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Seed polymorphism and effect of GA_3 on seed germination and seedling growth in *Eclipta alba*

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ABSTRACT A study on the seed morphology and influence of gibberellic acid (GA_3) on the germination (%) and seedling growth of *Eclipta alba* was conducted. Results revealed that seeds were polymorphic in nature. Three types of seeds, *viz.* black, dark brown and light brown were observed, which differed in their size, weight, viability and germination behaviour. Among these, black seeds showed maximum size, weight, viability, germination (%) and seedling growth. 5 mg l-1 concentration of GA_3 was most suitable for obtaining highest germination (%), shoot and root lengths, germination value (GV) and vigour index (VI) in fresh and 6 month old seeds.

Keywords: *Eclipta alba*, seed germination, polymorphism, germination value, seedling vigour, GA_3

Eclipta alba (Linn.) Hassk.is locally known as Bhrinaraj or Bhangra, belongs to family Asteraceae and a common weed in moist localities throughout India. Vagbhatta ascribed to the plant, the property of increasing longevity of a person [1]. The fresh herb is chiefly used as a tonic and deobstruent in enlargement of the liver and spleen and also in various chronic skin diseases. The plant is considered as an astringent and is also used as a cure for asthma, bronchitis and rheumatism [2]. It is also antimyotoxic and antihemorrhagic in nature [3]. Dry plant is used in viral hepatitis, asthma, memory disorders, minor cuts and burns, eye disease and plant juice in catarrhal jaundice [4].

Seed germination responses have a direct impact on a species distribution and abundance, since it is a key element affecting population dynamics, especially in semi-arid environments. The ecology and physiology of seed germination is fundamental for the growth and development of plants and is an essential basis for formation of most desirable means of determining the plant producing value of seeds. The main aim of a laboratory germination test is to estimate the maximum number of seeds, which can germinate is a optimum conditions. It under very important criteria to gain information with respect to the field planting values of a seed [5]. So, looking into the immense medicinal properties of *E. alba*, the present studies were aimed to find out the effect of GA_3 on seed germination, seedling growth, R/S ratio, GV and VI under controlled laboratory conditions for its large-scale multiplication.

MATERIALS AND METHODS

The seeds of *E. alba* were collected from natural habitats at three different sites, *viz.* Jat Hostel, Near Bhagat-Ki-Kothi Railway Station (site-I), Language Wing Campus (site-II), and Science Faculty Campus, JNV University, Jodhpur (site-III) during March-April 2004-05. The data on seed morphological parameters, *viz.* shape, size, colour, weight, volume and density were studied.

The shape of seeds was observed under dissecting microscope, while size (length and breadth) of 20 seeds with the help of Vernier Calipers. The weight of 100 seeds was taken with electronic balance. The volume of 100 seeds was calculated using water

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displacement method. Seed density was measured by using following formula:

$$\text{Seed density (g cc - 1)} = \frac{\text{Seed weight (g)}}{\text{Seed volume (cc)}}$$

Seeds collected from different sites exhibited polymorphism, but that of site-II showed maximum values of different seed parameters. Various seed parameters were taken in triplicate and confirmed twice. The seed viability was tested in fresh as well as 6 and 24 months stored seeds [6-7]. The seeds were stored in plastic containers with parad tablets to protect them from insects.

Seed germination behaviour was studied in fresh as well as 6 and 24 months stored seeds. They were presoaked for 24 h in different concentrations (0-50 mg l-1) of GA_3 and IAA. Thereafter, they were placed for germination studies under controlled laboratory conditions. Germination studies were performed in sterilized petridishes lined with single layer of filter paper moistened with distilled water from time to time. The experiments were carried out in alternate white light and dark conditions (12 h) obtained from 3 fluorescent tubes of 40 W each, fitted at a height of half meter from the petridishes (1000 lux) in seed germinator at 28°C temperature. Each petridish contained 10 seeds in triplicate and confirmed thrice.

The observations were recorded daily for a period of 10 days. After 10 days, the germination (%) and root & shoot lengths of seedlings were recorded. No germination was observed in IAA treatment, so the data for only GA_3 treatments are presented here in tabular form.

Germination value (GV) and vigour index (VI) of seedlings were calculated [8-9], respectively. The mean values of two years (2004 and 2005) for each parameter were analyzed [10] and presented in tabular form.

RESULTS AND DISCUSSION

Seed morphology

The data on various morphological parameters, *viz.* colour, weight, size, volume, density and viability of the seeds collected from different sites are given in Table 1. The seeds from different sites were light brown to black coloured. The seeds collected from site-II were heaviest and longest than other sites. Seeds of site-II having maximum (0.145) breadth, while seeds from site-III (0.133) were next to it. The values of seed volume at all sites were same, *i.e.* 0.04 CC. Seeds colleted from site-II showed the highest density, followed by site-III and lowest from site-I lot. The fresh as well as 24 months old seeds collected from different sites exhibited a cent percent viability. The data for seed weight

Table 1. Data on seed morphology, volume, density and viability in *E. alba* collected from sites I-III.

Sites	Colour	Morphological parameters					
		Weight of 100 seeds (g)	Size (cm)		Volume of 100 seeds (cc)	Density (g cc^{-1})	Viability
			Length	Breadth			
I	Light brown to black	0.031	0.225	0.115	0.04	0.775	100.00
II	-do-	0.035	0.268	0.145	0.04	0.875	100.00
III	-do-	0.032	0.258	0.133	0.04	0.800	100.00
CD		0.0027*	0.028**	NS	NS	NS	NS

NS= Non-significant; * & ** = Significant at P = 5 & 1%, respectively.

and length were significant at 5 and 1 percent probability levels, respectively. The maximum values for various morphological parameters were observed at site-II followed by site-III and minimum at site-I. So, the seeds collected from site-II were used for detailed germination studies.

Seed polymorphism

It is evident from Table 2 that the seeds collected from site-II were light brown, dark brown and black in colour. Significant differences were observed in their weight and size. The weight of 100 seeds ranged from 0.027 to 0.032 g. Black seeds had the maximum length (0.271 cm) and breadth (0.142 cm). Dark brown seeds showed maximum (0.05) volume. Light brown and black coloured seeds showed maximum density (0.695) with 90 and 100 percent viability, respectively. The data for seed weight and size were significant at 1 percent probability level, while non-significant for other parameters. Dimorphic and polymorphic seeds have been reported in a number of plant species such as *Evolvulus alsinoides* [11], *Commiphora wightii and Salvadora persica* [12] and *Suaeda fruticosa* and *Trianthema triquetra* etc. [13]. Owing to this variability in seeds, the occurrence of polymorphism can lead to better establishment of the plant species in varied ecological conditions, especially in desert and hence a preliminary step towards evolution [14].

Seed germination behaviour

It is evident from Table 3 that black seeds showed a cent percent germination and maximum root & shoot lengths and R/S ratio than other polymorphic seeds, when they were pre-soaked with 5 mg l-1 GA_3. Light and dark brown seeds showed 76.66 and 93.33 percent germination, respectively. The data were significant at 1% probability level, except for R/S ratio, which was non-significant.

Table 2. Colour, weight, size, volume, density and viability in polymorphic seeds of *E. alba* collected from site-II.

Sites	Colour	Morphological parameters				
		Weight of 100 seeds (g)	Size (cm)		Density of 100 seeds (cc)	Viability (g cc^{-1})
			Length	Breadth		
Light brown	0.027	0.206	0.115	0.04	0.695	90.00
Dark brown	0.030	0.268	0.137	0.05	0.612	100.00
Black	0.032	0.271	0.142	0.04	0.695	100.00
CD	0.0012**	0.0288**	0.0153**	NS	NS	NS

NS= Non-significant; and ** = Significant at P =1%.

Table 3. Effect of 5 mg l^{-1} of GA_3 (soaked for 24 h) on seed germination and seedling growth in polymorphic seeds of *E. alba*.

Seed colour	Germination (%)	Seedling growth (cm)		R/S ratio
		Root	Shoot	
Light brown	76.66	1.13	1.56	0.766
Dark brown	93.33	1.55	1.87	0.768
Black	100.00	1.95	2.53	0.787
CD	13.086**	0.2928**	0.2358**	NS

NS = Non-significant; and ** = Significant at P = 1%.

Thus, the data presented in Table 2 and 3 clearly showed that black seeds exhibited maximum values for various parameters studied as compared to light and dark brown seeds collected from site-II. So, black coloured seeds should be used for its large-scale multiplication under field conditions.

The data on effect of different concentrations of GA_3 on seed germination (%), seedling growth (root & shoot lengths), R/S ratio, GV and VI are presented in Table 4. Fresh seeds showed 66.66 percent germination under controlled laboratory conditions. Whereas, 73.33 and 43.33 percent germination was observed after 6 and 24 months of storage period, respectively under controlled conditions. Germination percent significantly increased when seeds were pre-treated with different concentrations of GA_3, being maximum in 5 mg l-1 GA_3. In fresh seeds, maximum root & shoot lengths and R/S ratio were observed in the same treatment. In 6 months old seeds, maximum root & shoot lengths and R/S ratio were observed in 5 and 10 mg l-1 GA_3 pretreatments, respectively. Data clearly revealed that 5 mg l-1 GA_3 treatment is the best for obtaining maximum germination and seedling growth in fresh and 6 month old seeds. After storage, germination (%) and seedling growth decreased significantly, so fresh seeds should be used for raising of nursery in large-scale multiplication. The data were significant at 5 and 1% probability levels except for germination (%) after 24 months, which were non-significant. Seed germination and seedling growth of many plants have responded positively to the application of growth regulators. The treatment of GA_3 gave highest germination with vigorous seedling growth in *Hippophaeti betana* [15]. Similar results were observed in *Podophyllum hexandrum* [16]. Gibberellin like substances have been reported in a number of seeds, which play an important role in *de novo* synthesis of a-amylase, responsible for hydrolysis of starch reserves in the endosperm [17]. GA_3 controls mobilization of starch, which acts as a respiratory substrate, as a raw material for synthesis of other growth regulators and increases the somatic pressure of cell, leading to immediate enhancement in cell elongation [15]. Promoting effects of GA_3 on seed germination by lowering the endogenous abscisic acid content of seed has been reported [18].

Germination Value and Vigour Index

The data presented in Table 4 clearly revealed that maximum values of GV and VI were observed in fresh seeds in 5 mg l-1 GA_3 treated seeds, while minimum in control. 6 and 24 months old seeds also showed similar results, but these values decreased significantly with ageing of seeds. Thus, fresh seeds should be used for obtaining maximum & speedy

Table 3. Effect of 5 mg l^{-1} of GA_3 (soaked for 24 h) on seed germination and seedling growth in polymorphic seeds of *E. alba*.

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germination and healthy seedlings. The seeds of *Withania somnifera* pre-treated with 1000 ppm of GA_3 resulted in vigorous growth of seedlings [19]. It was observed that seeds of *Alstonia scholaris* treated with 100 ppm of GA_3 showed highest vigour index [20]. The highest vigour index and germination velocity index in *Ocimum basilicum* was obtained in seeds pre-treated with 20 ppm of GA_3 [21]. This may be related with increased metabolic activity during germination due to GA_3 treatment. The higher seedling height with GA_3 presoaking treatment may be attributed to the cell multiplication and elongation in the cambium tissue of the internodal region, because gibberellic acid apparently activates the metabolic processes or nullifies the effect of growth inhibitors [22].

From the present studies, it is concluded that maximum germination (%) and seedling growth in *E. alba* can be obtained by pre-soaking with 5 mg l-1 GA_3 for 24 h. Speedy germination and vigrous seedlings growth were also observed in the same concentration of GA_3.

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