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# Effects of light and drought stress on germination of *Artemisia sieberi* Besser

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Preservation and development of plant cover are major factors in the management of range ecosystems. *Artemisia sieberi* is one of the native dominant species of vast areas in the Irano-Turanian bioclimatic region. This species is very tolerant to drought stress and grazing pressure. Therefore, it can be used to rehabilitate degraded rangelands of dry areas within this region. Understanding the germination characteristics of this species is important for the conduction of revegetation projects. Seeds of *A. sieberi* were collected randomly from 20 plants of existing vegetation along 6 kilometers transect at Isfahan Kolah Ghazi National Park located at 51°45'E and 35°15'N. To pass the dormant period, seeds were pre-chilled for nine days at 0 to 5°C. Two germination experiments were conducted in complete factorial block design. Moisture stress test was carried out with 0, -0.2, -0.4 and -0.6 MPa treatments using polyethylene glycol (PEG) solutions in Petri dishes. The light treatment test was conducted with 12 h light and dark versus a continual dark condition. Results indicated that, an increase in the drought level lead to a decrease in the percentage and rate of germination, plumule length and allometry ratios, whereas the radicle length increased. In comparison with the dark treatment, 12 h of light treatment increased the percentage of germination and radicle length, while allometry ratios and plumule length decreased. Continual dark treatment compared with the 12 h light and dark photoperiod significantly reduced radicle and increased plumule lengths. Drought tolerance of *A. sieberi* seeds during germination showed the high potential of this species for vegetation rehabilitation in dry regions.

**Key words:** *Artemisia sieberi*, seed germination, drought tolerance, light treatments, Iran.

## INTRODUCTION

The preservation and development of rangeland plant covers as natural ecosystems are important practices for prevention of desertification. The most suitable species for the reclamation of degraded vegetations are native species. Early stages of plant development, resistance to drought during the first year and intra-specific and extra-specific competition are important issues for plant establishment in natural habitats. *Artemisia* spp. (sagebrushes), as the most adaptable native plant can be used for vegetation rehabilitation in Iran's harsh steppe conditions. Sagebrushes are considered genetically important species in our country which regenerate by seeds and are resistant to heat, drought and pests

(Moghimi, 2005). In addition to controlling water and wind erosion, they also provide forage during the rainfall and winter. Sagebrushes also exhibit medicinal properties (Mozafarian, 1999). Survival mechanisms of *Artemisia sieberi* to severe conditions include the germination of a large number of seeds with high viability and resistance to wetting and thawing (Moghimi, 2005). *Artemisia* seeds generate a transparent gelatin cover around the seeds that make contact with moisture and somehow prevents germination in unfavorable conditions (Mozafarian, 1999). According to available literature, this species has not been widely used for reclamation of degraded lands in arid regions. The fruit of *A. sieberi* is a nutlet and ovoid shape, weighing 0.25 to 0.35 g (Moghimi, 2005). *A. sieberi* has after-ripening seeds with a short dormant period which breaks by being exposed to cold weather (Meyer and Monsen, 1992). According to Modares Hashemi (2003), enhancement of seed germination and

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a break in dormancy occurs with seed exposure to light while immersed in water, Modares Hashemi also reported that, seeds of *A. sieberi* after 9 days of exposure to 0 to 5°C in moist Petri dishes followed by 21 day exposure to alternate temperatures of 15 to 25°C showed significant increase in germination.

Seed germination is critical in life cycle of plants, yet often subjected to high mortality rates (Geraldine and Lisa, 1999). Although many factors can affect seed germination, light and soil moisture are the most important ones. Light serves as an important regulatory environmental signal in the seed germination of desert plants because seeds may undergo a transition from primary to secondary dormancy and then, develop a light requirement to trigger germination (Baskin and Baskin, 1995). The requirement for light is a genetic characteristic mediated by phytochrome (Jones and Hall, 1979) which stimulates the synthesis of growth-promoting substances to initiate germination (Okusanya and Ungar, 1983; Gul and Weber, 1999). Light alone affects germination in some species, while in others the light response is temperature dependent (Khan and Ungar, 1997). Huang and Gutterman (1999) have reported germination strategies of *Artemisia sphaerocephala* and concluded that achenes germinated in light conditions but not in the dark.

One of the most important problems which affect different aspects of plant growth and establishment in dry and semi-dry regions is drought stress which reduces and delays germination as well as growth and production (Hartmann and Kester, 1983). Fyfield and Gregory (1989) have found that, both the seed germination rate and final percent of germination decrease with reduced soil water potential. Studies regarding the impact of soil water potential on germination are often conducted with the use of polyethylene glycol (PEG) (Carpita et al., 1979; Hardegree and Emmerich, 1990). The results of the drought stress experiment with a PEG 6000 solution on two species of *Agropyron afghanicum* and *Agropyron cristatum* have shown that, drought stress negatively impacted the percentage of germination and these plants had a low resistance to drought (Shahryari and Javadi, 2005). Ronnenberg et al. (2007) assessed germination and seed viability of five perennials (*Allium polyrrhizum*, *A. cristatum*, *Arenaria meyeri*, *Artemisia frigida* and *Artemisia santolinifolia*) at three levels of osmotic stress (0, -0.5 and -1 MPa) and under conditions of alternate light/darkness versus complete darkness. The results showed that osmotic stress reduced seed viability and total germination in all five species. Darkness had no influence on viability, but positively affected seed germination of *A. polyrrhizum* and *A. cristatum*. In another study, Azarnivand et al. (2007) assessed the physiological effect of five levels of water stress (0, -0.3, -6, -9 and -1.2 MPa) on seed germination of *Artemisia spicigera* and *Artemisia fragrans*. The maximum germination rate was obtained at -0.3 MPa. Germination rate, as well as tigella and radicle growth lengths declined with decreasing water potential level. *A. spicigera*

was more resistant to water stress in comparison with *A. fragrans*. A literature review has indicated the lack of comprehensive work on seed germination characteristics of this species. Therefore, the main objective of this study was to investigate the effects of drought stress and light treatment on germination behavior of *A. sieberi* seeds.

## MATERIALS AND METHODS

### Seed collection and method of selection of *A. sieberi* plant groups

The required *A. sieberi* seeds were collected from Isfahan Kolah-Ghazi National Park located at 51°45'E and 35°15'N with an elevation of 1690 m from sea level. The climate of this region is dry, with a hot, dry summer season based on Koppen's climate classification method (Koppen and Geiger, 1936) with a mean annual precipitation of 145 mm, minimum and maximum temperatures of -16 and 42°C, respectively. Precipitation in the year of seed collection was 165.1 mm (IRIMO, 2010). Elevation of the study area varies from 1650 to 2534 meters from the plains to mountain peak (Khajedin and Soltani, 1998). The vegetation cover is very low and varies from 0 to 5%, at most (Khajedin, 2000).

Seeds were collected from 20 individual *A. sieberi* plants randomly selected along a six kilometer transect. In November 2006, prior to seed production, a light transparent cloth bag was placed over each plant and the opening of the bag was firmly closed under a canopy such that all the seeds were collected in bags. The seeds were collected in January 2007 and air-dried. Seeds from the ripened inflorescences were separated from other debris by a sieve and wind flow. The ISTA (1985) method was used to determine the 1000 seed weight. Twenty selected individual plants were grouped using cluster analysis based on the plants' heights and crown diameters as well as the estimated number of seeds of each individual plant. To calculate similarities between individual plants, the Motyka similarity index ( $SI_{MO}$ ) was employed as follows (Muller-Dombois and Ellenberg, 1974):

$$SI_{MO} = \frac{200 \sum MW}{MA + MB}$$

Where, MW is the minimum desired value for each common factor among A and B plants; MA is the total valued factor for plant A and MB is the total valued factors for plant B. Four plant groups were selected for the use of their seeds in this study.

### Cultivation of the seeds in petri dishes

As *A. sieberi* seeds require a chilling period for germination, the seeds of four selected plant groups were pre-chilled at 0 to 5°C for about nine days before the experiment (Khavazeh, 1998; Modares Hashemi, 2003). All equipments of the experiment were disinfected with alcohol and the seeds were disinfected by vita wax 2/1000 fungicide. Twenty five seeds were appropriately spaced on Watman 40 mm filter paper in each petri dish and the treatments were randomly allocated to Petri dishes. Petri dishes were placed in the incubators at temperature of 25°C. Plants were watered daily with distilled water such that seeds were not immersed in water. The number of the germinated seeds was recorded daily.

### Drought resistance of germinating seeds

Different water potentials were applied on Petri dishes using PEG

6000 to study the effect of water stress. The experiment was carried out with four replicates of 25 seeds in an incubator with a temperature of 25°C and 12 h alternate light and dark cycles. Water potentials of 0.0 (control group), -0.20, -0.40 and -0.6 MPa were produced by different concentrations of PEG 6000 solutions according to Michel and Kaufmann (1973). A total of 64 Petri dishes for 4 replicates of 25 seeds, 4 plant groups and 4 moisture stress levels were employed. To prevent water evaporation, the Petri dishes were placed in plastic bags. The solutions inside the Petri dishes were watered every three days to prevent an increased concentration of PEG. All seeds were kept under the same conditions for four weeks. Seeds were considered to have germinated when the radicles emerged (1 to 2 mm in length out of the tegument) (Ren and Tao, 2004).

### The effect of light and dark treatments on germinating seeds

Light and dark treatments included 24 h dark and 12 alternate light and dark periods. The experiment was carried out in an incubator at 25°C. Eight Petri dishes containing 25 seeds were used for each plant group.

### Data analysis

In the seed germination experiment, the following traits were measured and studied.

The final germination percentages (GF) and the rate of germination (RG) were determined by the following formula (Timson, 1965), respectively:

$$GF (\%) = [a / b] \times 100,$$

$$RG = \sum G/t,$$

Where "a" is the total number of seeds germinated for a given time; "b" is the total number of seeds; G is the percentage of daily seed germination; and t is the total germination period. At the end of this period, the length of the plumule and radicle after the emergence of the first two new leaflets were recorded. The important index of allometry was determined by calculating the length ratio of the plumule to the radicle. The arcsine square root transformation of GF was used to ensure normal distribution and homogeneity of variance (Gulzar and Khan, 2001; Ren and Tao, 2004).

Drought resistance and photoperiod experiments were conducted in a completely randomized factorial design. To study the differences among *A. sieberi* plant groups for germination traits, an analysis was carried out by means of two-way ANOVA (SAS version 9.1 programs) and the LSD test ( $P < 0.05$ ) was used to test differences between treatment means.

## RESULTS AND DISCUSSION

### Seed size and weight

The 1000 seed weight of the *A. sieberi* plants was 0.19 g. The mean length and width of the seeds were 1.7 and 0.6 mm, respectively.

### Selection of *A. sieberi* plant groups

Cluster analysis was performed on 20 *A. sieberi* plants selected on the basis of plant height, sizes of the two

diameters of the plant crowns and the number of estimated seeds of each plant. In the resulted dendrogram, four clusters were recognized on a similarity coefficient (SiC) of 83%, marked by a horizontal line and the letters from A through D (Figure 1) and the four major groups were separated. Plant numbers 10, 16, 17 and 20 from each cluster with the highest number of seeds were selected for seed collection and named plants 1, 2, 3 and 4.

### Drought treatment effect on seed germination

In dry and semi-dry regions, drought stress delays and reduces the rate of germination (Young et al., 1983). In this experiment, the effect of PEG solutions resulting -0.2, -0.4, -0.6 MPa water potential (WP) on seed germination traits was studied. The drought stress experiment showed that, the percentage germination in all drought treatment levels had significant differences of a probability level of 1%. Lengths of the radicle, plumule and allometry ratios were not significantly affected in all plants (Table 1).

### Interaction effects of plants and drought levels

The interaction effects of four plants and drought levels on the percentage germination and the rate of germination at the  $P = 1\%$  level and on the length of radicle and plumule were significant at  $P = 5\%$ , but no significant interactions were observed on allometry ratios. Results indicated that, the different levels of drought on different plants resulted in different percentages and rates of germination and different lengths of radicle and plumule. Reduction in percentage and rate of germination as well as plumule length were observed in all plants by increasing drought levels. Plant no. 3 had the highest percentage of germination in all treatment levels. This might denote more resistance of this plant to drought. The lowest percentage and rate of germination were observed at -0.6 MPa in plant no. 4. Plant no. 3 showed the highest rate of germination in the control compared with the other plants (Figure 2).

LSD test showed that the highest percentage and rate of germination were observed in the control (92.25%). Zheng et al. (2005) reported that, the rate and percentage of germination of *A. sphaerocephala* were reduced under drought stress and a small number of seeds were germinated in WP of -1.4 MPa. Villalobos and Pelaez (2001) studied the effects of drought stress on germination and plant establishment of *Prosopis caldenia* and showed that, the highest rate of germination in the control treatment occurred at 30°C and the least at -1 MPa treatment at 35°C. Their results also showed that, the percentage of germination in -1 MPa was significantly reduced at a variable temperature range from 20 to 35°C ( $P < 5\%$ ) which confirmed the results of this study.

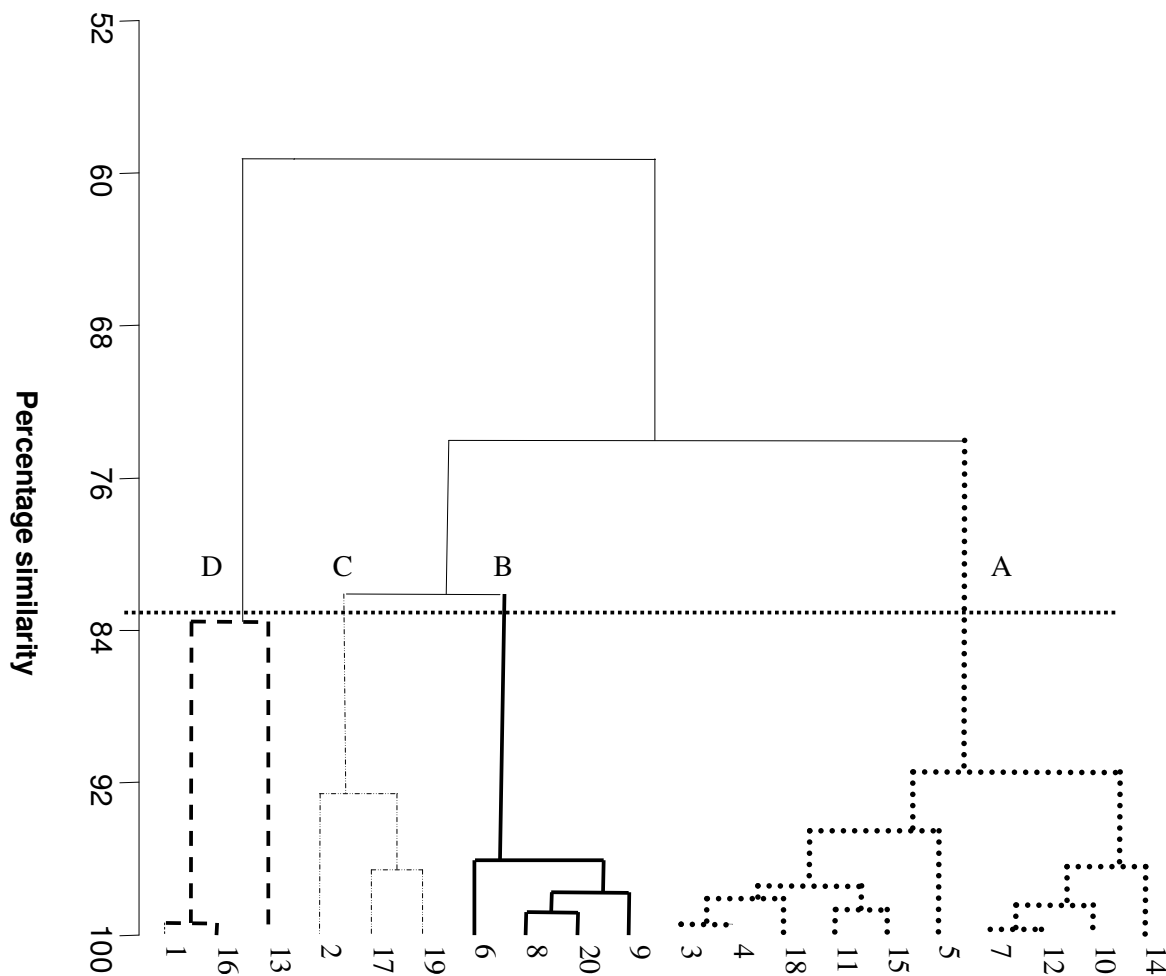


Figure 1. Denderogram resulting from cluster analysis of the grouping of randomly selected plants.

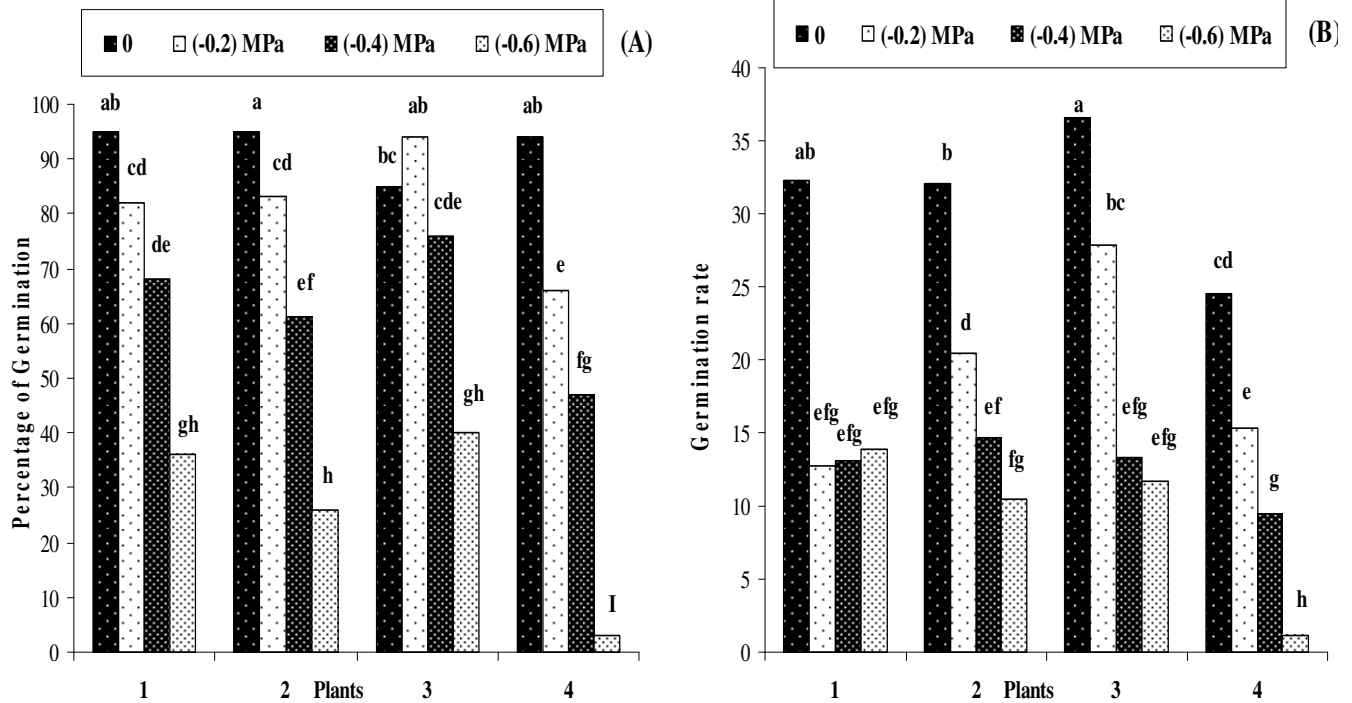
Table 1. ANOVA for drought resistance experiment.

Source of variation	df	Mean squares (cm)				
		Germination (%)	Germination rate	Radicle length (cm)	Plumule length (cm)	Allometry ratio
Treatment	3	127.79**	379.4**	29.09**	1.01**	0.012**
Plants	3	14.27**	66.31**	4.23 ns	0.017 ns	0.001 ns
Treatment and plants	9	4.41**	13.07**	3.44*	0.03*	0.001ns
Error	48	49.9	2.65	1.52	0.011	0.0003
Coefficient variation (%)	-	12.72	17.07	28.64	21.68	3.01
R <sup>2</sup>	-	0.91	0.92	0.64	0.86	0.77

\*P < 0.05, \*\*P < 0.01; ns: Non- significant.

In this research, the rank of germination percentage of plants from high to low was as follows: nos. 3, 1, 2 and 4. Plants no. 3 and 4 were significantly different from each other and from the others for the rate of germination whereas, no difference was seen in plants 1 and 2, relative to each other. The effect of drought treatments on

the radicle length of plants 1 to 4 was not significantly different, however, in plant no. 4 the increase of drought from -0.4 to -0.6 MPa resulted in a significant reduction of the radicle length. The highest and lowest radicle lengths were observed in plant no. 2 at the -0.2 MPa treatment and in plant no. 4 at the -0.6 MPa treatment, respectively



**Figure 2.** Mean interaction effects of different plants and drought treatments on: (A) percentage of germination and (B) germination rate.

(Figure 3).

A non-significant increase was observed in the plumule length in plant no. 4 at -0.2 MPa WP. With an increase in drought from 0 to -0.2 MPa, the length of the radicle significantly increased (in all plant) and the highest radicle length with a mean of 5.94 cm was observed. Thus, it can be said that at -0.2 MPa drought conditions, this plant increased its root growth. Plant no. 4 was most affected by -0.6 MPa drought treatments in all of the parameters studied in this research (Figures 2 and 3). Balestri and Cinelli (2004) studied *Pancretium maritimum* and showed that, drought was a preventing factor for germination. At -0.6 MPa WP, radicle length was significantly reduced compared with other drought levels. Results also showed that plants were generally very sensitive to -0.6 MPa WP.

An increase in drought resulted in the reduction of the plumule length and allometry ratios; plants retained their radicle length compared with the plumule length to obtain more water (Figure 3). The results also showed that, plant no. 4 was significantly different from plants 2 and 3 for radicle length, but not significantly different from plant no. 1. In regards to plumule length, plants were not significantly different and reacted similarly under drought stress. Increasing water stress reduced plumule length. Therefore, reduction of allometry ratios is an indication of growth priority for radicles under unfavorable moisture conditions. These results are comparable with the results obtained by Finch et al. (2001) on the *Dacus carota* plant.

They have reported that the plant plumule length reduced as drought stress increased.

**Effects of light treatment on germination characteristics**

Alternate light and dark and continuous dark treatments were imposed on germinating seeds. Germination rates at all treatment levels was not significantly affected however, with the other factors under study, significant differences ( $P = 1\%$ ) were observed (Table 2).

**Interaction effects of plants and light treatments**

Results indicated that, the continuous dark condition significantly reduced germination percentage and length of radicles compared with the 12 h photoperiod. The highest germination percentage and radicle length was observed in the 12 h photoperiod in plant no. 2. Additionally, the highest germination rate, plumule length and allometry ratios were observed under dark treatment in plant no. 2.

In the light treatment, plant no. 1 showed a high percentage of germination. In plant no. 4, the lowest allometry ratios, percentage and rate of germination were observed with dark treatment

Germination rate was not significantly affected by light.

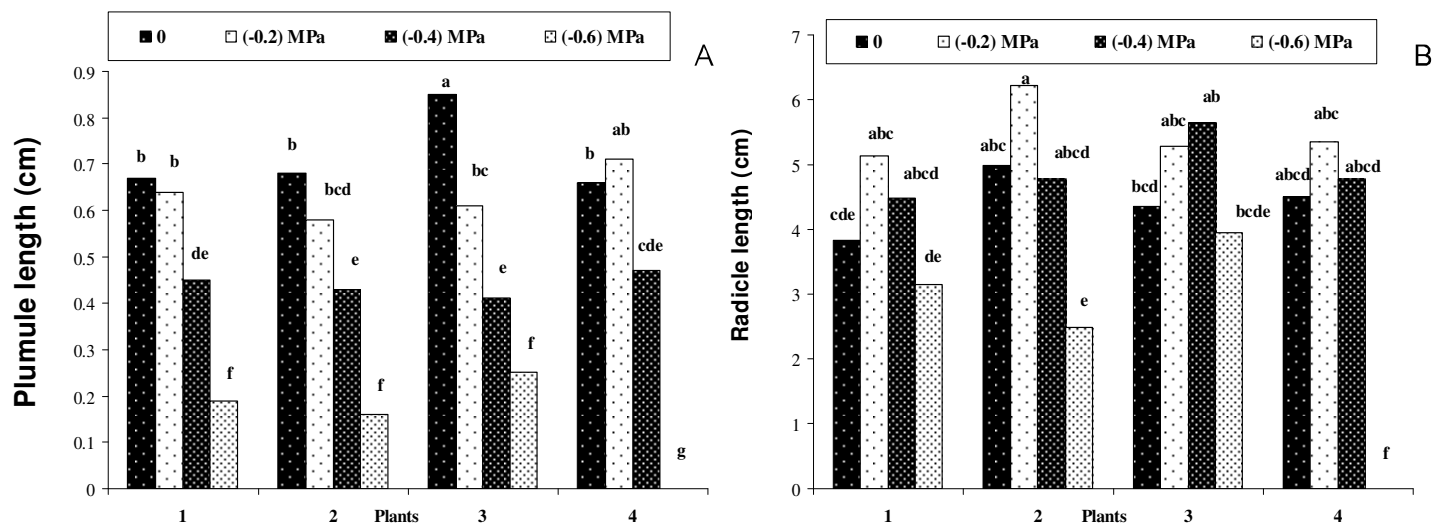


Figure 3. Mean interaction effects of different plants and drought treatments on: (A) plumule length and (B) radicle length.

Table 2. ANOVA for the light experiment.

Source of variation	df	Mean squares (cm)				
		Germination (%)	Germination rate	Radicle length (cm)	Plumule length (cm)	Allometry ratio
Treatment	1	4106.2**	0.09ns	58.83**	1.79**	0.63**
Plants	3	360.37**	122.49**	0.96ns	0.04ns	0.007ns
Treatment and plants	3	456.76**	56.75**	0.34ns	0.1ns	0.008ns
Error	24	40.69	9.37	0.37	0.04	0.008
Coefficient variation (%)	-	10.05	18.97	19.85	21.62	12.6
R <sup>2</sup>	-	0.88	0.7	0.88	0.68	0.77

\*P < 0.05, \*\* P < 0.01; ns: non-significant.

treatments. Therefore, it can be said that the length of the plumule and allometry ratios are reduced in light, but the length of the radicle is increased (Table 3). Javadi and Azarnivand (2003) have stated that, the percentage of germination in *Artemisia aucheri* was higher in light than dark treatment, but germination rate was higher in continuous dark treatment. In another study, Huang and Gutterman (1999) have reported germination strategies of *A. sphaerocephala* and concluded that, achenes germinated in light but not in dark. While Cinelli and Balestri (2004) stated that, germination of *P. maritimum* seeds in dark was as good as the germination in a 12 h alternate dark and light cycle.

LSD test on germination means indicated that, plant no. 2 and 4 were significantly different (Table 4). The plumule and radicle length and allometry ratios of different plants were not significantly different. Plant no. 2 had the highest germination percentage and rate as well as longest radicle and plumule lengths. In this experiment plants no. 2 and 4 had the highest and lowest germination percentage and germination rate, respectively (Table 4).

## Conclusions

The origin of the observed differences among the plants may be from the genetic variations among *A. sieberi* species populations. The results of germination experiments has shown that, by increasing the concentration of PEG, the germination percentage, germination rate, plumule length and allometry ratios reduced in all of the four groups of seeds. Since plumule growth reduced in response to water stress, therefore, allometric ratios reduced.

The radicle length under drought conditions increased at -0.2 MPa, probably due to reduction in plumule growth which allocated more photosynthesis to the radical. The results of this research showed that, *A. sieberi* seeds germinated better in natural and appropriate photo-periods compared with darkness. In all plants, the allometry ratios and plumule lengths were reduced, but germination percentage and radicle lengths increased in light (similar to natural conditions) compared with darkness. Germination rate in the two light treatments were

**Table 3.** Mean interaction effects of plants and light treatments for all factors.

Treatment	Plant	Germination (%)	Germination rate	Radicle length (cm)	Plumule length (cm)	Allometry ratio
Light	1	95 <sup>a</sup>	32.24 <sup>bc</sup>	3.82 <sup>b</sup>	0.67 <sup>d</sup>	0.19 <sup>b</sup>
Darkness	1	58 <sup>e</sup>	27.23 <sup>c</sup>	1.72 <sup>c</sup>	1.05 <sup>bc</sup>	0.62 <sup>a</sup>
Light	2	95 <sup>a</sup>	32 <sup>bc</sup>	4.97 <sup>a</sup>	0.68 <sup>d</sup>	0.14 <sup>b</sup>
Darkness	2	83 <sup>cd</sup>	47.73 <sup>a</sup>	2.12 <sup>c</sup>	1.37 <sup>a</sup>	0.66 <sup>a</sup>
Light	3	85 <sup>bc</sup>	36.64 <sup>b</sup>	4.35 <sup>ab</sup>	0.85 <sup>cd</sup>	0.2 <sup>b</sup>
Darkness	3	72 <sup>de</sup>	31.51 <sup>ob</sup>	1.37 <sup>c</sup>	1.05 <sup>bc</sup>	0.77 <sup>a</sup>
Light	4	94 <sup>ab</sup>	24.51 <sup>cd</sup>	4.5 <sup>ab</sup>	0.66 <sup>d</sup>	0.15 <sup>b</sup>
Darkness	4	33 <sup>f</sup>	18.07 <sup>d</sup>	1.57 <sup>c</sup>	1.3 <sup>ab</sup>	0.88 <sup>a</sup>

Treatment means followed by the same letter are not significantly different at P < 0.05 according to the LSD test.

**Table 4.** Mean interaction effects of light and darkness on different plants for all factors.

Plant	Germination (%)	Germination rate	Radicle length (cm)	Plumule length (cm)	Allometry ratio
1	76.5 <sup>b</sup>	29.73 <sup>b</sup>	2.77 <sup>b</sup>	0.86 <sup>a</sup>	0.4 <sup>a</sup>
2	89 <sup>a</sup>	39.86 <sup>a</sup>	3.55 <sup>ab</sup>	1.03 <sup>a</sup>	0.4 <sup>a</sup>
3	78.5 <sup>b</sup>	34.08 <sup>ab</sup>	2.86 <sup>b</sup>	0.95 <sup>a</sup>	0.48 <sup>a</sup>
4	63.5 <sup>c</sup>	21.29 <sup>c</sup>	3.04 <sup>ab</sup>	0.98 <sup>a</sup>	0.52 <sup>a</sup>

Within treatment means followed by the same letter are not significant at P < 0.05 according to LSD test.

not significantly different. The most and the least resistant individuals to drought regarding germination traits were from plants no. 3 and 4, respectively. It appeared that seeds from plant no. 2 were the least affected, whereas seeds of plant no. 4 were the most affected by light treatments. Variations in response to drought and light treatments on germinating seeds of these plants is a promising phenomenon for investigators to search for most successful genomes in germination stages for rehabilitation programs in dry regions.

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