Effects of seed priming with gibberellin on germination of safflower under salinity stress

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Abstract

Salinity is one of the most important factors limiting plant growth and production around the world. Gibberellic acid is known as a strong growth stimulator, effective on seed germination and breaking of seed dormancy in various plant species. In order to study the response of Safflower cultivar Sofeh to salinity stress, a factorial experiment was conducted based on a randomized complete block design with three replications in Pardis Agricultural Research and Education Center, Saveh, Iran. The first factor was salinity at three levels including 0, 100, and 200 mM NaCal. The second factor was seed priming with gibberellin at three levels including 0, 20, and 40 mgl^{-1}. Findings suggested that the salinity and seed priming improved biochemical indexes of proline and peroxidase in the seedlings under study, increasing their mean germination time, germination rate, germination percentage, and chlorophyll contents. Maximum proline contents were observed in the seedlings treated with 200 mM salinity and primed with 49 mgl^{-1}gibberelin, 3.48 mg per gram fresh weight. Maximum mean germination time, germination rate, germination percentage, and chlorophyll contents were related to 0 mM salinity and 40 mgl^{-1}gibberellin at 2.86 days, 26.16%, 76.6%, and 8.05%, respectively. In sum, the findings suggested that priming of safflower seeds with gibberellin under saline conditions improves the plants' resistance against salinity and their germination and growth.

Keywords: Peroxidase; priming; proline; safflower; salinity

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Introduction

Despite remarkable developments in expansion of agriculture and food production in recent years, the number of people who suffer from malnutrition around the world is on the increase. The rapid growth in the world population and the concomitant needs for the production of more food have led to an increased tendency to exploit saline soil and water resources and this in turn has resulted in accumulation of lots of experiences around the world on making use of these disadvantageous resources (Hashemi Nia et al., 1997). Wise and carefully managed use of saline water can increase the availability of irrigation water as there is a considerable body of salty water around the world (Rhoads, 1992).

Plants' response to abiotic stresses vary and their ability to adapt to such stresses depend

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on the duration of stress, the species under stress, and the stage of stress (Munns and Tester, 2008). Plants' resistance against salinity varies and safflower is generally considered as a salinity resistant crop (Shannon, 1997). In fact, it is possible to use saline water for irrigation purposes in safflower farms provided that the salinity level is kept below 8 dS/m (Bassil and Kaffka, 2002). The aim of safflower cultivation is oil extraction. Therefore, the effect of salinity on the oil yield of this species is important (Nalz, 1989).

So far, researchers have done lots of work to help improve seed germination under farm conditions. One of the achievements have been the proposal to manage germination through pre-sowing treatment of the seeds which is technically called seed priming (Harris, 2006). Through this procedure, seeds are soaked in water, growth stimulating or inhibitory hormones, or various osmotic solutions before they are dried to the original moisture level. Soaking the seeds in water induces some biochemical processes required for the start of germination process e.g., breaking seed dormancy, hydrolysis, metabolism of the inhibitory substances, absorption of water, and enzymatic activities. Some or all of these processes, which facilitate germination, occur as a result of seed priming and their effects are still observed in the seeds after drying them again (Asgedom and Becker, 2001). By its positive effects on facilitating the seedling's growth and its better and faster establishment, faster coverage of the soil surface, the improved power of competing with unwanted weeds, improved root development, and therefore its more water and nutrient absorption capability, priming can improve crop performance and particularly under unfavorable abiotic conditions, its positive influences are better revealed (Eisvand et al., 2010).

Soaking the seeds in plant growth regulating substances such as gibberellin, salicylic acid, auxin, ethephon, and abscisic acid is called hormonal priming. Gibberellic acid is considered as a strong stimulator effective on seed germination and breaking of seed dormancy in various plant species (Kochaki and Sarmadnia, 1999). Therefore, considering the significance of the issue the present study was conducted in Saveh, Markazi Province, central Iran with the aim of assessing and analyzing the effects of priming safflower seeds under salinity stress and at different stages of the plants' development.

Materials and Methods

In order to investigate the response of Safflower plants, Sofeh cultivar, to salt stress, a factorial experiment was conducted based on a randomized complete block design with three replications in the Laboratory of Pardis Agricultural Research and Education Center, Saveh, Iran (1108 m above sea level; 50/20 °N, 35/03 °E) during 2017-2018. The first factor was three concentrations of salinity (0. 100, and 200 mM) and the second factor was seed priming with three levels of gibberellin (0, 20, and 40 mg/l). Seeds of safflower cultivar Sofeh were obtained from Karaj Seed and Plant Improvement Institute, Oil Seeds section. Sofeh is a cultivar based on the selection of single plants from the local safflower plants in Isfehan through the selection method of special lines with an emphasis on the homogeneity of the red color of florets, the number and size of bolls, lack of thorns, early maturity, the adjacency of the angle between the main stem and the sub-branches, and suitable height of the plants for mechanized harvest.

The study was carried out in sterilized petri dishes, 9 cm in diameter in the laboratory. Three concentrations of gibberellin (0, 20, and 40 mg/l) were used in petri dishes for priming treatment and in each replication, 30 primed seeds were put in a germinator set at 25 ℃ for 14 days. All dishes and seeds were sterilized prior to the experiment using sodium hypochlorite solution 3%. Priming solutions were prepared by adding 1 l distilled water to each of the three levels of gibberellin concentrations in the study to obtain 0, 20, and 40 mg/l solution. After the priming period, the seeds were thoroughly washed several times with distilled water and were kept in room temperature and under dark conditions to dry to their original weights. The seeds were observed on a daily basis and the number of germinated seeds was recorded. Emergence of radicles 2 mm in length was considered as germination. Counting and recording of the germinated seeds were continued by day 7 when no more changes were observed in the number of germinated seeds.

Analysis of the seedlings started on day 14. Each level of the saline solution was prepared by adding 1 ml distilled water to the relevant amount of NaCl and the solution was added to the pots. The seeds' mean germination time, germination rate, and germination percentage along with the seedlings' proline, peroxidase, and chlorophyll contents were assayed in the study. Bates (1973) was used in order to assay the seedlings' proline contents and a number of the primed seeds were randomly selected for this purpose. Peroxidase contents of the seedlings were also assayed based on the method of Fayer (1976) again with a number of seeds randomly selected after priming. Mean germination time was calculated using the following equation (Matthews and Khajeh Hosseini, 2006):

$$MGT = \Sigma (nt) / \Sigma(n) \quad (1)$$

where n is the number of germinated seeds in each day and t is the number of day when the counting was done. Germination rate was assayed based on Agrawal (2004):

$$GR = \Sigma (GT / DT) \quad (2)$$

where GR, GT, and DT are germination rate, the number of seeds germinated on day t, and the number of days after sowing based on GT, respectively. Germination percentage (GP) was obtained based on Lanucci et alo. (2000):

$$GP= (GS/ TS) \times 100 \quad (3)$$

where GP, GS and TS are germination percentage, the number of germinated seeds in e ach day, and the total number of seeds, respectively. Finally, chlorophyll contents were assayed using the method of Arnon (1967) and 0.2 g leaf samples were used in acetone 80% for this purpose. The resulting extract was then put on the filter paper and some acetone was added to the solution to adjust it to 25 ml in order to extract the leaf chlorophyll thoroughly. SAS was used for statistical analyses of the obtained data based on Duncan's test for comparisons of means among treatments ($p \leq 0.05$).

Results

Physiologic features

Analysis of variance (Table 1) showed that the interaction of effects of salinity and seed priming were significant on the proline contents of the seedlings ($p \leq 0.01$). In fact, under 200 mM salinity, gibberellin priming resulted in an increase in the proline contents of the seedlings under study; however, no significant difference was observed under 100 mM salinity and priming and non-priming condition. Maximum proline contents were related to the 0 mM salinity and 20 mg/l gibberellin (3.42 mg per gram fresh weight) and also the treatment involving 200 mM salinity and 40 mg/l gibberellin (3.50 mg per gram fresh weight). On the other hand, as Table (2) shows, Minimum proline contents were observed in the treatment involving 200 mM salinity and no gibberellin (control)(1.98 mg per gram fresh weight), the treatment with 100 mM salinity and 0 mg/l gibberellin (2.04 mg per gram fresh weight), and also the plants treated with 0 mM salinity and 0 mg/l gibberellin (1.98 mg/g fresh weight).

Analysis of variance showed that the interaction of effects of salinity and seed priming was significant at $p \leq 0.01$ (Table 1). Under zero salinity stress and also 100 mM salinity conditions,

Table 1
Analysis of variance of mean germination time, germination rate, germination percentage, and proline and peroxidase content of the seedlings and seed yield in safflower

		Square Means					
S.O.V	df	MGT	GR	GP	Proline	Peroxidase	Chlorophyll
Blocks	٢	0.02346790$^{n.s}$	0.3364198$^{n.s}$	15.110927$^{n.s}$	0.09414938$^{n.s}$	0.06580370*	0.03454198$^{n.s}$
EC	٢	0.26704568**	102.9845679**	568.833486**	0.08950494$^{n.s}$	0.12033704*	18.04904198**
Error	٤	0.00984383	0.5401235	49.229412	0.04683642	0.02001296	0.03101975
Priming	٢	0.05305309*	422.2623457**	3671.823857**	12.80074568**	10.93911481**	9.66196420**
EC*P	٤	0.05334012*	92.1327160**	191.982948**	0.31220494**	0.40671852**	2.65155864**
C.V.(%)		4.367453	3.395479	8.187508	7.667877	0.50971481**	2.527074

**: significant at $p \leq 0.01$; *: significant at $p \leq 0.05$; ns: not significant; GP: germination percentage; GR: germination rate; MGT: mean germination time

seed priming and raising the level of gibberellin concentration increased the seedlings' peroxidase contents while no significant change in the peroxidase levels was observed under 200 mM salinity and priming and non-priming conditions. Maximum peroxidase levels were observed in seedlings treated with 0 mM salinity and 40 mg/l gibberellin (3.48 mg/g fresh weight) and also those treated with 200 mM salinity and 40 mg/l gibberellin (3.47 mg/g fresh weight). The minimum peroxidase content (1.97 mg/g fresh weight) was related to the highest salinity level, i.e. 100 mM NaCl, and 0 mg/l gibberellin (control). At each level of salinity, seed priming with gibberellin resulted in higher peroxidase contents compared with non-priming treatments (Table 2).

The interaction of effects of salinity and priming on chlorophyll contents was also significant at p≤0.01 (Table 1). At each salinity level, seed priming with gibberellin led to the increased chlorophyll contents as compared with the control and the maximum chlorophyll content (8.05%) was recorded under 0 mM salinity and 40 mg/l gibberellin while the minimum content (5.19%) was related to the treatment involving 0 mM salinity and 0 mg/l gibberellin (Table 2).

Germination indexes

Findings showed that the interaction of effects of salinity and seed priming treatments on mean germination time of safflower seeds under study was significant at p≤0.05 (Table 1). With the reduction of salinity and increased gibberellin concentration used in priming, the mean germination time at each level of salinity increased so that generally, the highest mean germination time (2.86 days) was recorded under 0 mM salinity and 40 mg/l gibberellin treatment while the lowest value (2.49 days) was related to 200 mM salinity and 40 mg/l gibberellin treatment (Table 2).

Germination rate showed significant difference (p≤0.01) under the interaction of effects of salinity and priming treatments (Table 1). Maximum germination rates were related to the seeds treated with 0 mM NaCl and 40 mg/l gibberellin (28.16%) and 200 mM NaCl and 40 mg/l gibberellin (19.16%). On the other hand, the minimum germination rate (15.11%) was observed under 200 mM salinity and 0 mg/l gibberellin (Table 2).

Significant differences (p≤0.01) were observed in germination percentages under combined effects of salinity and gibberellin priming treatments (Table 1). At each level of salinity, seed priming with gibberellin increased germination percentage while salinity alone reduced germination percentage so that maximum (76.6%) and minimum (44.4%) germination percentages were recorded under 0 mM salinity and 0 mg/l gibberellin and also 200 mM salinity and 0 mg/l gibberellin treatments, respectively (Table 2).

Discussion

Salinity and gibberellin priming treatments significantly affected the proline contents of the safflower seedlings under study.

Table 2
Comparison of mean effects of salinity and priming interactions on mean germination time, germination rate, germination percentage, seedling proline, seedling peroxidase, and safflower seed yield

Priming	MGT (day)	GR (per day)	GP (%)	Proline (mg/g)	Peroxidase (mg/g)	Chlorophyll (%)
1	2.61^{b}	21.55^{d}	60.3^{d}	1.89^{c}	2.51^{d}	5.19^{h}
1	2.58^{bc}	22.66^{b}	64.0^{d}	2.04^{c}	1.97^{f}	5.64^{f}
1	2.49^{c}	15.11^{g}	44.4^{f}	1.98^{c}	3.19^{c}	5.36^{g}
2	2.86^{a}	20.05^{c}	52.5^{e}	3.42^{a}	2.31^{e}	5.97^{e}
2	2.69^{b}	19.94^{e}	59.9^{d}	2.95^{b}	3.32^{bc}	6.01^{e}
2	2.74^{b}	^{c}21.77	72.20^{c}	2.98^{b}	3.38^{ab}	7.47^{b}
3	2.89^{a}	28.16^{a}	76.6^{a}	3.01^{b}	3.48^{a}	8.05^{a}
3	2.74^{b}	18.5^{h}	52.2^{f}	2.84^{b}	3.34^{ab}	6.7^{c}
3	2.71^{b}	19.16^{a}	76.2^{b}	3.50^{a}	3.47^{a}	6.25^{d}

Similar letters in each column and row do not differ significantly. Salinity 1, 2, and 3 are 0. 100, and 200 mM, respectively. Gibberellin 1, 2, and 3 are 0, 20, and 40 mg/l, respectively.

Increasing salinity also improved proline contents in this study. These are in agreement with the findings of Gibon et al. (2002) on canola, Yamada and Matssumono (1989) on barley, and Ghoulam et al. (2002) on sugar beet. Accumulation of proline is common in the plants under salinity stress as it plays a role in osmotic adjustments and enzymatic activities of the plants (Greenway and Munns, 1980).

Research showed that 100 mM salinity reduced enzymatic activities such as those of peroxidase in barley (Tracey et al., 2003). Jokar Tang Karami et al. (2016) in their study on two genotypes of marigold seeds reported that peroxidase enzyme activity in the primed seeds increased at germination stage and under salinity stress, activities of peroxidase and ascorbate peroxidase in the primed seeds were more than the non-primed seeds.

The change in the concentration of chlorophyll is considered as a short term reaction against stress. Under saline conditions, due to too much increase in the Na^+ ions, the chlorophyll damages while priming can reduce the devastating effects of salinity (Ahraf et al., 1997). Eisvand et al. (2010) found that gibberellin increased chlorophyll contents in the tall wheatgrass seedlings under study. Under salinity stress, chlorophyll and carotenoid contents of chickpeas reduced while proline content and activities of peroxidase enzyme increased. They also rep found that in the chickpeas under gibberellic acid and salinity treatment, chlorophyll contents and catalase enzyme activities increased and this is in agreement with the findings of the present study. The germination index of marigold seeds primed with gibberellin and under various levels of salinity stress was found to increase (Rezaei et al., 2013). Also, 150 mM salinity reduced mean germination time and growth of wheat seedlings (Afzal et al., 2007).

Various methods of priming can increase the rate and homogeneity of germination in seeds under stress conditions(Heydecker and Coolbear, 1978). Priming the safflower seeds with gibberellin mitigated the deleterious effects of salinity stress. Priming was also reported to improve the percentage and rate of germination in wheat seeds under stress (Zarafshaniet al., 2013). Finally, Eisvand et al. (2010) found that priming the tall wheatgrass seeds with gibberellin improved germination indexes and led to improved growth of the seedlings.

Conclusion

Based on the findings of the present study, under 100 and 200 mM salinity, increasing the concentration of gibberellin in priming increased enzymatic activities in safflower seedlings; however, under control salinity level, no significant changes were observed in the enzymatic activities. Generally, the highest levels of proline, peroxidase, and chlorophyll were related to the increased levels of priming and with an increase in the concentration of priming solution (gibberellin) the seedlings' tolerance against salinity stress also increased. Reduced salinity and increased priming resulted in the increased mean germination time, germination rate, and percentage in safflower. Priming with gibberellin mitigated the adverse effects of salinity stress on the growth and development of safflower seedlings. For example, 20 mg/l gibberellin played a role in adjusting the seedlings' response to salt stress and was able to improve the growth and nutrition in safflower seedlings under salinity stress.

References

Afzal, I. S. M. A., Basra, T., ElahiLodhi and **S. Jaaved Butt.** 2007. ' Improving germination and seedling vigour in wheat by halopriming under salt condition'. *Pakestan Journal Agriculture* .Sci, 44: 40-49.

Arnon, A. N. 1967. 'Method of extraction of chlorophyll in the plants'. *Agronomy Journal*. 23: 112-121.

Agrawal, R. L. 2004. 'Seed technology'. Oxford and IH Publishing Co. LTD. New Delhi.

Ashraf, M. and **M. R. Foolad**. 2007. 'Pre sowing seed treatment Ashotgun approach to improve germination, plant growth, and crop yield under saline and non saline conditions'. **Advance in Agro**, 88: 223-265.

Asgedom, H. and **M., Becker**. 2001. 'Effects of seed priming with different nutrientsolutions on germination seedling growth and weed competitiveness of cereals in Eritreain Prroc

Deutscher Tropentag '. University of Bonn and ATSAF Margraf Publishers Press Weickersheim, 76 :282-299.

Bates, L. 1973. 'Rapid determination of free proline for water stress stadies'. *Plant & Soil*, 39, 205-207.

Bassil, E. S. and **S. R. Kaffka**. 2002. 'Response of safflower to saline soils and irrigation: II. Crop response to salinity'. *Agricultural Water Management*, 54, 81-92.

Eisvand, H. R. R. Tavakkol Afshari, F. Sharifzadeh, H. Maddah Arefi, and **S. M. Hesamzadeh**. 2010. 'Effects of hormonal priming and drought stress on activity and isozyme profiles of antioxidant enzymes in deteriorated seed of tall wheatgrass (*Agropyron elongatum* Host)'. *Seed Sci. Technol*. 38(2): 280-297.

Eisvand, H. R., M. A. Alizadeh and A. **Fekri, A**. 2010. 'How hormonal priming of aged and nonaged seeds of bromegrass affects seedling physiological characters'. *Journal of New Seeds*, 11(1): 52-64.

Fayer, M. D. 1983. 'New approach to probing polymer and polymer blend structure using electronic excitation transport'. Department of Chemistry, Stanford University, Stanford, California 94 :305-314.

Gibon, Y., R. Sulpice and **F. Larher**. 2002.' Proline accumulation in Canola leaf discs subjected to osmotic related to stress in the loss of chlorophylls and to the decrease of mitochondrial activity'. *Plant Physiol*. 110: 469-476.

Ghoulam, C., A. Foursy and **K. Fares.** 2002. 'Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars'. *Environmental and Experimental Botany* 47(1):39-50 .

Greenway, H. R. and **R. Munns**. 1980. 'Mechanisms of salt tolerance in nonhalophytes'. *Annu. Rev. Plant Physiol*. 31: 149-190.

Heydecker, W. and **P. Coolbear**. 1978. 'Seed treatment for improved performance: Surving and attempted prognosis'. *Seed Sci. Technol*. 5: 353-425.

Harris, D. 2006. 'Development and testing of 'on-farm' seed priming Advan'. Agron. 90: 129-178.

Matthews, S. and **M. , Khajeh Hosseini**. 2006. 'Mean germination time as an indicator of emergence performance in the soil of seed lots of Maize (*Zea mays*)'. *Seed Science and Technology*, 34: 339-347.

JokarTang Karami. M. A., Ghanbari and F. Moradi. 2016. 'Evaluation of the effect of salinity stress on the activity of some antioxidant enzymes in plants obtained from marigold seeds *Marianum silybum. Iranian Seed Science and Research* 40: 11-21.

Rezaei, M. M. , Sedghi and **R. , Seyed Sharifi**. 2013. 'Effect of various priming on germination characteristics and use of marigold seeds of *Calendula officinalis* L. under salinity conditions'. *Second National Congress of Organic Agricultur*, 167: 149-156.

Rhoades, J.D., A. Kandiah and **A. M. Mashali**. 1992. 'The use of saline waters for crop production'. *FAO irrigation and drainage*, 48: 115-125.

Shannon, M.C. 1997. 'Adaptation of plants to salinity'. *Adv. Agron*. 60(243): 75-120.

Tracey, A. C., A. J. Miller, S. A., Laurie and **R. A. Leigh**. 2003. 'Potassium activities in cell compartments of salt- grown barley leaves'. *J Exp Bot.*54(383):657-61.

Yamada, T. and **H. , Matssumono**. 1989. 'Accumulation of asparagines in NaCl stressed barley seedlings'. *Ber. Ohara Inst. Landwirtsch. Biol.* 19: 181-190.

Zarafshani, K. Sharifi, I., H., Azadi, G.H.,Hosseininia and **P., de Maeyer**. 2012. 'Drought vulnerability assessment: the case of wheat farmers in western Iran'. *Global and planetary change*, 98-99: 122-130.