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RESEARCH ARTICLE

Effects of 6-Benzylaminopurine (BAP) Treatment on Seed Germination and Seedling Vigour of Endemic Herb *Exacum trinervium* L. in Sri Lanka: Conservation strategy

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Abstract: Based on the scientific information available, *E. trinervium* is currently of conservation concern since it shows poor seedling growth even under satisfactory seed germination. Ex-situ cultivation is therefore recommended as a conservation strategy. This study aimed to (i) investigate the effective pre-sowing 6-Benzylaminopurine (BAP) treatment that enhances the seed germination and seedling vigour and (ii) to study morphological traits that can be used in assessing healthy seedlings of *E. trinervium.* The experiment was conducted in a completely randomized design under both laboratory and green house conditions with BAP concentrations of 1.0, 2.0 and 3.0 mgL^{-1} as pre-sowing treatments and distilled water as the control with different exposure durations *i.e.* 18, 24 and 30 hours. The results showed that there was no significant effect of BAP treatments on seed germination percentage under laboratory and nursery conditions. The lowest average mean germination time (MGT) was obtained 24 h exposure duration for the green house condition. Seedling dry weight and seedling Vigour Index (SVI) were significantly ($P<0.05$) higher for BAP concentration of 3.0 mgL^{-1} under 24 h exposure duration. Growth parameters i.e. number of leaves, plant height, root length, number of primary roots, dry weight and fresh weight of *E. trinervium* were significantly ($P<0.05$) induced by BAP concentration and exposure duration. Accordingly, BAP concentration of 3.0 mgL^{-1} under 24 h exposure duration proved to be the optimum BAP treatment for the increase of seedling vigour of *E. trinervium* under the green house conditions. Therefore, the present study suggests that BAP treatment may be involved metabolically in the stimulation of germination and then to increase the seedling vigour of *E. trinervium.*

Keywords: *Exacum trinervium*, BAP, mean germination time, seedling vigour index

Introduction

Exacum trinervium is a slender wild herbaceous plant which is considered as a threatened endemic medicinal herb (Jayaweera, 1981; IUCN, 1999) in Sri Lanka. It is locally known as "Binara or Ginihiriya". The herb grows about 1m in height at an altitude up to 2000m. The entire herb is used as a tonic for curing of mild fever (Jayaweera, 1981). The distribution of this species is confined to wet zones of low-country and up-country in Sri Lanka (Dassanayake, 1999). Further, the distribution is restricted on account of the destruction and conversion of its natural habitats and may also be due to climatic factors. This eventually results in the occurrence of a very small population of this endemic plant in Sri Lanka. Blue flowers with brilliant yellow anthers and herbaceous nature gave this plant an important horticultural value. The flowering generally commences in September and continues to bloom for several months (Dassanayake, 1999). Therefore, a group of scientists in Royal Botanic Garden, Peradeniya, Sri Lanka, has prioritized

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the efforts for conservation of *E. trinervium* through ex-situ cultivation and then this was introduced into floriculture industry as an ornamental plant (Krishnarajah, et al., 2002). Based on the early trials carried out by Royal botanical garden at Peradeniya, it has been shown that the main challenge in accomplishing this purpose was to enhance seed germination and develop vigorous seedlings as it showed poor germination (less than 30%) and weak seedlings at initial testing. Pre-sowing BAP treatment is in the common practice in enhancing seed germination and increasing seedling vigour for instance in *Amaranthus cruentus* L. (Tiryaki, et al., 2009). Literature evidently illustrates that the attempts made to improve seed germination and enhance quality of *E. trinervium* is scanty. To the best of our knowledge, this was the first investigation on the impact of the pre-sowing BAP treatment on seed germination and seedling vigour of *E. trinervium*. Therefore, the main objectives of the present study were to develop an effective pre-sowing BAP treatment to enhance the seed germination and seedling vigour as well as to identify morphological traits for the assessment of healthy seedlings of *E. trinervium*.

Materials and methods

Germination test for laboratory verification

Seeds of *E. trinervium* were collected from wild plants maintained in the green house of Royal Botanic Garden, Peradeniya. Twenty dried seeds were used to determine average dry weight and mean length. Healthy seeds i.e. not shrunken, free of insect damages were used for all the treatments. For germination tests air-dried seeds were washed thoroughly with double distilled water (DW), and dipped in different pre-chilled (40C) BAP pre-sowing treatment solutions (0, 1.0, 2.0 and 3.0 mgL-1), for different time durations (18, 24 and 30 h). Control was maintained using distilled water. Treated seeds were washed two-three times with distilled water and placed in plastic plates lined with Qualigens (6150A) filter paper. Three replicates per treatment with 20 seeds in each replicate were used and they were distributed according to a completely randomized design and enclosed in a polythene propagator (16 in width, 24 in height, 200 gauges) under laboratory conditions (average minimum and maximum temperatures were 270C, 370C respectively) and monitored daily. The filter papers were moistened daily with DW. Seeds were observed after three days and five days after sowing to count germinated seeds and seed germinating stage was considered upon their radical emergence. The Mean Germination Time (MGT) was calculated by using the relation: $MGT = \sum(fx)/\sum x$, where x is the number of newly germinated seeds on each day, and f is the number of days after seeds were set to germinate (Butola & Badola, 2004).

Nursery Condition

As the germination tests in the laboratory verified that seed lot is healthy and no negative effect of BAP on seed germination, the same lot of seeds was used for the nursery test. Nursery pots containing equal volumes of well drained sterilized river sand were moistened with equal amounts of water and allowed to drain to field capacity. The replicates that were distributed according to a completely randomized design and were treated with different concentration of BAP for different exposure times in the same manner described in germination test. Each sample of treated seeds was then surface sown (distance between seeds 0.5 cm) in nursery pots (11 cm diameter, 9 cm height) and then each pot was enclosed in a polythene propagator (8 in width, 14 in height, 200 gauges) and equal watering was done in every-other day. Instead of BAP, distilled water was used in the control experiment. After 12 weeks, seedling growth was assessed by harvesting all individuals under each treatment and different growth parameters i.e. fresh weight (g/plant), root length (cm/plant), number of leaves per plant and dry weights (g) of seedlings were determined. The dry weight of seedlings was obtained by drying seedlings at 700C to a constant weight. A method was developed to determine the Seedling Vigour Index (SVI) using dry matter accumulation and MGT as below (Butola & Badola, 2004).

$$SVI = (\text{Dry weight per seedling}/MGT)*100$$

Data were analyzed with the help of MINITAB 14 statistical software. Correlation coefficient was determined by pooling data from all the treatments and the relationship amongst different seedling traits was examined.

Results

Mean dry weight and mean length of *Exacum trinervium ssp. trinervium* seeds were 0.5 mg and 0.3 mm respectively. None of the three concentrations of BAP used in this experiment as well as none of the exposure periods showed a significant effect ($P>0.05$) on the germination of seeds. Further among the nine laboratory and nursery treatments, the effect of BAP concentration and exposure duration were not significant ($P>0.05$) on seed germination percentage over the control (Table 1 & 2). However, for laboratory experiment, the highest germination

percentage i.e. 100% was obtained for 1.0 mgL-1 at 24h and 30h exposure times (Table 1) while for nursery condition, the highest germination percentage i.e. 98.33% was obtained for the control with 18h exposure time. The first germination was observed after five days of sowing and that was in the control after 24h and 1 mgL-1 after 30 h of exposure. In contrast to the expectation mean germination time (MGT) of *E. trinervium* was significantly reduced ($P<0.05$) in control with 30h exposure duration in laboratory condition (Table 1). Although the effect of exposure duration had no significant effect on MGT under nursery conditions (Table 2), 24h exposure duration reduced mean germination time under nursery conditions. Untreated seeds started germinating only after the other treatments reached the 95% level of germination (Table 2).

Number of leaves, plant height, root length and number of primary roots were considered as the growth parameters. The effect of BAP concentration and exposure duration significantly increased ($P<0.05$) the plant height, root length, number of primary roots and fresh weight (Table 3). BAP concentration and exposure duration showed significant effect ($P<0.05$) on fresh weight, dry weight and hence on SVI. BAP treatments of 2 mgL-1 and 3 mgL-1 with 24h exposure had shown significantly higher ($P<0.05$) SVI and dry weights (Table 4). The highest SVI was obtained for 3 mgL-1 of BAP for 24 h exposure while the lowest was for 1 mgL-1 / 30 h (Figure 1).

Table 1. Effect of pre-sowing BAP treatment on seed germination under laboratory condition

BAP Concentration/ mgL-1	Time Duration/ hours	Germination% (Mean ± SD)	Mean Germination Time (Mean ± SD)
Control (Distilled water)	18	91.67a ± 7.64	3.977a ± 0.086
	24	98.33a ± 2.89	3.356abc ± 0.051
	30	96.67a ± 2.89	3.105ab ± 0.105
1	18	96.67a ± 2.89	4.070ab ± 0.061
	24	100.00a ± 0.00	3.567ab ± 0.419
	30	100.00a ± 0.00	3.696ab ± 0.095
2	18	98.33a ± 2.89	4.471abd ± 0.288
	24	96.67a ± 5.77	4.243abc ± 0.439
	30	96.67a ± 2.89	3.943ab ± 0.685
3	18	90.00a ±13.23	4.740abd ± 0.608
	24	96.67a ± 5.77	4.087ab ± 0.326
	30	96.97a ± 5.77	4.000ab ± 0.250
P -value		A = 0.373 B = 0.218 A × B = 0.826	A = 0.000 B = 0.000 A × B = 0.804
α-value		0.05	0.05

Bold values correspond to treatments with the highest germination percentage and lowest mean germination time. Values followed by the same letters are not significantly different, according to TSRT at $P \leq 0.05$.

Table 2. Effect of pre-sowing BAP treatment on seed germination under green house condition

(A.)BAP Concentration/ mgL-1	(B.)Time Duration/ hours	Germination% (Mean ± SD)	Days taken for 1st seed germination (Mean ± SD)	Mean Germination Time (Mean ± SD)
Untreated seeds	-	80.00b ± 3.16	7.000b ± 0.0000	14.000b ± 0.000
Control (Distilled water)	18	98.33a ± 2.58	5.167a ± 0.4082	5.974a ± 0.224
	24	95.00a ± 3.16	5.000a ± 0.0000	5.833a ± 0.224
	30	92.50a ± 11.73	5.167a ± 0.4082	5.894a ± 0.316
1	18	86.67a ±6.83	5.500a ± 0.5477	6.203a ± 0.354
	24	95.00a ± 6.32	5.167a ± 0.4082	5.848a ± 0.221
	30	96.67a ± 8.16	5.000a ± 0.0000	5.771a ± 0.343
2	18	93.33a ± 5.16	5.833a ± 0.4082	6.285a ± 0.143
	24	90.83a ± 5.85	5.333a ± 0.5164	6.010a ± 0.389
	30	92.50a ± 8.22	5.167a ± 0.4082	6.005a ± 0.317
3	18	94.17a ± 9.70	5.167a ± 0.4082	6.257a ± 0.175
	24	89.17a ± 20.10	5.167a ± 0.4082	5.819a ± 0.491
	30	85.83a ± 25.58	5.333a ± 0.8165	6.222a ± 0.408
P-value		A = 0.552 B = 0.932 A × B = 0.552	A = 0.165 B = 0.091 A × B = 0.317	A = 0.128 B = 0.005 A × B = 0.466
α-value		0.05	0.05	0.05

Bold values correspond to treatments with the highest germination percentage, lowest days for 1st seed germination and lowest mean germination time. Values followed by the same letters are not significantly different, according to TSRT at $P \leq 0.05$

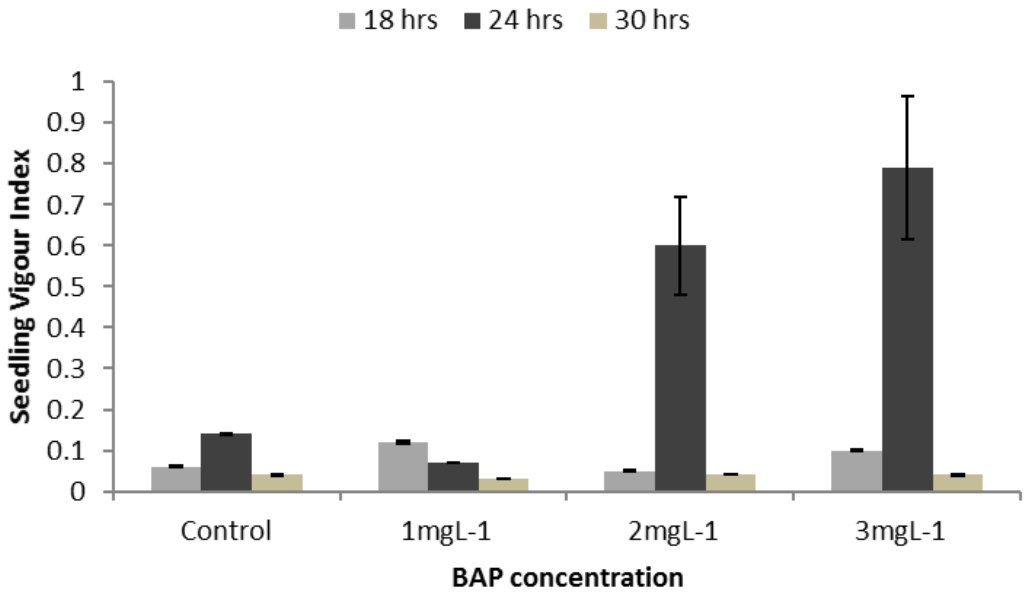

Figure 1. Effect of pre-sowing BAP concentration and time duration on seedling vigour index under green house condition (mean value ± SD).

Table 3. Effect of pre-sowing BAP treatment on seedling growth of *E.trinervium* under greenhouse condition

(A.)BAP/ mgL-1	(B.)Time Duration/ hours	No of leaves (Mean ±SD)	Plant height (Mean ±SD)	Root length (Mean ±SD)	No. of Iry roots (Mean ±SD)	Fresh weight per seedling (Mean ±SD)
Control	18	10.053± 1.404	3.255 ± 0.739	1.9719 ± 0.4678	12.757 ± 2.662	0.040478 ± 0.002689
	24	9.450 ± 1.630	3.155 ± 1.344	2.4443 ± 0.5959	11.212 ± 3.367	0.019692 ± 0.008573
	30	8.093± 1.464	2.337 ± 1.058	2.4574 ± 0.6634	10.781 ± 2.506	0.011937 ± 0.005167
1	18	8.488± 1.493	2.046± 0.880	2.3897 ± 0.5539	12.031 ± 3.968	0.064400 ± 0.009463
	24	7.926± 1.280	2.068± 0.603	2.5218 ± 0.6843	9.550 ± 1.492	0.018489 ± 0.003376
	30	6.873± 1.639	1.808± 1.140	2.1747 ± 0.4200	7.638 ± 1.932	0.046826 ± 0.012958
2	18	8.098± 1.331	2.199± 0.720	2.2180 ± 0.4522	9.818 ± 1.779	0.028863 ± 0.007828
	24	9.361± 1.792	3.385± 2.509	2.3530 ± 0.5389	10.582 ± 2.831	0.022227 ± 0.005983
	30	8.045± 1.445	2.220± 1.219	2.5948 ± 0.6631	9.065 ± 2.592	0.020528 ± 0.008723
3	18	9.190± 1.451	2.726± 1.339	2.3844 ± 0.6332	10.129 ± 2.967	0.018368 ± 0.013032
	24	8.351± 1.915	3.084± 2.578	2.2839 ± 0.5979	10.385 ± 3.196	0.045179 ± 0.018104
	30	8.234± 1.413	1.840 ± 0.455	2.2965 ± 0.7112	8.727 ± 2.732	0.032281 ± 0.013877
P-value		A = 0.000 B = 0.000 A × B = 0.000	A = 0.000 B = 0.000 A × B = 0.000	A = 0.232 B = 0.001 A × B = 0.000	A = 0.000 B = 0.000 A × B = 0.000	A = 0.000 B = 0.004 A × B = 0.000
α-value			0.05	0.05	0.05	0.05

Bold values correspond to treatments with the maximum number of leaves, plant height, root length, number of primary roots and fresh weight per seedling.

Table 4. Effect of pre-sowing BAP treatment on seedling growth of *E.trinervium* under green house condition

(A.)BAP Concentration/ mgL-1	(B.)Time Duration/ hours	Dry weight per seedling (Mean ±SD)	SVI (Mean ±SD)
0	18	0.004133a ± 0.000547	0.06820a ± 0.01108
	24	0.008500a ± 0.001262	0.14550a ± 0.01699
	30	0.002133a ± 0.000677	0.03612a ± 0.01400
1	18	0.007400a ± 0.002790	0.11850a ± 0.04243
	24	0.004233a ± 0.000137	0.07015a ± 0.00433
	30	0.001700a ± 0.000590	0.02970a ± 0.00872
2	18	0.002967a ± 0.000712	0.04683a ± 0.01042
	24	0.036767b ± 0.006519	0.62760b ± 0.12055
	30	0.002117a ± 0.001040	0.03552a ± 0.01837
3	18	0.006150a ± 0.001608	0.09837a ± 0.02421
	24	0.045917c ± 0.009700	0.81682c ± 0.18113
	30	0.002183a ± 0.000574	0.03553a ± 0.00915
P-value		A = 0.000 B = 0.000 A × B = 0.000	A = 0.000 B = 0.000 A × B = 0.000
α-value		0.05	0.05

Bold values correspond to treatments with the maximum dry weight per seedling and SVI Values followed by the same letters are not significantly different, according to TSRT at $P \leq 0.05$.

Discussion

The effects of various seed priming techniques i.e. hydro-priming, halo-priming, osmo-priming, thermo-priming, solid-matrix priming, etc. (Tiryaki, et al., 2009) depend upon plant species, stage of plant development, concentration/dose of priming agent and incubation period (Tzortzakis, 2009). In previous studies, it has been revealed that priming solution with BAP improved germination in seeds of *Amaranthus sp*. (Tiryaki, et al., 2009). In this study, sterilized river sand was used as the potting medium since *E. trinervium* can naturally be seen on exposed roadside embankment close to water (Cramer, 1981). Further, the potting medium was sterilized to avoid the contamination of soil-borne pathogens (Masterlarz, 1977). According to the results obtained, there were no significant effects of BAP concentration and exposure time on seed germination. Although not significant, it showed higher germination percentages under both laboratory and green house conditions. Therefore, it is obvious that BAP treatments did not negatively affected on seed germination. Moreover, the obtained results showed that MGT was reduced in some treatments for instance control/30 h and 3 mgL-1 /24 h. Therefore, it is suggested that these treatments may help early seed germination providing them a higher competitive ability (Zhang & Maun, 1990) and hence reducing the chances of mortality. In addition, results indicated that germination percentage and MGT under laboratory condition was different than that of nursery condition. That could be due to the difference of other factors such as environmental conditions and the potting medium used, etc. This study revealed that growth parameters significantly increased i.e. early seedling growth: number of leaves, plant height, root length and number of primary roots with the BAP treatment thereby that can increase seedling vigour of *E. trinervium*. This might be due to altered physiology of embryos and liberation of enzymes, so that the developmental processes occur more rapidly after sowing (Kattimani, et al., 1999). Interestingly, control with distilled water also shown a significant effect on some growth parameters such as number of leaves and number of primary roots, probably due to its similarity to hydro-priming treatment. BAP concentration and exposure time significantly affected number leaves and increasing number of leaves may increase the quality of an ornamental plant.

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Results revealed that the BAP concentration has a significant correlation with the seedling dry weight (r2 = 0.377), SVI (r2 = 0.377) and MGT (r2 = 0.249). According to the data, there was a significant correlation between dry weight per seedling and SVI (r2 = 0.997). Negative correlation was obtained for seedling dry weight with that of "days for first seed germination" (r2 = -0.020) and MGT (r2 = -0.149) under greenhouse condition. This indicates that when the "time duration spent for the seed germination" and MGT decrease, the seedling dry weight tend to increase. Thus, early germinated seedlings should be healthier and may be retained during thinning to mitigate competition (Butola & Badola, 2004). During the study period, no insect/pest attacks were observed. Pandey et al. (2000) reported that the BAP enhanced seed germination in A. hererophyllum an important medicinal herb of the Himalayan region. Therefore, the BAP treatment might be involved metabolically in the stimulation of germination and then to increase the seedling vigour of *E. trinervium*. According to the results in this experiment, treatment of 3 mgL-1 BAP concentration with 24 h exposure duration can be considered as the suitable treatment for obtaining *E. trinervium* seedlings with high vigour. Seedlings were harvested at three months of post-sowing. Further studies are required for alternative treatments (Optimizations of temperatures, substrates, etc., and/or combining of seed priming techniques with using chemicals such as gibberalic acid, ethanol, KNO_3 and $NaHClO_3$ etc.) for obtaining more vigorous plants.

Conclusion

Based on the results BAP treatment at 3 mgL^{-1} concentration with 24h exposure duration can be recommended as the optimum BAP treatment for increasing seedling vigour of *Exacum trinervium* ssp *trinervium* under greenhouse condition. Among the early growth parameters, number of leaves, plant height, root length and number of primary roots can be used as morphological traits in assessing healthy, high vigour seedlings of *E. trinervium*.

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