

## **Osmopriming Effects on Safflower (*Carthamus tinctorius* L.) Seed Germination and Seedling Growth**

**Esmail Nabizadeh<sup>1</sup>, Farid Yousefi<sup>2</sup> and Farzad Gerami<sup>3\*</sup>**

<sup>1</sup>Faculty of Agriculture and Natural Resources, Mahabad Branch, Islamic Azad University, Mahabad, Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, Agriculture Faculty, Shahid Chamran University, Ahvaz, Iran

<sup>3</sup>Young Researchers Club, Mahabad Branch, Islamic Azad University, Mahabad, Iran

---

### **ABSTRACT**

*In order to evaluation the effect of seed osmo-priming by polyethylene glycol (PEG 6000) on safflower (cv. Pdideh) seed germination and seedling growth, a laboratory experiment was carried out at the Agronomy Department of Agriculture Faculty of Shahid Chamran University, Ahvaz, Iran, during 2011. Seeds primed with four osmotic potential levels (i.e. distilled water; OP<sub>0</sub>, -2 MPa; OP<sub>-2</sub>, -4 MPa; OP<sub>-4</sub> and -6 MPa; OP<sub>-6</sub>) for 12 (D<sub>12</sub>), 24 (D<sub>24</sub>) and 36 (D<sub>36</sub>) hours priming duration. Experiment was arranged factorial (4 × 3) in a completely randomized design with three replications. In this study, seed germination percentage, germination rate, plumule and radicle length, radicle to plumule ratio, seed vigor index as well as plumule and radicle weight were investigated. Our results showed that, all studied traits significantly affected by osmotic potential levels except germination percentage and plumule weight. Also, osmo-priming duration had significant effect on all germination and seedling growth characteristic except plumule weight. Plumule and radicle lengths, seed vigor index and radicle weight significantly affected by interaction of treatments. All studied traits decreased by decreasing of osmotic potential levels. Therefore, OP<sub>-6</sub> and D<sub>36</sub> had the most inhibitory effect on studied characteristics. In general, results of present study suggest that, osmotic potential increasing and reduction of priming duration could improve seed germination and seedling growth parameters of safflower.*

**Keywords:** Radicle, Seed vigor, Plumule, Weight, Length.

---

### **INTRODUCTION**

Safflower (*Carthamus tinctorius* L.) is a member of the family *Compositae* cultivated for its seed, which is used as edible oil. This crop was also grown for its flowers, used for colouring and flavouring foods and making dyes. It is a rich source of oil (35-40 %) and linoleic acid content (75-86 %). Safflower flowers are known to have many medicinal properties for curing several chronic diseases [1]. They are rich in vitamin A, iron, phosphorus, and calcium [2]. In addition, it also contains a small amount of oleic acid, linolenic acid, flavonoids, amino acids and polysaccharides [3]. Safflower is a highly branched, herbaceous, thistle-like annual, usually with many long sharp spines on the leaves.

In recent years, a lot of research has been done on the invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of some field crops [4]. Furthermore, the invigoration persists under less than optimum conditions such as salinity [5]. Seed invigoration treatments such as hydro-priming, osmo-priming, hardening, matirpriming and growth regulators have been successfully employed in many parts of the world.

There is different treatment for seed priming which polyethylene glycol (PEG) is one of the most prevalent treatment. This treatment does not have any physiological responses to seed and in order to having more molecular weight cannot pass the cell wall and so it be used for water potential adjustment in germination experiments [6, 7]. Osmoticums such as mannitol, polyethylene glycol and sodium chloride (osmo-priming) and in water (hydro-priming) has been reported to be an economical, simple and a safe technique for increasing the capacity of seeds to osmotic adjustment and enhancing seedling establishment and crop production under stressed conditions. This could be due to faster emergence of roots and shoots, lower incidence of resowing, more vigorous plants, better drought tolerance, early flowering, early harvest and higher grain yields under adverse conditions [8].

Ghiyasi *et al.* [9] demonstrated that, osmo-priming of *Zea mays* L. seeds with polyethylene glycol 8000 at -5 MPa osmotic potential had the positive effect on emergence, grain and biological yield compared with other treatments. Result of an experiment showed that, soybean seeds that primed with -1.2 MPa osmotic potential and 12 hours priming duration had most progressive effect on seed germination and vigor in compared with higher and lower treatments [7]. Golshani *et al.* [10] reported that, osmo-priming at lower concentration could improve rice (*Oryza sativa* L.) seed germination and seedling parameters. In this research, -3 MPa osmotic potential had better results in compared with -6 and -9 MPa. In other experiment, seed osmopriming improve final germination percentage, germination rate, seedling length and seed vigor index of bersim clover and maximum invigoration observed in seed osmoprimed at -8 MPa for 8 hours [11].

Therefore, the aim of present study was to evaluation the effect of osmopriming by PEG 6000 with different concentrations and priming duration on safflower seed germination and seedling growth.

## MATERIALS AND METHODS

A laboratory experiment was carried out at the Agronomy Department of Agriculture Faculty of Shahid Chamran University of Ahvaz, Iran, during 2011, to evaluation the osmo-priming effects on safflower (*Carthamus tinctorius* L.; cultivar Padideh) seed germination and seedling growth. The experimental design was completely randomized, with three replications. The treatments were arranged in a 4 × 3 factorial design.

For this purpose, initially seeds sterilized with ethanol 5% for 30 second and then maintained in sodium hypochloride 1% for 10 min. Then seeds washed with distilled water and dried between two paper towels. Sterilized seeds primed by different polyethylene (PEG 6000) levels or osmotic potentials (i.e. distilled water; OP<sub>0</sub>, -2 MPa; OP<sub>-2</sub>, -4 MPa; OP<sub>-4</sub> and -6 MPa; OP<sub>-6</sub>) for 12 (D<sub>12</sub>), 24 (D<sub>24</sub>) and 36 (D<sub>36</sub>) hours priming duration. After priming, seeds were washed with distilled water and then dried. 20 seeds from each of the treatments were placed in 9 mm diameter petri dishes on whatman filter paper that was moistened with 10 ml distilled water, and then petri dishes were maintained in a germinator at 25 °C for germination. Germination progress was measured daily at 24 hours intervals and contained for 7 days. Germination was considered when the radicles were 2 mm long. Final germination percentage, radicle and plumule length (mm) and weight (mg) were measured after the 7th day. Germination percentage [12], Germination rate [13] and seed vigor index [14] were calculated according to the following formula:

Germination percentage (%) = (Number of germinated seeds / Total number of seeds) × 100

Germination rate = Number of germinated seeds / Days of first count + ... + Number of germinated seeds / Days of final count

Seed Vigor Index = (Plumule length + Radicle length) × Germination percentage

The data collected were processed by analysis of variance (ANOVA) and analyzed with SAS software and charts were drawn using Excel program. Then treatment means were compared using the least significant difference (LSD) at 5% probability levels.

## RESULTS AND DISCUSSION

### Germination percentage

Statistical analysis of variance results (Table 1) demonstrated that, safflower germination percentage significantly affected by priming duration ( $p < 0.01$ ). In contrast, this trait not influenced by osmotic potential levels and interaction of treatments. Mean comparison of germination percentage showed that, this trait decreased by increasing of priming duration from 12 to 36 hours (Table 2). But, had no significant differences from D<sub>24</sub> to D<sub>36</sub> (Table 2). Therefore, the highest (92.5 %) and lowest (71.25 %) germination percentage obtained by D<sub>12</sub> and D<sub>36</sub>, respectively

(Table 2). Also, result of Figure 1 indicated that, this trait not affected by various osmotic potential levels. Sadeghi *et al.* [7] reported that, soybean seed germination significantly affected by osmo-priming duration. 24 hours priming duration had the most inhibition effect on germination percentage as compared with 6, 12 and 18 hours. Reduction in germination percentage by the effects of PEG 6000 may be due to decrease in water potential gradient among seeds and their surrounding medium adversely affects seed germination percentage. Thus, the mobilization of stored reserves and synthesis of proteins in germinating embryos are not able to begin subsequent growth processes [15].

### Germination rate

Analysis of variance results indicated that, germination rate significantly affected by osmotic potential levels and priming duration at 1 and 5% probability levels, respectively (Table 1). In spite, interaction of treatments had no significant effect on this trait. Trend of germination rate changes under different osmotic potential levels revealed that, This trait had no significantly affected by decreasing of osmotic potential levels from 0 to -4 MPa. In spite, decreasing of osmotic potential from -4 to -6 MPa caused significant decrease in germination rate. Of course, no significant differences observed between OP<sub>-2</sub> and OP<sub>-6</sub> (Figure 2). So that, Maximum (12.85) safflower germination rate observed in OP<sub>0</sub> (without osmotic potential) and minimum (10.45) value observed in OP<sub>-6</sub> (Figure 2). Also, evaluation of different osmo-priming duration revealed that, this trait was significantly decreased by increasing of priming duration from 12 to 24 hours. But, increasing of priming duration from 24 to 36 hours had no significant effect on this trait safflower seeds that primed 12 hours (D<sub>12</sub>) had the highest (13.86) germination rate and 36 hours priming duration (D<sub>36</sub>) had the lowest (10.0) germination rate (Table 2). According to the results of Mubshar *et al.* [16], osmoprimed seeds exhibited germination rate in comparison to non-primed seeds. Osmopriming affected seed water absorption during germination. So that, the seed before being placed on their bed, it will absorb water. Then, metabolic reactions of germination are started. This leads to the synthesis or re-activating enzymes are effective in germination. In addition to, gene expression and synthesis of nucleic acids before planting are begun. These series of reactions are leading to improved germination of primed seeds [17].

### Plumule length

Based on statistical analysis results, plumule length significantly influenced by osmotic potential levels ( $p < 0.05$ ), priming duration ( $p < 0.01$ ) and interaction of treatments ( $p < 0.01$ ). Investigation of plumule length under different osmotic potential levels showed significant decrease as affected by decreasing of osmotic potential from -4 to -6 MPa (Figure 3). Highest (49.46 mm) plumule length obtained by OP<sub>0</sub> and lowest (39.87 mm) value for this trait observed in OP<sub>-6</sub>. Also, plumule length increased by increasing of osmo-priming duration from D<sub>12</sub> to D<sub>24</sub> and then significantly decreased by increasing of priming duration to 36 hours (D<sub>36</sub>) (Table 2). While, D<sub>36</sub> had the most inhibitory effect on this plumule length. Interaction of treatments showed that, OP<sub>0</sub> (control) with D<sub>24</sub> cause maximum (54.55 mm) plumule length, while, D<sub>36</sub> with OP<sub>-6</sub> cause minimum (12.18 mm) plumule length (Figure 9). Kadkhodaie and Bagheri [18] reported that, osmo-priming leads to the significant increase in the plumule length and plumule to radical ratio in the linseed seeds. According to the obtained results on examining seed treatment and drought stress, significant hydro-priming treat against osmo-priming with germination percentage and rate features and plumule length can be the result of quick water absorption in hydro-priming seeds, and this process in the seed will be done with a more speed. As a result of the water absorption cell dividing will be done with more speed and this will increase the germination percentage and rate and radicle and plumule length.

### Radicle length

Analysis of variance showed that safflower radicle length significantly affected by osmotic potential ( $p < 0.01$ ), priming duration ( $p < 0.05$ ) and interaction of them (OP  $\times$  D) ( $p < 0.01$ ). Mean comparison results revealed that, radicle length significantly decreased by decreasing of osmotic potential from -2 to -6 MPa and safflower maximum (46.14 mm) radicle length obtained in OP<sub>-2</sub> and lowest (30.1 mm) radicle length obtained in seeds that primed with OP<sub>-6</sub> (Figure 4). Osmo-priming duration data showed that, D<sub>12</sub> and D<sub>36</sub> hours had the highest (42.56 mm) and lowest (35.82 mm) radicle length, respectively (Table 2). OP<sub>-2</sub> improved radicle length in compared with other osmotic potential levels (Figure 4). Interaction of treatments indicated that, OP<sub>0</sub> with D<sub>36</sub> improved radicle length and cause maximum (49.47 mm) of this trait. Also, OP<sub>-6</sub> with D<sub>36</sub> had the most inhibition effect on this trait and minimum (9.64 mm) radicle length obtained by this treatment (Figure 10). Basra *et al.* [19] reported the increase of radicle and plumule growth of primed onion seeds in 7 days after sowing and the primary seedling growth.

### Radicle to plumule ratio

Analysis of variance (ANOVA) results showed that, radicle to plumule ratio high significantly ( $p < 0.01$ ) affected by osmotic potential levels (OP) and priming duration (D). In spit, interaction of treatments (OP  $\times$  D) had no significant effect on this trait (Table 1). By increasing of osmotic potential from zero to -2 MPa this trait improved from 0.9 to 1.3. Then by increasing osmotic potential from -2 to -6 MPa R/P significantly decreased and lowest R/P obtained by -6 MPa osmotic potential. Maximum (1.03) radicle to plumule ratio obtained by OP<sub>-2</sub> and minimum (0.76) value observed in seeds that affected by OP<sub>-6</sub> (Figure 5). Also, this trait significantly decreased by increasing of osmo-

priming duration from 12 to 24 hours and then decreased by increasing of osmo-priming duration from 24 to 36 hours. Therefore, D<sub>36</sub> and D<sub>24</sub> had the maximum (0.97) and minimum (0.76) radicle to plumule ratio, respectively (Table 2). Similar to our result, Neamatollahi *et al.* [20] reported that, root to shoot ratio decreased by increasing of PEG concentration. Also, in their experiment maximum and minimum root to shoot ratio were obtained at -0.3 and -0.9 MPa, respectively.

### Seed vigor index

The ANOVA results for safflower seed vigor index showed that, this trait high significantly affected by osmotic potential levels, priming duration and interaction of treatments at 1% probability level (Table 1). Similar to other traits, seed vigor index significantly decreased by decreasing of osmotic potential from -4 to -6 MPa. Of course, no significant effect observed as affected by osmotic potential from 0 to -4 MPa. Highest (81.73) seed vigor index observed in OP<sub>0</sub> and lowest (52.28) value obtained in OP<sub>-6</sub> (Figure 6). Seed vigor index significantly decreased by increasing priming duration. Maximum (86.21) and minimum (55.37) seed vigor index obtained by D<sub>12</sub> and D<sub>36</sub>, respectively (Table 2). Interaction of treatments showed that, OP<sub>0</sub> with D<sub>12</sub> and OP<sub>-6</sub> caused maximum (92.67) and minimum (14.83) seed vigor index. Rouhi *et al.* [21] mentioned that, vigor index was higher in the primed seed with -0.8 MPa of PEG 6000 for 16 hours was higher in compared with other treatments.

### Plumule weight

Analysis of variance results about plumule weight demonstrated that, this trait no affected by osmotic potential levels (OP), priming duration (D) and interaction of them (OP × D) (Table 1). Also, no differences observed between various osmotic potential levels (Figure 7). Therefore, this trait showed tolerance to increasing of polyethylene concentration and priming duration.

### Radicle weight

Radicle weight was significantly affected by osmotic potential (OP), priming duration (D) and interaction of treatments (OP × D) ( $P < 0.01$ ) (Table 1). This trait significantly decreased with decreasing of osmotic potential from OP<sub>0</sub> to OP<sub>-2</sub>. While, no significant differences observed among osmotic potential levels from OP<sub>-2</sub> to OP<sub>-6</sub>. The highest (0.36 mg) radicle weight obtained in OP<sub>0</sub> (control) and lowest (0.28 mg) value for this trait observed in OP<sub>-6</sub> (Figure 8). Also, evaluation different priming period showed that, D<sub>24</sub> improved radicle weight and caused highest (0.36 mg) radicle weight. Also, safflower in D<sub>36</sub> with no significant differences with D<sub>12</sub> had the lowest (0.27 mg) radicle weight (Table 2). Investigation of osmotic potential levels and priming duration revealed that, in OP<sub>0</sub> by increasing of priming duration radicle weight increased, while in other in other osmotic potential levels, this trait increased by increasing of priming duration from 12 to 24 hours and then decreased by increasing of priming duration from 24 to 36 hours. Similar to our result, Rouhi *et al.* [22] reported that, osmo-priming treatments had positive effects on seedlings dry weight in all of the investigated Bersim clover species (primed and non primed).

**Table 1: Analysis of variance results of safflower germination and seedling growth as affected by different osmotic potential levels and priming duration.**

S.O.V.	d.f.	Mean of square							
		GP (%)	GR	P length	R length	R/P	SVI	P weight	R weight
OP	3	137.96 <sup>ns</sup>	9.4*	165.46*	467.04**	0.14**	1415.1**	0.1 <sup>ns</sup>	0.01**
D	2	1393.75**	55.22**	945.31**	136.41*	0.17**	2871.76**	0.09 <sup>ns</sup>	0.03**
OP × D	6	34.49 <sup>ns</sup>	0.52 <sup>ns</sup>	345.87**	311.78**	0.02 <sup>ns</sup>	628.1**	0.1 <sup>ns</sup>	0.01**
Error	24	129.55	3.11	43.47	30.66	0.41	2652.15	0.08	0.001

\*, \*\*, ns, Significant at  $P = 0.05$ ,  $P = 0.01$  and non-significant, respectively. d.f. degree of freedom. S.O.V: source of variation. GP: germination percentage. GR: germination rate. P: plumule. R: radicle. SVI: seed vigor index.

**Table 2: Safflower seeds germination and seedling growth parameters as affected by different priming duration**

Treatment	GP (%)	GR	P length (mm)	R length (mm)	R/P	SVI	P weight (mg)	R weight (mg)
D <sub>12</sub>	92.5 a	13.86 a	48.25 a	42.56 a	0.86 a	86.21 a	1.39 a	0.3 b
D <sub>24</sub>	78.75 b	10.32 b	53.5 a	39.33 ab	0.74 b	68.67 b	1.26 ab	0.36 a
D <sub>36</sub>	71.25 b	10 b	36.19 b	35.82 b	0.97 a	55.37 c	1.23 b	0.27 b

In each section, means followed by the same letter within columns are not significantly different ( $p = 0.05$ ) according Duncan test D: priming duration (hours), GP: germination percentage. GR: germination rate. P: plumule. R: radicle. SVI: seed vigor index.

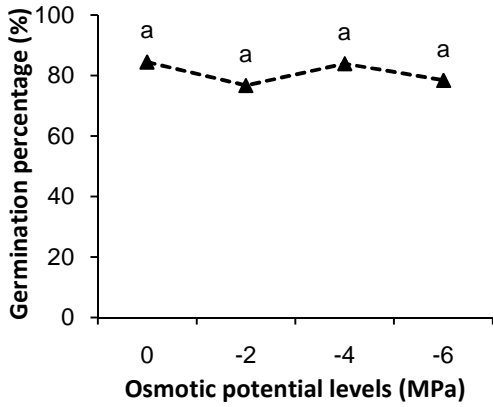


Figure 1: Safflower seed germination percentage as influenced by osmotic potential levels.

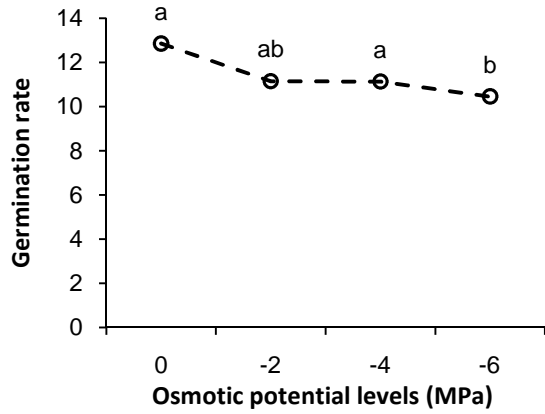


Figure 2: Safflower seed germination rate as influenced by osmotic potential levels.

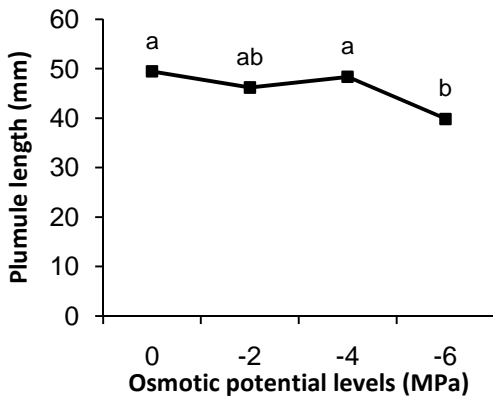


Figure 3: Safflower plumule length as influenced by different osmotic potential levels.

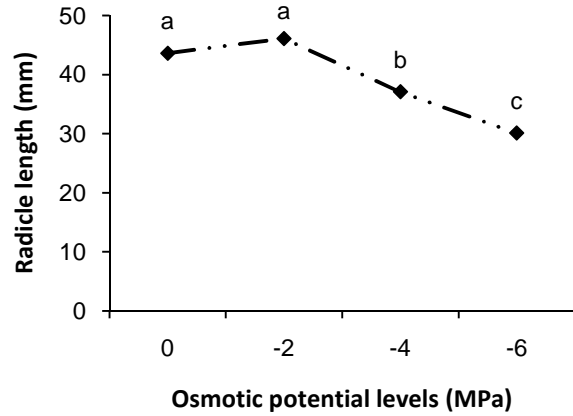


Figure 4: Safflower radicle length as influenced by different osmotic potential levels.

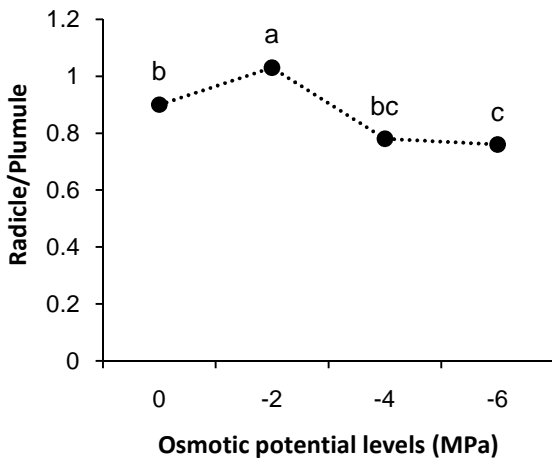


Figure 5: Safflower radicle to plumule ratio length as influenced by different osmotic potential levels.

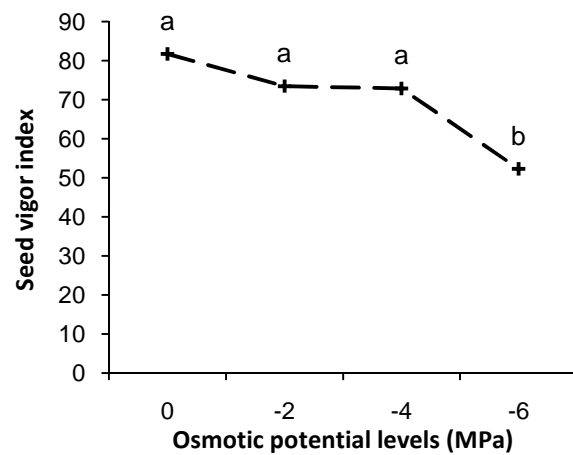


Figure 6: Safflower seed vigor index as influenced by different osmotic potential levels.



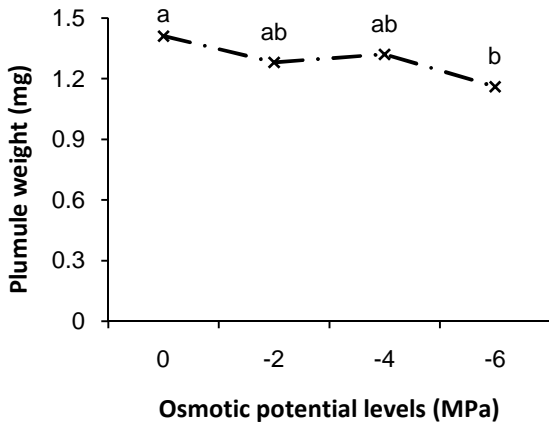


Figure 7: Safflower plumule weight as influenced by different osmotic potential levels.

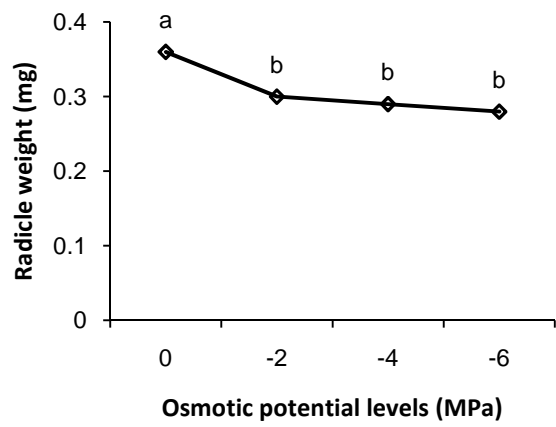


Figure 8: Safflower radicle weight as influenced by different osmotic potential levels.

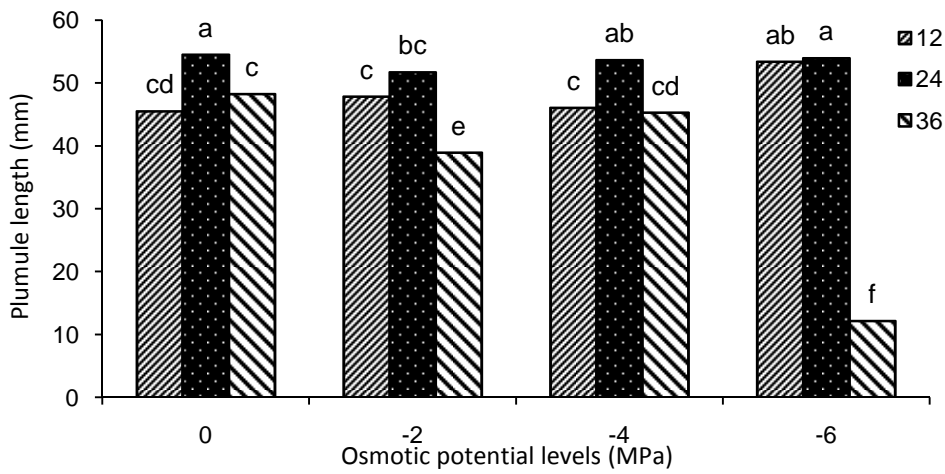


Figure 9: Comparison of safflower plumule length as influenced by different osmotic potential levels and priming duration.

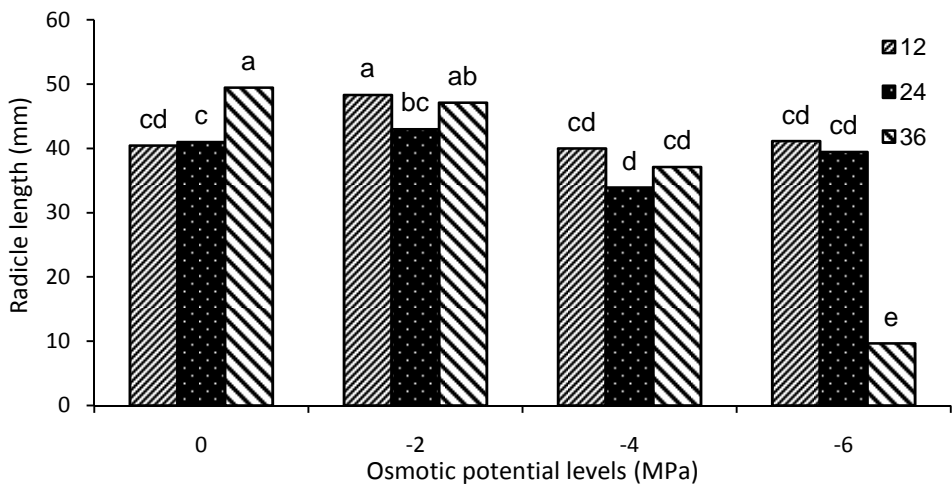
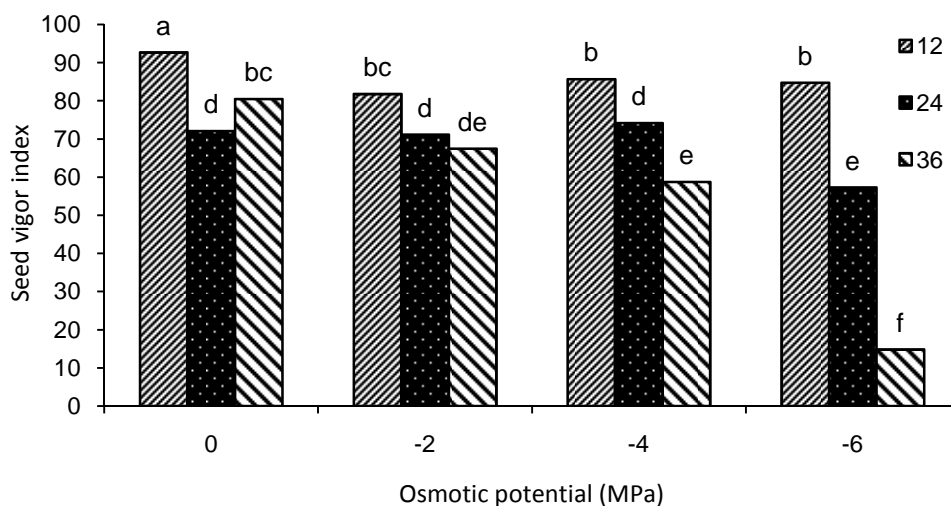
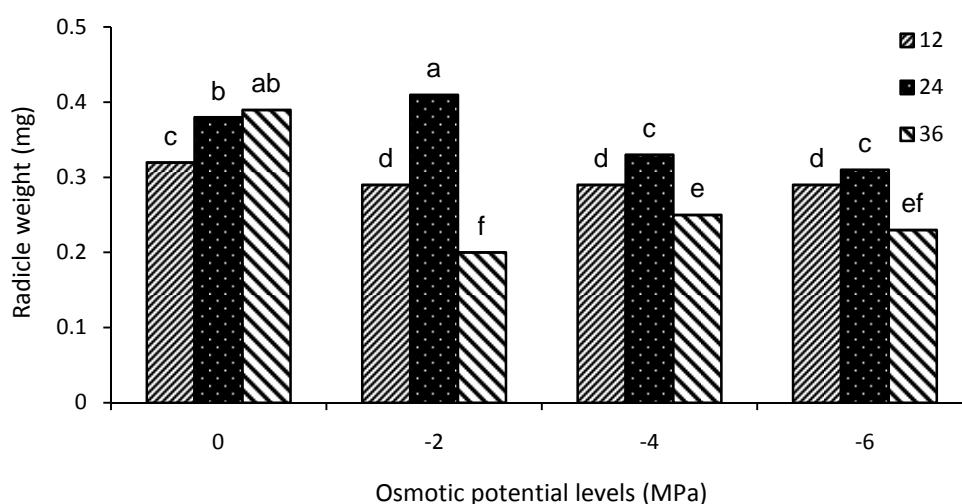


Figure 10: Comparison of safflower radicle length as influenced by different osmotic potential levels and priming duration.



**Figure 11: Comparison of safflower seed vigor index as influenced by different osmotic potential levels and priming duration.**



**Figure 12: Comparison of safflower radicle weight as influenced by different osmotic potential levels and priming duration.**

### CONCLUSION

According to the results, all studied traits significantly affected by osmotic potential levels except germination percentage and plumule weight. Also, priming duration had significant effect on all germination and seedling growth characteristic except plumule weight. Plumule and radicle lengths, seed vigor index and radicle weight significantly affected by interaction of treatments. All studied traits decreased by decreasing of osmotic potential levels. Results of present study suggest that, osmotic potential increasing and reduction of priming duration could improve seed germination and seedling growth parameters of safflower.

### REFERENCES

- [1] H. H. Mündel, *Madras Agric. J.*, **1969**, 56,772-777.
- [2] N. Nimbkar, *Times Agric. J.*, **2002**, 2, 32-36.
- [3] J. Zheng; Functional food. Chemical Industry Press, Beijing, pp. 730-733, **1999**.
- [4] S. Basra, E. Ullah, E.A. Warriach, M.A. Cheema, L. Afzal, *Int. J. Agric. Biol.*, **2003**, 5: 117-120.
- [5] T. Muhyaddin, H.J. Weibe, *Seed Sci. Technol.*, **1989**, 17, 49-56.
- [6] W.E. Emmerich, S.P. Hardgree, *Agron. J.*, **1990**, 82, 1103-1107.
- [7] H. Sadeghi, F. Khazaei, L. Yari, S. Sheidaei, *ARPN J. Agric. Biol. Sci.*, **2011**, 6, 39-43.
- [8] H.C. Passam, D. Kakouriotis, *Hortic. Sci.*, **1994**, 57, 233-240.

- [9] M. Ghiyasi, M. Pouryousef Myandoab, M. Tajbakhsh, H. Salehzadeh, M.V. Meshkat, *Res. J. Biol. Sci.*, **2008**, 3, 1452-1455.
- [10] M. Golshani, H. Pirdashti, K. Saeb, B. Babakhani, A. Heidarzade, *World Appl. Sci. J.*, **2010**, 9, 221-225.
- [11] H.R. Rouhi, M.A. Aboutalebian, F. Sharif-Zadeh, *Int. J. AgriScience.*, **2011**, 1, 701-774.
- [12] C.A. Parera, D.J. Cantliffe, *Hort. Review.*, **1994**, 16, 109-139.
- [13] AOSA; Seed vigor hand testing book. Contribution No. 32 to the handbook on seed testing, Association of official seed analysis, Springfield, IL, **1983**.
- [14] A.A. Abdul-Baki, J.D. Anderson, *Crop Sci.*, **1973**, 13, 630-637.
- [15] S. Ramagopal, *J. Plant Physiol.*, **1990**, 136, 621-625.
- [16] H. Mubshar, M. Farooq, S.M.A. Basra, N. Ahmad, *Int. J. Agri. Biol.*, **2006**, 8 (1): 14-18.
- [17] M. Ghiyasi, M. Tajbakhsh, R. Amirnia, *Adv. Environ. Biol.*, **2011**, 5, 3540-3542.
- [18] A. Kadkhodaie, M. Bagheri, *World Acad. Sci. Eng. Tech.*, **2011**, 73, 373-377.
- [19] A.S. Basra, B. Singh, C.P. Malik, *Biol. Plantarum.*, **1994**, 36, 365-371.
- [20] E. Neamatollahi, M. Bannayan, A. Ghanbari, M. Haydari, A. Ahmadian, *Not. Bot. Hort. Agrobot. Cluj.*, **2009**, 37, 190-194.
- [21] H.R. Rouhi, R. Tavakkol Afshari, S.A. Moosavi, M.H. Gharineh, *Not. Sci. Biol.*, **2010**, 2, 59-63.
- [22] H.R. Rouhi, M.A. Aboutalebian, A. Sepehri, F. Karim, S.A. Moosavi, *Afr. J. Biotechnol.*, **2010**, 10, 19084-19088.