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Research Article

Impact of Habitat Variability on Phenotypic Traits and Seed Germination Performance of *Euphorbia helioscopia* L.; a Case Study from the Kashmir Himalaya, India

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Abstract: *Euphorbia helioscopia* L. (Euphorbiaceae), is an important medicinal plant species of the Himalayan region. The species showed wide variability in its phenotypic traits under different environmental conditions. The purpose of this study was to determine the impact of elevational gradient on the morphological traits and reproductive features of the species. The plants grown in the Kashmir University Botanical Garden were more vigorous and taller than the plants of high-altitude sites. Principal component analysis (PCA) revealed that the habitat of KUBG and Kangan (Ganderbal) were comparatively facilitated the growth of *E. helioscopia*. The regression analysis showed a positive correlation between various morphological traits. Our attempted results highlighted an elaborated account of the variation of phenotypic traits and seed germination performance in response to different environmental conditions along an elevational gradient.

Keywords: Analysis, Elevational gradient, *E. helioscopia,* Habitat, Kashmir Himalaya, Phenotypic traits, Variability.

1. INTRODUCTION

Plant species occupy different geographical areas covering contrasting habitats characterized by different environmental conditions. The adaptability of plant populations to varying environmental conditions reflects its survival and success in those particular habitats [1]. Various environmental factors may affect plants directly or indirectly by changing plant responses to other factors. To understand the response of plants to varying growth conditions over a small geographical scale, different elevational ranges are worthy model systems. Elevational gradients thus provide an appropriate scenario to study these responses and important characteristics that are required for the proper functioning of plants in varying habitats [2]. The study of seed germination methods and seed dormancy are important in understanding survival, regeneration, plant community establishment, and adaptations of plants to changing climatic conditions [3–7]. The association between plant regeneration from seeds and climate has resulted in the evolution of distinct germination needs across species [8], and this, in turn, plays a critical role in establishing vegetation type and plant distribution [9]. *Euphorbia helioscopia*, a member of the family Euphorbiaceae, is widely distributed in Europe and cooler regions of Asia, where it is a cosmopolitan weed growing in crop fields, gardens, and roadsides [10]. It is a green, fleshy annual herb growing up to 50 cm in height, with an alternate arrangement of sessile leaves; inflorescence compound umbel, cyathium subsessile; involucre campanulate (Figure 1). Male flowers many, exserted from involucre, ovary nearly reaching up to the margin of the cup. Fruit triangular capsule; seeds ovoid and dark brown. *E. helioscopia* has great medicinal importance and is a potent source of secondary metabolites such as tri-terpenoids, di-terpenoids, flavonoids, and tannins [11]. The whole plant is used to treat various diseases such as arthritis,

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edema, pulmonary tuberculosis, malaria, bacillary-dysentery, osteomyelitis, and cervical tuberculosis lymphadenitis [12, 13]. The leaves and stems are used as febrifuge and vermifuge [14], roots as anti-helminthic, and seeds mixed with roasted pepper are used to treat cholera [15]. In addition, the milky juice from the leaves and fresh stems are used to release pus [16]. *E. helioscopia* grows along an elevational gradient ranging from 1500–2450 m asl and in different habitats such as open, sloppy, etc. The present study was the first attempt to understand the degree of variation in phenotypic traits and seed germination performance in different habitats along an elevational gradient.

2. MATERIAL AND METHODS

2.1 Survey, Collection, and Documentation

Extensive field surveys were carried out across different habitats of Kashmir Himalaya to identify the specific areas. Five distinct sites including four natural sites (Kangan (1850 m asl), Drang (2230 m asl), Betab valley (2350 m asl), Gulmarg (2450 m asl), and one control site at Kashmir University Botanical Garden (KUBG) — 1595 m asl were selected for the present study. Geographical coordinates, characteristics, and distribution of these selected sites (Table 1; Figure 2). To study the phenotypic traits (microscopic and macroscopic characteristics), 10 plants were selected and tagged from each selected population site [17]. The marked individuals across different study sites were monitored on regular basis. Further, the plants were collected at the reproductive stage for analyzing the selected traits.

For statistical analysis, OriginPro (version 2019b) software was used. Multivariate ordination was performed to depict the impact of habitat variability on different phenotypic traits of the plant while regression analysis was performed to analyze the correlation between two different traits. Different populations were statistically analyzed for various phenotypic traits such as stem length, leaf length, number of branches per plant, plant height, and branch length. The propagules and seedlings were transferred to the Kashmir University Botanical Garden (KUBG).

2.2 *In vitro* Seed Germination

Studies on the germination of seeds were undertaken to examine the impact of various pre-sowing seed treatments using different concentrations of various chemicals to ascertain the best treatments for breaking dormancy and accelerating the germination rate. All germination tests were conducted according to the following protocol: seeds were sterilized and placed in sterilized petriplates having Whatman No.1 filter paper wetted with distilled water or given solution. Randomly collected air-dried seeds from the selected populations were used for *in vitro* seed germination studies. Each treatment consisted of 3 replicates, each with ten seeds. The seeds were sterilized for 5–7 minutes with 0.1 percent mercuric chloride ($HgCl_2$), then rinsed five times with double distilled water to eliminate any remaining $HgCl_2$. At an average temperature of 18–24 ^{0}C, the seeds were exposed to both physical (freezing for various periods) and chemical (Gibberellic acid, Thiourea, and kinetin) treatments. Seeds were kept in petriplates on moist Whatman No.1 filter papers moistened with 10 ml of the given treatment solution or distilled water (control). Radicle emergence of 1 mm was set as the germination indicator, and total germination was recorded at the end of the experiment when no new seeds germinated in respect of all the treatments. Three replicates, each with a control set for comparing mean germination time (MGT) and percentage germination of seeds were used for each treatment. Percentage seed germination was obtained by using the below formula:

$$\% \text{ Seed Germination} = \frac{\text{Number of seeds germinated}}{\text{Total no. of seeds kept for germination}} \times 100$$

3. RESULTS

The species shows significant variability in phenotypic characters under varying environmental conditions. This phenotypic variability was observed in all the selected study sites including four natural populations i.e. Kangan, Betab valley, Drang, Gulmarg, and one control site at KUBG Srinagar. Plant height (PH), leaf length (LL), branch length (BL), number of branches per plant (NBP), and stem length (SL) were among the

Table 1. The geocoordinates and characteristics of study sites.

Study sites	Altitude (masl)	Longitude (E)	Latitude (N)	Habitat	Threat factor
Gulmarg	2450	74.3642	34.05065	Open slope	Landslides
Betab-valley (Pahalgam)	2350	75.3617	34.05406	Sunny open slope	Landslides and construction of roads
Drang (Tangmarg)	2230	74.4027	34.04009	Sunny Open slope	Landslides
Kangan (Ganderbal)	1850	74.8953	34.26194	Open field across roadside	Construction of roads
KUBG*	1595	74.8343	34.12797	Sunny open field	Nil

*Kashmir University Botanical Garden, Srinagar

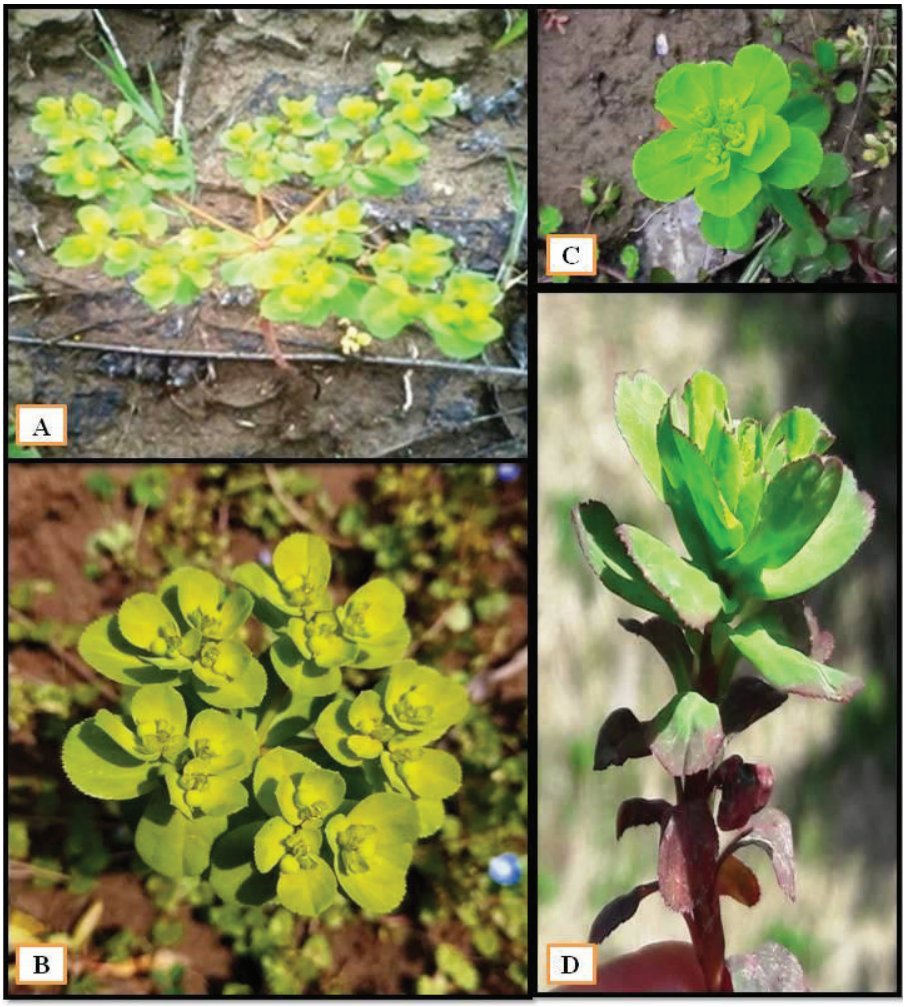


Fig. 1. *Euphorbia helioscopia* L.: A. Habit; B − C. Inflorescence; D. Leaves.

morphological parameters evaluated for phenotypic variability. Table 2 shows the differences in various phenotypic traits studied during the peak flowering period, across and within populations. The plant height ranges from 29.1–39.6 cm in all studied populations. A significant trend of a gradual decrease in plant height was observed along an elevational gradient. The present study depicted that the plant population at KUBG showed more height whereas the population at Gulmarg had less height among all populations.

The number of branches per plant also showed variation among and within studied populations. The highest and lowest number of branches per plant was recorded at KUBG and Gulmarg respectively. Thus branch number per plant showed a decreasing trend from high to low altitude. Branch length also shows a decreasing trend with an increase in altitude. Further, leaf length decreases with an increase in altitude. The population growing at high altitudes i.e., the population at Gulmarg showed smaller leaf lengths (2.4 cm long) while populations growing at lower altitudes had larger leaf lengths (3 cm long).

The impact of various pre-treatments (physical & chemical) on the germination of seeds recorded for fresh seeds is shown in Table 3. The best seed germination (60 %) was recorded in gibberellic acid (50 ppm). Though, no seed germination was observed in control.

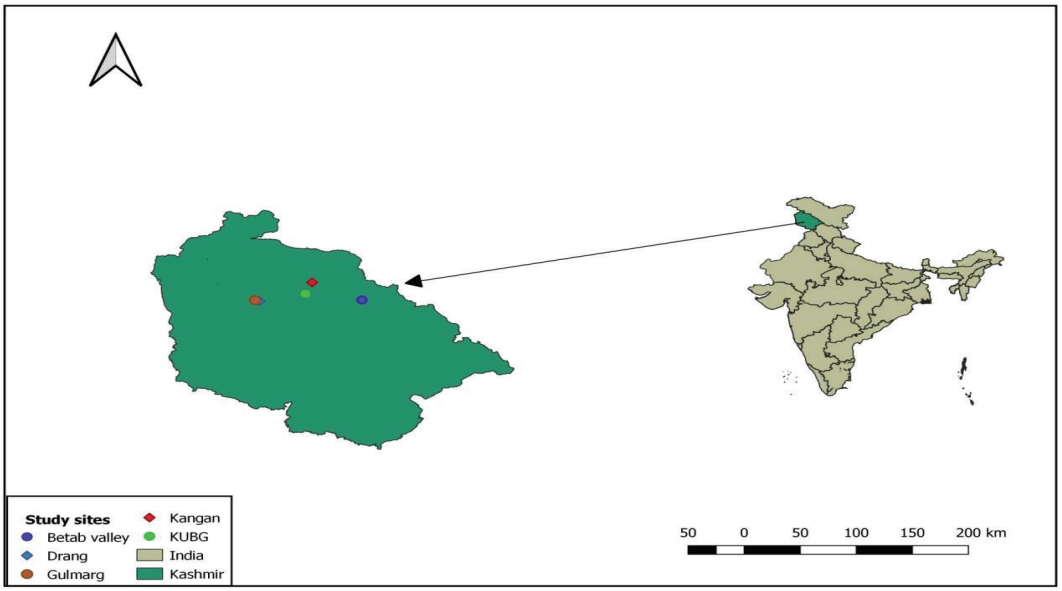


Fig. 2. Map showing the location of different study sites in Kashmir Himalaya (QGIS version 3.20).

Table 2. Phenotypic variability of *E. helioscopia* across different populations.

Phenotypic Characters	**KUBG**	**Drang**	**Betab valley**	**Gulmarg**	**Kangan**
Mean plant height (cm)	39.6	34.2	29.1	25.4	33.4
Mean length of stem (cm)	23.4	18	16.4	13.1	21.2
Mean No. of branches/plant	6	5	5	4	6
Mean branch length (cm)	10.5	10.5	8.3	7	11
Mean leaf length (cm)	3	2.6	2.5	2.4	2.8

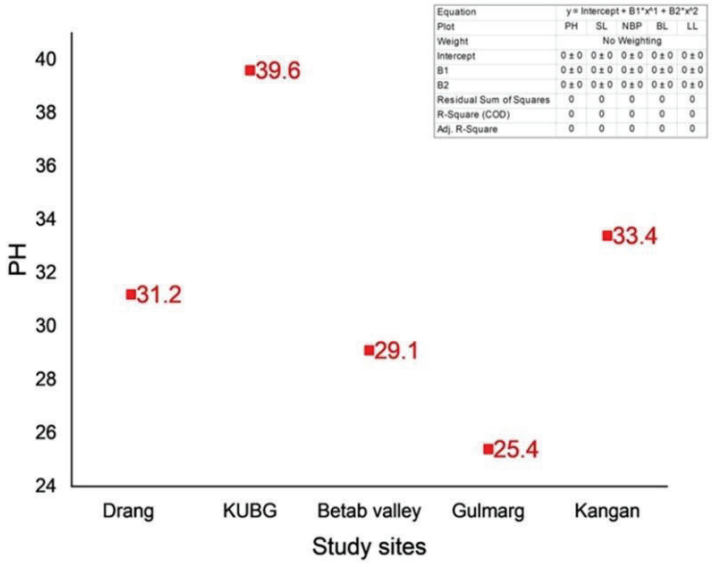
Table 3. Effect of different chemical treatments on seed germination.

Dry chilling (60 days) + different hormones	Conc. (ppm)	Seed germination	No. of days taken for 1st germination	No. of seeds kept for germination	Germination (%)
Gibberellic acid	50	6	15	10	60
	100	2		10	20
	200	3		10	30
Thiourea	50	2	15	10	20
	100	-		10	0
	200	-		10	0
Kinetin	50	-	19	10	0
	100	1		10	10
	200	1		10	10
Potassium nitrate	50	1	19	10	10
	100	1		10	10
	200	-		10	0
Control		-	-	10	0

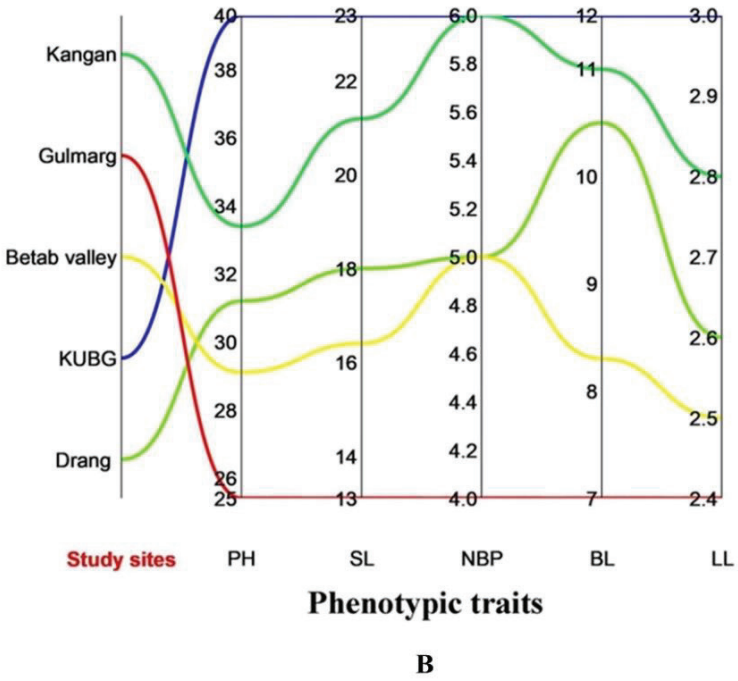
4. DISCUSSION

The present study revealed that *E. helioscopia* grows both in plains and sub-alpine regions of Kashmir Himalaya. It prefers sandy and clay loams and is often associated with light-textured soils [18]. The populations at Gulmarg mostly prefer humus moist soils. However, populations at Kangan and other sites growing across roadsides and cultivated fields prefer sandy and little moist soils. Studies on plant morphology of different populations reveal that the analyzed phenotypic characters vary considerably across different populations viz. Gulmarg, Betab valley, Drang, and Kashmir University Botanical Garden (KUBG). Figures 3A & B depict the mean value of phenotypic characters at different study sites. Both in natural and transplant populations, the plants show a high degree of variability in morphological traits, i.e plant height, length of leaves, length of branches, length of the stem, etc, along the elevational gradient. The regression analysis between different morphological characters (Figures 4A − C) revealed that there is a positive correlation (r^2 = 0.82234) between branch length (BL) and the number of branches per plant (NBP), similarly positive correlation (r^2 = 0.96528) between plant height (PH) and leaf length (LL). Further a positive correlation (r^2 = 0.94613) between stem length (SL) and plant height (PH). Scale interval (Figure 5) showed the range value and mean of different phenotypic traits. The multivariate ordination (Principal Component Analysis) (Figure 6) shows that the plant population at Kangan and KUBG encompass an environment favorable for most of the plant characteristics. This phenotypic variability allows the adaptation of species in tackling the change in environmental conditions. [19]. Similar findings were reported by several workers [20, 21]. Korner and Cochrane [22] stated that the decrease in plant height along with elevational gradient is advantageous for the species as it prevents the plant from strong winds. There is an increase in leaf dimensions in plants growing at lower altitudes as compared to those growing at higher altitudes. The results show conformity [1, 23], who reported that leaf dimensions generally decrease with an increase in altitude.

Further, chemical and physical treatments have been found to enhance seed germination in plant species. The seeds kept in the gibberellic acid (under a concentration of 50–200 ppm) show a better response towards germination, while the seeds which were kept in other chemicals (hormones) show little or negligible response towards germination. In the case of control (distilled water) treatment, the seeds show no response (Figure 7).



A



B

Fig. 3 (A & B). Parallel plots showing mean values of phenotypic traits at different study sites.

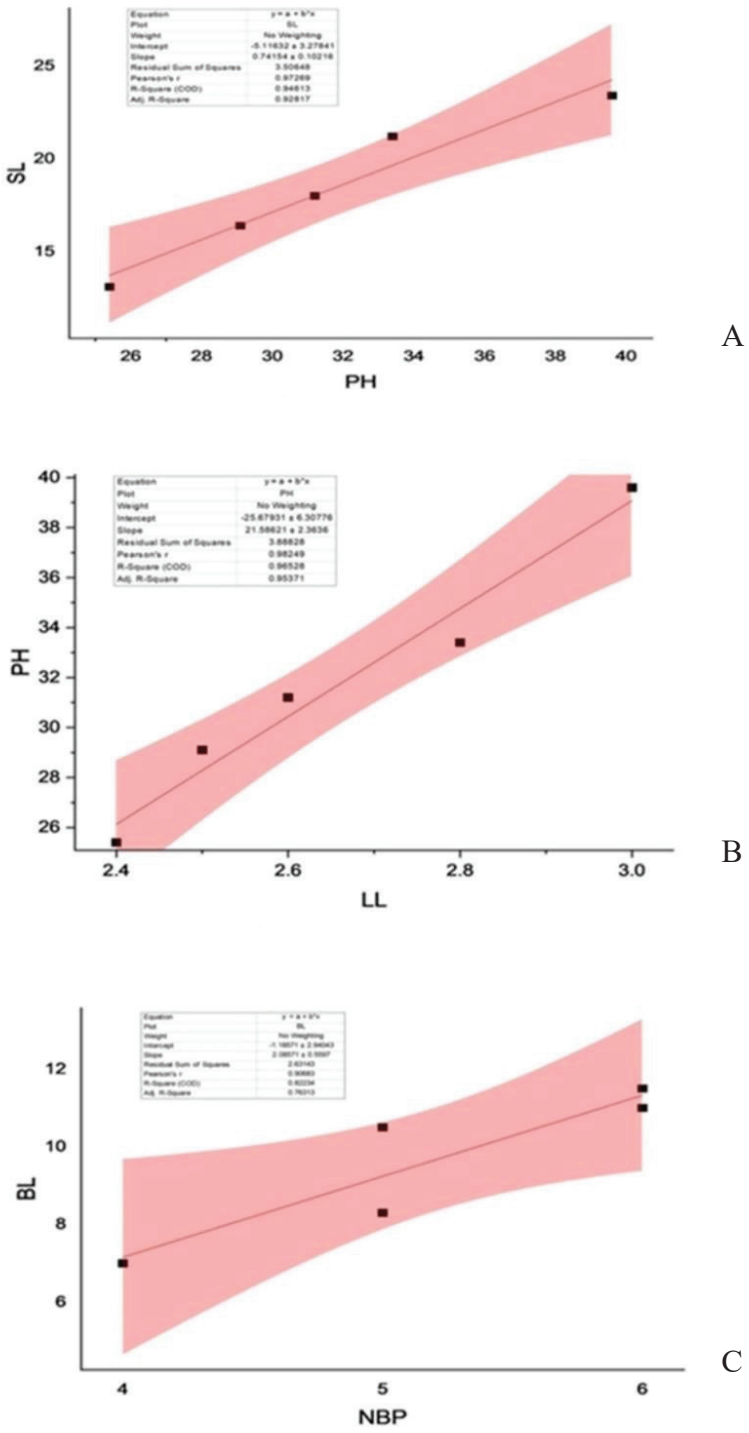


Fig. 4 (A−C). Regression analysis and scale interval between various morphological traits of *E. helioscopia.* **BL.** Branch length; **NBP.** No. of branches/plant; **PH.** Plant height; **LL.** Leaf length; **SL.** Stem length.

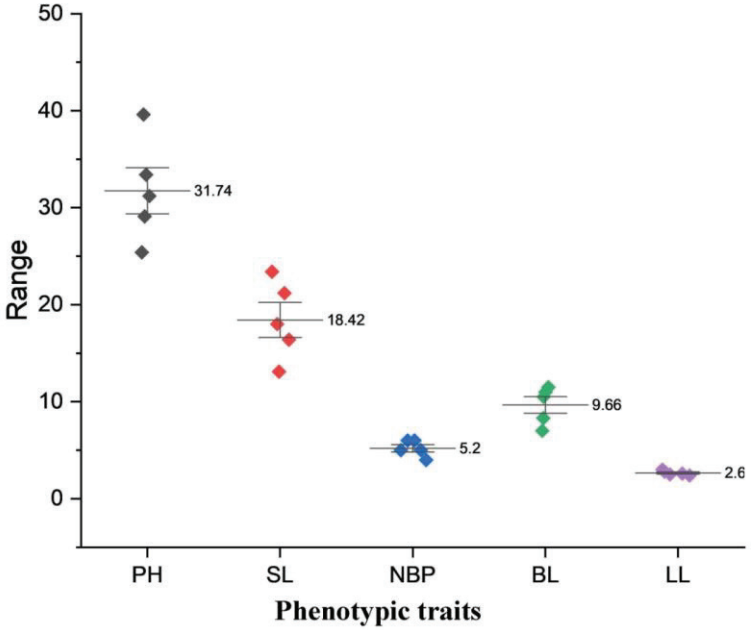


Fig. 5. Scale interval showing the mean and range of all phenotypic traits.

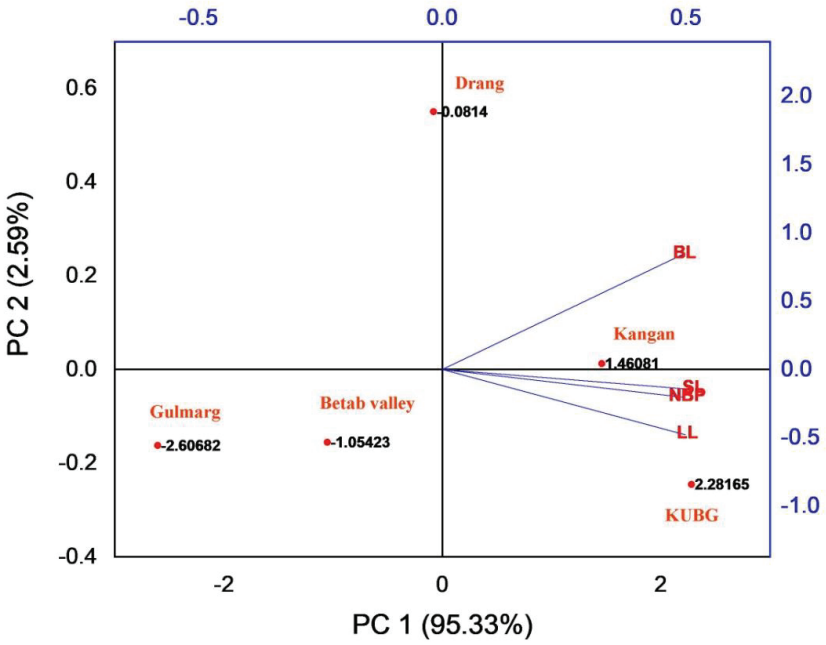


Fig. 6. Principal component analysis of morphological traits of *Euphorbia helioscopia* L. across selected study sites. **PH.** Plant height; **SL.** Stem length; **NBP.** No. of branches/plant; **BL.** Branch length; **LL.** Leaf length.

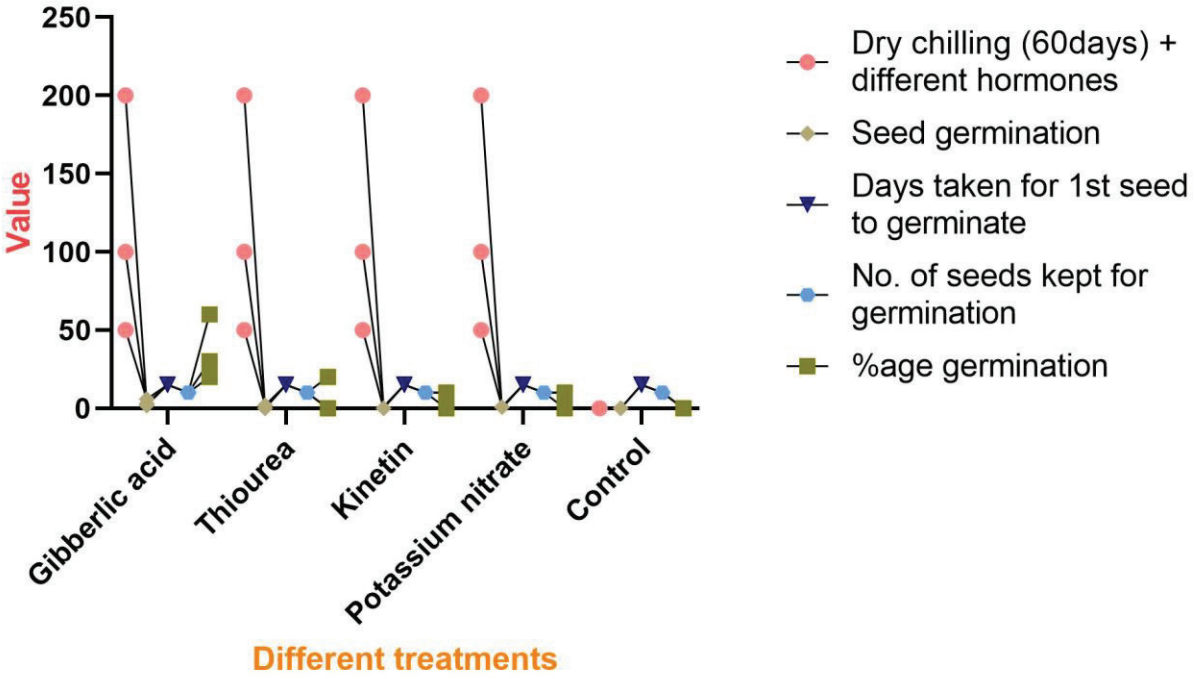


Fig. 7. Effect of hormone & chemical treatments on seed germination of *E. helioscopia.*

4. CONCLUSION

The present study revealed a high degree of variability in morphological traits i.e. plant height, length of leaves, length of branches, length of the stem, and other traits, in natural as well as transplant populations in different habitats along the elevational gradient. These variations in phenotypic traits of *E. helioscopia* may be attributed to the varying environmental conditions across habitats along elevational gradients. The negative correlation of morphological features with increasing altitude suggests that this species grows better at lower altitudes. The present results also revealed that *E. helioscopia* had a very low percentage of seed germination. Among the treatments given, gibberellic acid shows promising results in terms of the percentage germination as well as survival percentage. It can be suggested from the present study that the plant species growing at low altitudes were comparatively much vigorous in respect of the various morphological features. Further, these variations in phenotypic traits and seed formation may be due to the impact of various environmental factors, seasonal variations, and competition for resources or herbivory.

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6. CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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