5	1	3
3	0.05	5	2	4
3	0.05	5	3	3
3	0.05	5	4	2

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.05	6	1	9
3	0.05	6	2	5
3	0.05	6	3	9
3	0.05	6	4	3
3	0.05	6	5	5
3	0.05	6	6	5
3	0.05	6	7	9
3	0.05	6	8	3
3	0.05	6	9	6
3	0.05	6	10	2
3	0.1	1	1	0.58
3	0.1	1	2	0.76
3	0.1	1	3	0.72

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.1	2	1	0.93
3	0.1	2	2	0.42
3	0.1	2	3	0.48
3	0.1	3	1	0.81
3	0.1	3	2	0.72
3	0.1	4	1	1
3	0.1	4	2	1
3	0.1	4	3	2
3	0.1	4	4	2.5
3	0.1	4	5	1
3	0.1	4	6	1
3	0.1	4	7	1
3	0.1	4	8	1
3	0.1	4	9	1.5
3	0.1	4	10	1.5
3	0.1	4	11	0.5

Table 6.9 Various root length due to the effect of colchicine (Continued).

Month (after colchicine treatment)	Colchicine Concentration (%)	Replicate	Plant number	Length of the longest root
3	0.1	5	1	1.5
3	0.1	5	2	0.5
3	0.1	5	3	0.5
3	0.1	5	4	1
3	0.1	5	5	1
3	0.1	5	6	0.5
3	0.1	6	1	3
3	0.1	6	2	0.5
3	0.1	6	3	1
3	0.1	6	4	1
3	0.1	6	5	0.5

APPENDIX D: *Eulophia* chromosomes

Table 6.10 Gametophyte chromosomes of *Eulophia* spp.

Species	Gametophyte chromosomes	Source
Eulophia aculeata subsp. huttonii (Rolfe) A. V. Hall	27	CCDB chromosome count data base
Eulophia angolensis (Rchb.) Summerh.	34	CCDB chromosome count data base
Eulophia angolensis (Rchb.) Summerh.	35	CCDB chromosome count data base
Eulophia angolensis (Rchb.) Summerh.	36	CCDB chromosome count data base
Eulophia angolensis (Rchb.) Summerh.	37	CCDB chromosome count data base
Eulophia angolensis (Rchb.) Summerh.	38	CCDB chromosome count data base
Eulophia campestris Wall.	24	IPCN chromosome report
Eulophia cristata (Afzel.) Steud.	23	CCDB chromosome count data base
Eulophia dabia (D. Don) Hochr.	24	CCDB chromosome count data base
Eulophia dabia (D. Don) Hochr.	27	CCDB chromosome count data base
Eulophia dentata Ames	27	CCDB chromosome count data base
Eulophia dentata Ames	28	CCDB chromosome count data base
Eulophia ensata Lindl.	27	CCDB chromosome count data base
Eulophia epidendraea (J. Koenig) C. E. C. Fisch.	27	CCDB chromosome count data base
Eulophia epidendraea C.E.C. Fisch.	27	IPCN chromosome report

Table 6.10 Gametophyte chromosomes of *Eulophia* spp. (Continued).

Species	Gametophyte chromosomes	Source
Eulophia euglossa (Rchb. f.) Rchb. f.	22	IPCN chromosome report
Eulophia euglossa (Rchb.) Rchb. ex Bateman	22	CCDB chromosome count data base
Eulophia euglossa (Rchb.) Rchb. ex Bateman	20	CCDB chromosome count data base
Eulophia exaltata Rchb.	16	CCDB chromosome count data base
Eulophia foliosa (Lindl.) Bolus	27	CCDB chromosome count data base
Eulophia fridericii (Rchb.) A. V. Hall	24	CCDB chromosome count data base
Eulophia gracilis Lindl	22	PROTA4U
Eulophia graminea Lindl.	28	CCDB chromosome count data base
Eulophia graminea Lindl.	27	CCDB chromosome count data base
Eulophia graminea Lindl.	21	CCDB chromosome count data base
Eulophia graminea Lindl.	24	CCDB chromosome count data base
Eulophia graminea Lindl.	22	CCDB chromosome count data base
Eulophia graminea Lindl.	23	CCDB chromosome count data base
Eulophia graminea Lindl.	48	CCDB chromosome count data base
Eulophia hians Spreng.	50	CCDB chromosome count data base
Eulophia hians var. nutans (Sond.) S. Thomas	25	CCDB chromosome count data base
Eulophia hians var. nutans (Sond.) S. Thomas	47	CCDB chromosome count data base

Table 6.10 Gametophyte chromosomes of *Eulophia* spp. (Continued).

Species	Gametophyte chromosomes	Source
Eulophia hormusjii A.V. Duthie	27	IPCN chromosome report
Eulophia horsfallii (Bateman) Summerh.	31	CCDB chromosome count data base
Eulophia leachii Greatrex ex A. V. Hall	26	CCDB chromosome count data base
Eulophia leontoglossa Rchb.	27	CCDB chromosome count data base
Eulophia macowanii Rolfe	28	CCDB chromosome count data base
Eulophia macrostachya Lindl.	17	IPCN chromosome report
Eulophia nuda Lindl.	27	IPCN chromosome report
Eulophia nuda var. andersonii Hook. f.	28	IPCN chromosome report
Eulophia ochreata Lindl.	27	CCDB chromosome count data base
Eulophia ovalis Lindl.	21	CCDB chromosome count data base
Eulophia ovalis Lindl.	40	CCDB chromosome count data base
Eulophia ovalis var. bainesii (Rolfe) P. J. Cribb & L. Croix	42	CCDB chromosome count data base
Eulophia paniculata Rolfe	30	IPCN chromosome report
Eulophia parviflora (Lindl.) A. V. Hall	25	CCDB chromosome count data base
Eulophia petersii (Rchb.) Rchb.	24	CCDB chromosome count data base
Eulophia promensis Lindl.	19	CCDB chromosome count data base
Eulophia pulchra (Thouars) Lindl.	16	CCDB chromosome count data base

Table 6.10 Gametophyte chromosomes of *Eulophia* spp. (Continued).

Species	Gametophyte chromosomes	Source
Eulophia pulchra (Thouars) Lindl.	17	CCDB chromosome count data base
Eulophia R. Br. ex Lindl.	27	IPCN chromosome report
Eulophia ramentacea Lindl.	27	IPCN chromosome report
Eulophia speciosa (R. Br.) Bolus	20	CCDB chromosome count data base
Eulophia speciosa (R. Br.) Bolus	27	CCDB chromosome count data base
Eulophia spectabilis (Dennst.) Suresh	16	CCDB chromosome count data base
Eulophia spectabilis (Dennst.) Suresh	27	CCDB chromosome count data base
Eulophia spectabilis (Dennst.) Suresh	16	CCDB chromosome count data base
Eulophia streptopetala Lindl.	42	CCDB chromosome count data base
Eulophia streptopetala Lindl.	21	CCDB chromosome count data base
Eulophia streptopetala Lindl.	20	CCDB chromosome count data base
Eulophia tenella Rchb.	60	CCDB chromosome count data base
Eulophia tuberculata Bolus	50	CCDB chromosome count data base
Eulophia welwitschii (Rchb.) Rolfe	27	CCDB chromosome count data base
Eulophia zeyheriana Sond.	56	CCDB chromosome count data base
Eulophia zollingeri (Rchb.) J. J. Sm.	17	CCDB chromosome count data base