

Germination, seed reserve utilization and seedling growth rate of five crop species as affected by salinity and drought stress

¹Saeed Sharafi, ²Zaneyar Khaneh, ³Somaye Akhlaghi and ⁴Zeinolabedin Jouyban

1- Arak University Iran, 2- Islamic Azad University, Mahabad Branch, Mahabad, Iran, 3- Karaj Payamnoor University, and 4- Young Researchers Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran

Email: sharafi.saeed@gmail.com; Tell: 09183641420 fax: 0861-2761007

Abstract: This experiment was conducted to determine the effects of salinity (osmotic pressure of 0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 MPa) and drought stress levels (osmotic pressure of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 MPa) on percent germination, germination rate, time to 10% germination, germination uniformity, seed reserves depletion, seed reserves utilization rate and seedling growth rate in *Hordeum vulgare*, *Brassic napus*, *Zea maize*, *Medicago sativa* and *Medicago scutellata*. All of the indices were affected by salinity and drought stresses. In corn, rapeseed and barley, seedling growth rate, percent germination and germination rate increased at lower levels of salinity, however, no significant differences were observed among them at different drought stress levels. Seed reserve depletion percentage (SRDP) and seed reserve utilization rate (SRUR) were higher in corn compared to other seeds in salinity treatments, while barely was the leading crop for the same parameters at drought stress treatments.

[Saeed Sharafi, Hamid Fateh, Somaye Akhlaghi and Zeinolabedin Jouyban. **Germination, seed reserve utilization and seedling growth rate of five crop species as affected by salinity and drought stress.** *Life Sci J* 2012;9(1s):94-101] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 16

Key word: Seed, Salinity, Drought, Germination

Introduction

Barley, corn, rapeseed, alfalfa and annual medic are major crops not only in Iran, but all around the world. Barley, Corn, Alfalfa and Annual medic, are very important for animals feeding in Iran agriculture. Leguminous crops are mostly salt sensitive (Mass and Hoffman, 1977; Chandra, 1980; Saxena, 1984). Annual medics can be planted in pastures and dry lands (Sharafi et al., 2006). Since Iran is located in arid and semi-arid regions of the world, the climatic conditions of this country is very critical in crop production. Results of Sharafi et al., (2006), indicated that annual medics have the ability for sowing in dry and rain fed farming systems, whereas some of researchers believe that this plant is a typical range-plant.

Since 91% of our required edible oil is imported, rapeseed can play a very important role in providing edible oil in Iran. This plant is drought resistant and could be produced in large areas of the country in rotation with wheat at our dry farming system.

Salinity and drought are major environmental limiting factors in plant growth and productivity (Allakhverdive et al., 2000b). The detrimental effects of high salinity and drought on plants can be observed at the whole-plant as the death of plants and/or decreases in productivity. During the onset and development of salt and drought stress within a seed, all major processes such as protein synthesis and energy and lipid metabolism are affected. Carbohydrates and other substrates which are needed for cell growth are mainly supplied through the

process of photosynthesis, and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl.

Salinity and drought stress biology and plant or seed responses to these environmental stresses have been discussed over last two decades (Flowers et al., 1977; Greenway and Munns, 1980; Ehret and Plant, 1999; Hasegawa et al., 2000; Zhu, 2002).

Drought along with salinity stresses is the most important and effective environmental factors in agricultural practices in arid and semi-arid regions of the world. Regarding the extent of arid and semi-arid regions and saline soils in Iran (more than 70% of arid and semi-arid lands). Selection of tolerant crops to the existing environmental conditions is of especial importance in crop production practices. The proper understanding of interactive responses of different crops to adverse conditions at different growth stages is a necessity for a sustainable crop production under adverse environmental conditions. The goal of this experiment was to determine the effect of drought and salinity stresses on little oil and forage crops at germination and early growth stages.

Materials and methods

Two separate experiments were conducted at Seed Research Laboratory of Shahrood University of Agricultural Sciences, Shahrood, Iran.

Salinity experiment

Factorial combinations of 5 crop species (*H. vulgare*, *B. napus*, *Z. maize*, *M. sativa* and *M.*

scutellata) and seven salinity levels were the treatments of the first experiment. The experimental design was a randomized complete block design with three replications per treatment. Osmotic pressure (salinity levels) of 0 (control), 0.25, 0.50, 0.75, 1, 1.25 and 1.5 MPa were created using NaCl, according to the Van Hoff formula presented in Salisbury and Ross (1996). Seeds were germinated in 10 cm Petri dishes with one Whatman No.1 filter papers moistened with the appropriate solutions or distilled water for 0 MPa. Twenty seeds per dish were used for each treatment. Seeds were incubated in the dark at 25 ± 1 °C in a controlled temperature room.

Three replicates of 20 seeds were weighed (W_1), dried at 104°C for 24 h and then reweighed (W_2). Seed water content was calculated as $[(W_1 - W_2)/W_2]$. The initial seed dry weight was calculated using the data for seed water content and W_1 . The initial 20 seed dry weight for *H. vulgaris*, *B. napus*, *Z. maize*, *M. sativa* and *M. scutellata* was 0.53, 0.14, 5.3, 0.06 and 0.41 gr, respectively.

After five days, oven-dried weight of seedlings was determined. The weight of utilized (mobilized) seed reserve was calculated by the dry weight of the original seed minus the dry weight of the seed remnant. Conversion efficiency of mobilized seed reserve into plant tissue was estimated by dividing seedling dry weight (SLDW) by the utilized seed reserve. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve depletion percentage (SRDP).

Seeds were observed daily for up to 5 days and considered germinated when the radicle was approximately 2 mm long or more. After five days seedling shoot and root lengths were measured and seedling dry weight was obtained after oven drying at 70°C . Seedling growth rate (mg d^{-1}) was calculated by seedling dry weight divided by five. Seed reserve utilization rate (mg d^{-1}) was calculated as the dry weight of the original seed minus the dry weight of seed remnant divided by five. Conversion efficiency of seed reserves into plant tissue (g g^{-1}), i.e. production of seedling dry matter per unit of usage of seed reserves, was estimated by dividing seedling dry weight by the total seed reserve depletion.

Estimates of time taken for cumulative germination to reach 10% (D10), 50% (D50) and 90% (D90) of maximum at each treatment were interpolated from the germination progress curve versus time. Then germination rate (R_{50} , day^{-1}) and germination uniformity (GU, h) was calculated as:

$$R_{50} = 1/D50 \quad (\text{I})$$

$$\text{GU} = D10 - D90 \quad (\text{II})$$

Germination uniformity obtained from eq.II is negative and the lower the value the lower the germination uniformity; but ignoring the minus sign indicates the time taken (h) for cumulative germination to go from 10% to 90% of the maximum. D10 can be interpreted as the time lag for germination, or the time to the beginning of germination (Soltani et al, 2002).

Drought experiment

Drought levels of 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4 MPa were created using polyethylene glycol (PEG), based on equation suggested by Michel and Kaufman (1983). The seeds were *H. vulgare*, *B. napus*, *Z. maize*, *M. sativa* and *M. scutellata*. All measurements were similar to salinity experiment.

Results and discussions

Abiotic stress accounts for more crop productivity losses than any other factor. Yet our ability to improve plant stress tolerance remains limited due to our lack of understanding of the inherent complexity of stress signaling and adaptation processes (Bohnert et al, 2002; Comis, 1997).

A summary of the analysis of variance (ANOVA) and main effects of crop species and osmotic pressure (OP) on germination components, i.e. seedling growth rate, total germination, germination rate, time to 10% germination, germination uniformity, seed reserve depletion, seed reserve utilization rate, root and shoot length is presented in table 1.

Germination and its attributes responses to drought:

Significant differences were observed for all germination traits among crop species. The only significant ($P < 0.05$ and 0.01) interaction effects were crop species \times salinity for total germination (GT), seed reserve depletion percentage (SRDP), seed reserve utilization rate (SRUR), and for crop species \times drought for germination rate (GR), seed reserve depletion percentage (SRDP), seed reserve utilization rate (SRUR) and root length (RTL) (table 1). These results were similar to that reported by Kheradnam and Ghorashy (1973) and Ashraf and Waheed (1992). Total germination was decreased by increment in OP from 0.5 MPa to 1.5 MPa. Germination rate and time lag of germination were progressively affected by OP lower than 0.5 MPa, but germination uniformity was decreased equally by OP of 0.25 MPa to 1.5 MPa. This is in agreement with reports by Soltani et al., (2002) in chickpea, Nafziger (1994) in wheat, Beveridge and Wilise (1959) in emergence of purple-flowered alfalfa and Johnson and Luedders (1971) and Jhonson and Wax (1978) in germination and emergence of soybean.

Table 1. Main and interaction effects of crop species, salinity and drought stress on seedling growth rate (SLGR, mg d⁻¹), total germination (R50, d⁻¹), germination rate (GR, d⁻¹), time to 10% germination (D10, h), germination uniformity (GU, h), seed reserve depletion percentage (SRDP, %), seed reserve utilization rate (SRUR, mg d⁻¹), root length (RTL, mm), shoot length (STL, mm).

Summary of ANOVA	SLGR	GT	R50	D10	GU	SRDP	SRUR	RTL	STL
Salinity experiment:									
Seed crop species (SS)	**	**	**	**	**	**	**	**	**
Salinity (S)	**	**	**	*	n.s	**	**	*	n.s
SS×S	n.s	*	n.s	n.s	n.s	**	**	n.s	n.s
Drought experiment:									
Seed crop species (SS)	**	**	**	**	n.s	**	**	**	**
Drought (D)	*	**	**	n.s	n.s	**	**	*	**
SS×D	n.s	n.s	**	n.s	n.s	**	**	*	n.s

* and ** significant difference at P< 0.05 and 0.01; n.s, not significant.

Germination and its attributes responses to salinity:

The crop species × salinity and crop species interactions are shown in figures 1, 2. Legume seeds are more susceptible to salinity and drought stress compared to grasses. Kheradnam and Ghorashy (1973) and Ashraf and Waheed (1993) reported similar results. Seed reserve depletion percentage and seed reserve utilization rate progressively decreased with declining OP (figs 1, 2). Seed reserve utilization rate was 7.3, 3.7, 14.9, 1.5 and 7.4 in salinity stress and 14.5, 3.4, 10.5, 1.5 and 8.03 in drought stress for barley, rapeseed, corn, perennial medic and annual medic, respectively. This result is similar to what reported by Williams (1967) in Woolly pod vetch and by Qiu et al. (1994) in common vetch. Significant crop species×OP interaction were found for total germination, seed reserve depletion percentage and seed reserve utilization rate in salinity experiment and for germination rate, seed reserve depletion percentage, seed reserve utilization and root length in drought experiment (table 1; figs 1,2,3) (Soltani et al, 2002). Effects of OP were significant for root length and shoot length, seedling growth rate (p<0.01). Results showed that OP significantly reduced root length, shoot length and seedling growth rate from highest to lowest level. As OP regime reduced from 0.25 to 0.75 MPa root length reduced significantly.

Salinity treatment:

Changes in seedling growth, germination rate of dissected embryos

There was increase rapeseed seedling growth with increase in salinity levels rather than other seedling. Whereas, under mild salinity treatment a similar trend were observed in all germinated embryos (0.75 MPa). On the contrary, under more salinity stress maize seedling growth considerably reduced. Stressed endosperms, however, showed a rapid recovery in water potential. In perennial and annual alfalfa no tolerance to salinity stress was observed. Furthermore, under stress treatment there was no

significant differences between these species (fig 1-a). According to the results, (fig 1-b, c) during germination, no considerable change in endosperm growth was observed. Although, extra germination percent and growth rate in barely, maize and rapeseed seedling was observed. Germination of perennial and annual alfalfa seeds in distilled water reached (10%) after 57, 83 h. respectively. In contrast, germination values of alfalfa endosperm did show much difference in stressed condition, probably because cell expansion was not accompanied by cell division. In earlier research, Gill and Singh (1985) have reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stresses. Changes in anyone of these processes can affect other metabolic activities, particularly carbohydrate metabolism that plays an important role in germination and seed development. Germination uniformity of all seeds except for annual alfalfa seed was similar. Under osmotic stress, alfalfa Seed rigidity resulted in Heterogeneous seed germination (Heidari sharifabad, 2006). SRDP is an attribute that specify vigorous seed reserves.

Drought treatment:

Changes in seedling growth, germination rate of dissected embryos

In relation to growth, both germinated embryos and endosperm were suppressed by drought treatment. Differences were observed in SLGR, germination percent and germination rate (fig 2-b, c). Under stress conditions, maximum SLGR of endosperms was observed in corn and barely seeds (fig 2- a). Similar trend in *medicago spp.* was observed in salinity treatment that resulted in Maximum stoppages in germinations were observed in alfalfa species. (fig. 2-b, c).

Seed size affected SRUR process. In larger seeds higher quantity of reserves will be allocated to seedling growth. Other related traits such as SRDP can be affected in seeds that are subjected to Abiotic

stresses. Consistent with this conclusion, corn and barely seeds had uppermost resistance to drought

stress.

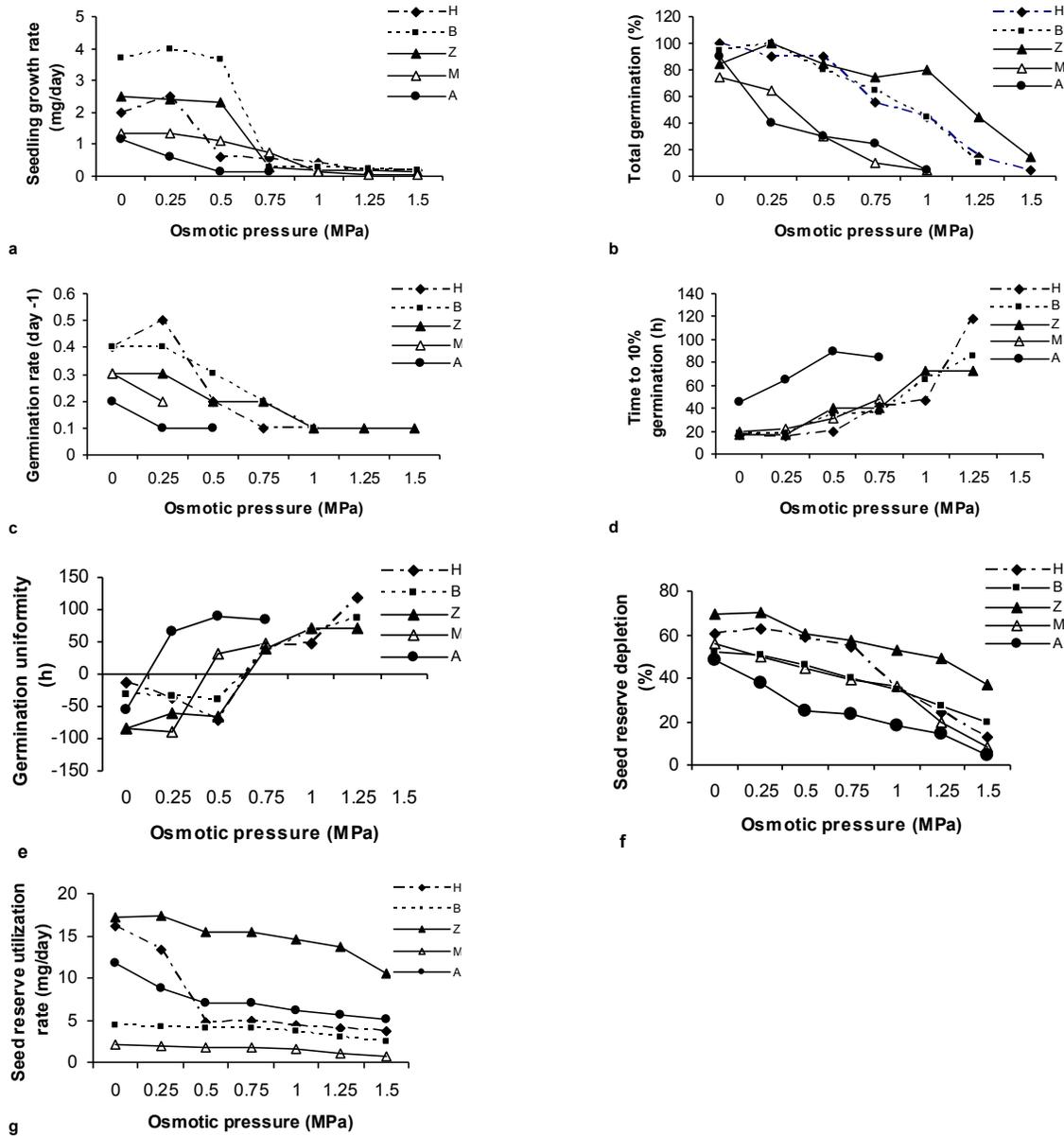


Fig 1. Effect of NaCl-induced salinity (MPa) on Seedling growth rate (a), Total germination (b), Germination rate (c), Time to 10% germination (d), Germination uniformity (e), Seed reserve depletion (f), Seed reserve utilization rate (g) in *H.vulgaris* (H), *B.napus* (B), *Z.maize* (Z), *M.sativa* (M) and *M. scutellata* (A).

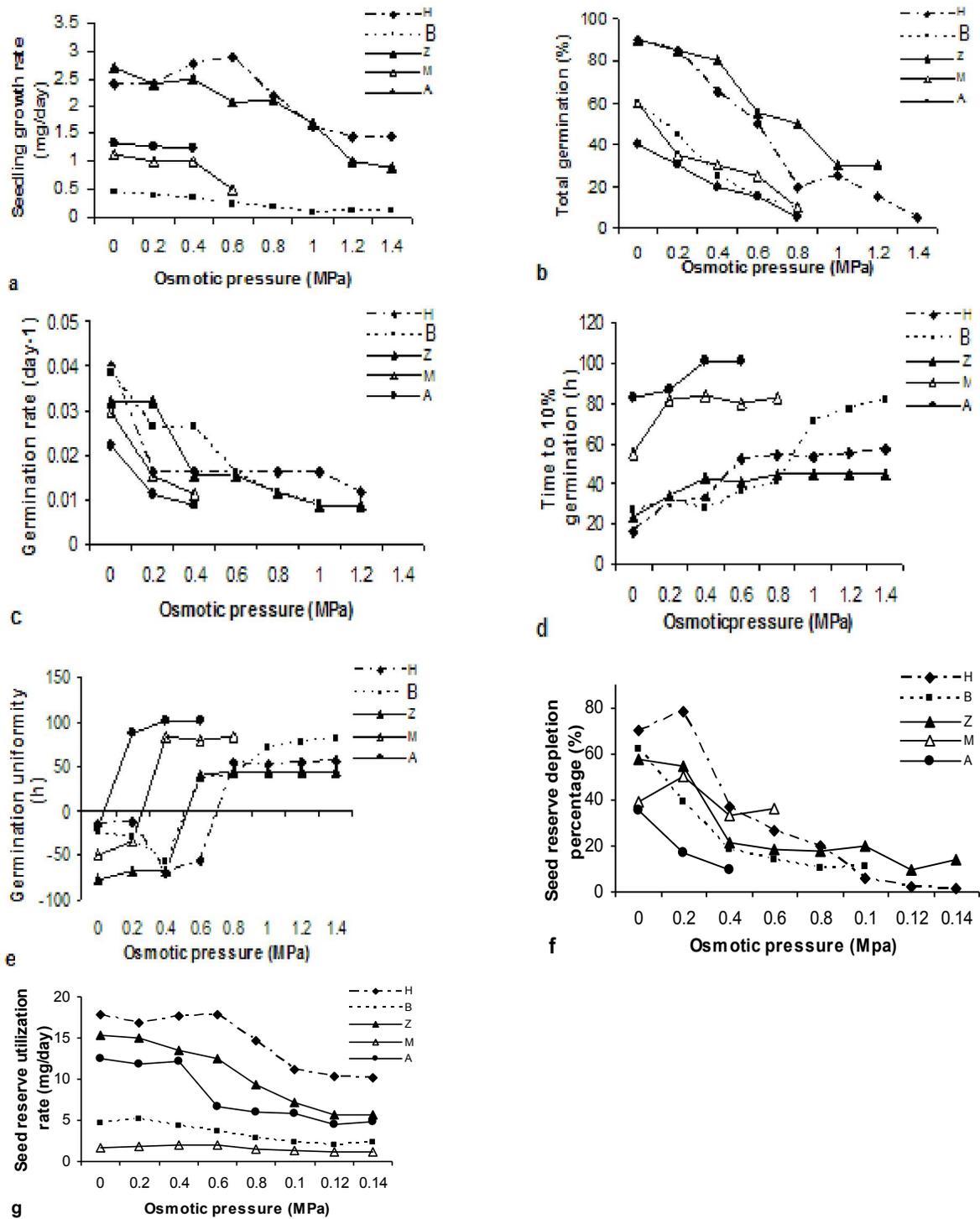


Fig 2. Effect of PEG-induced drought (MPa) on Seedling growth rate (a), Total germination (b), Germination rate (c), Time to 10% germination (d), Germination uniformity (e), Seed reserve depletion (f), Seed reserve utilization rate (g) in *H.vulgaris* (H), *B.napus* (B), *Z.maize* (Z), *M.sativa* (M) and *M. scutellata* (A).

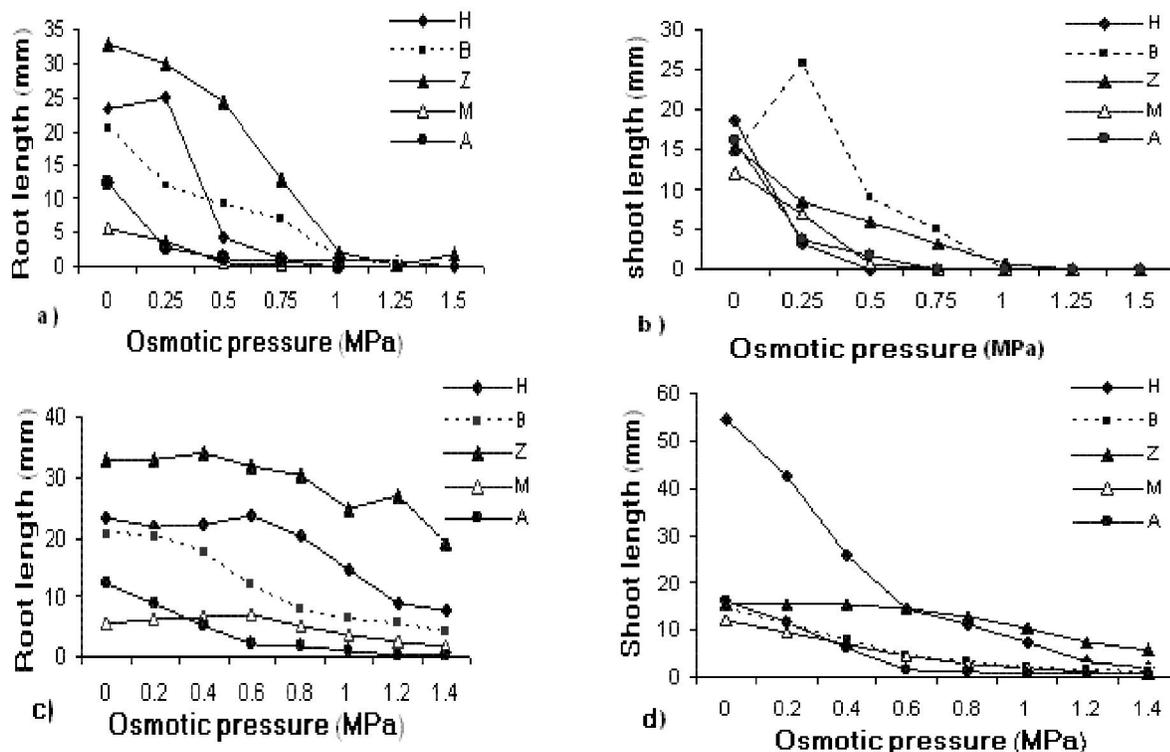


Fig 3. Effect of NaCl-induced salinity (Mpa) on root length (a) and shoot length (b) and effect of PEG-induced drought (MPa) on root length (c) and shoot length (d) in seed germination of *H.vulgaris*, *B.napus*, *Z.maize*, *M.sativa* and *M. scutellata*.

Conclusion:

The present investigation monitored changes caused by both salinity and drought stress in germination rate and some trait of *maize*, *rapeseed*, *barely* seed embryos and endosperm. Osmotic stress caused a considerable decrease in germination of control seeds. In earlier research, Gill and Singh (1985) have reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stresses. Changes in anyone of these processes can affect other metabolic activities, particularly carbohydrate metabolism that plays an important role in germination and seed development. In earlier research Schubert et al., (1995) and Kameli and Losel (1996) reported a linear correlation between water stress and dry matter accumulation in *Medicago sativa* and *Triticum durum* respectively. Our findings are similar to results of *M. sativa* and *T. durum*.

Upon relieving stress with distilled water, a high percentage of ungerminated seeds under drought and salinity treatment germinated. Similar declines and recoveries in seed germination have been reported in the literature (Khan and Ungar, 1984; Woodell, 1985; Gupta et al., 1993; Singh et al., 1996; Ungar, 1996). Reduced germination under water stress conditions may be attributed to the effect that seeds seemingly

develop an osmotically enforced “dormancy” under water stress conditions, which may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Singh et al., 1996, Prado et al., 2000). Furthermore, under osmotic stress a significant reduction in water potential and tissue water content of germinated embryos and endosperm was observed, indicating that these tissues were under stress. Similar observations of decreases in water level under stress conditions were made by Gill et al. (2001) in sorghum, Siddique et al. (2000) in wheat (*Triticum durum*), Prado et al. (2000) in *Chenopodium* (*Chenopodium quinoa*), Pennypacker et al. (1990) in alfalfa (*Medicago sativa*) and Gupta et al. (1993) in chickpea (*Cicer arietinum*).

The results of this study showed:

1. There is genetic variation for germination and seedling traits among different crop species.
2. Decreasing seedling growth rate as affected by OP is a result of declining seed reserve utilization rate.
3. Grass seeds are more tolerant to salinity and drought stress compared to legumes.
4. Plant cultivar affects germination traits.

Acknowledgements

We are grateful to Dr. M. Gholipoor for his valuable suggestions during the course of this study. The authors would like to thank Mr. H. Amiri, Miss A. Poor Asadolahi and S. Akhlaghi for their assistance in conducting the experiments.

References

- Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., Inaba, M., Murata, N., 2000b. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol.* 123, 1047–1056.
- Ashraf, M. and Waheed, A. (1992). Screening chickpea for salt tolerance. *Der. Tropen.*, 5, 45-55.
- Beveridge, J.L., Wilise, C.P., 1959. Influence of depth of planting, seed size and variety on emergence and seedling vigor in alfalfa. *Agron. J.* 51, 731–734.
- Bohnert HJ, CushmanJC (2002) Plant and environmental stress adaptation strategies. In “Plant Biotechnology and transgenic plants”, Oksman-Caldentey KM, and Barz W, eds. Marcel Dekkar, New York, NY. In press.
- Chandra, S. (1980). Effect of edaphic factors on Chickpea, Proceeding of the International Workshop on Chickpea Improvement, 28 Feb-2 Mar 1979, Hyderabad, India, pp. 33-42, ICRIASAT, Patancheru, India.
- Comis D (1997) Aerenchyma. *Lifelines for living underwater.* *Agric Res* 45 (8): 4-8.
- Ehret, D.L., Plant, A.L., 1999. Salt tolerance in crop plants. In: Dhaliwal, G.S., Arora, R. (Eds.), *Environmental Stress in Crop Plants*. Commonwealth Publishers, New Delhi, India, pp. 69–120 (Chapter 5).
- Flowers, T.J., Troke, P.F., Yeo., 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Phys.* 28, 89-121.
- Gill, K. S., O. S. Singh, 1985. Effect of salinity on carbohydrate metabolism during paddy (*Oryza sativa*) seed germination under salt stress condition. *J. Exp. Biol.*, 23, 384–386.
- Gill P. K., A. D. Sharma, P. Singh, S. S. Bhullar, 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulg. J. Plant Physiol.*, 27, 72–84.
- Greenway, H., Munns, R., 1980. Mechanisms of salt tolerance in non-halophytes. *Annu. Rev. Plant Phys.* 31, 149-190.
- Gupta, A. K., J. Singh, N. Kaur, R. Singh, 1993. Effect of polyethylene glycol induced-water stress on germination and reserve carbohydrates metabolism in Chickpea cultivars differing in tolerance to water deficit. *Plant Physiol. Biochem.*, 31, 369–378.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnet, H.J., 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Phys.* 51, 463-499.
- Johnson, D.R. and Luedders, V.D. (1974). Effect of planted seed size on emergence and yield of soybeans. *Agronomy Journal*, 66, 117-118.
- Johnson, D.R. and Wax, L.M. (1978). Relationships of soybean germination and vigor tests to field performance. *Agronomy Journal*, 70, 273-278.
- Kameli, A., D. M. Losel, 1996. Growth and sugar accumulation in durum wheat plants under water stress. *New Phytol.*, 132, 57–62.
- Khan, M. A., I. A. Ungar, 1984. Seed polymorphism and germination responses to salt stress in *Atriplex triangularis*. *Bot. Gaz.*, 145, 487–494.
- Kheradnam, M. and Ghorashy, S.R. 1973. Salt tolerance of chickpea varieties during germination. *Agronomy Journal*, 65, 329-330.
- Kindiger B, Sokolov V and Khatypova IV (1996) Evaluation of apomictic reproduction in a set of 39 chromosome maize-*Tripsacum* backcross hybrids. *Crop Sci* 36: 1108-1113.
- Kirst, G.O., 1989. Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 21–53.
- Mass, E.V. and Hoffman, G.J. 1977. Crop salt tolerance: Current assessment. *Journal of Irrigation and Drainage Division of American Society of Civil Engineers*, 103, 115-134.
- Pennypacker, B. W., K. L. Leath, W. L. Stout, R. R. Hill, 1990. Techniques for stimulating field drought stress in greenhouse. *Agron. J.*, 82, 951–957.
- Prado, F. E., C. Boero, M. Gallardo, J. A. Gonzalez, 2000. Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* wild seeds. *Bot. Bull. Acad. Sin.*, 41, 27–34.
- Qiu, J., Mosjidis, J.A. and Williams, J.C. (1994). Seedling growth as affected by seed weight in common vetch. *Agronomy Journal*, 86, 251-255.
- Salisbury, F.B. and Ross. C.W. (1996). *Plant physiology*. Wadsworth Publ. Co., Belmont, CA. SAS Institute, 1989. SAS/STAT user guide, Version 6, 4th editions, SAS Inst., Inc., Cary, NC.

26. Saxena, N.P., Saxena, M.C., Johansen, C., Virmani, S.M. and Harris, H. (1996). Future research priorities for chickpea in WANA and SAT. In *Adaptation of Chickpea in West Asia and North Africa Region*, (eds, N.P. Saxena, M.C., Saxena, C. Johnson, S.M. Virmani and H. Harris), pp. 257-263, ICARDA, Aleppo, Syria.
27. Schubert, S., R. Serraj, E. Plies-Balzer, K. Mengel, 1995. Effect of drought stress on growth, sugar concentrations and amino acid accumulation in N₂-fixing alfalfa (*Medicago sativa*). *J. Plant Physiol.*, 146, 541–546.
28. Sharafi, S., Ghasemi, S., and Gholipour, M. 2006. The affective ness of yield and some attributes of *Medicago scutellata* Var Rabinson from sowing date, sowing depth and seeding rate. 9 th congeries Agriculture and Plant breeding of Iran
29. Sharafi, S., Ghasemi, S., and Azizi, Kh. 2006. Evaluating effectiveness of fiber and protein percentage in *Medicago scutellata* from sowing date, seeding depth and seeding rate. 9 th congeries Agriculture and Plant breeding of Iran.
30. Siddique, M. R. B., A. Hamid, M. S. Islam, 2000. Drought stress effects on water relations of wheat. *Bot. Bull. Acad. Sin.*, 41, 35–39.
31. Singh, P., P. Bhaglal, S. S. Bhullar, 1996. Differential levels of wheat germ agglutinin (WGA) in germinating embryos of different wheat cultivars in response to osmotic stress. *Plant Physiol. Biochem.*, 34, 547–552.
32. Soltani, A., Galeshi, S., Zeinali, E., Latifi, n., 2002. Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. *Seed Sci. Technol.* 30, 51 to 60.
33. Soltani, A., Zeinali, E., Galeshi, S., Latifi, N., 2001. Genetic variation for and interrelationships among seed vigor traits in wheat from the Caspian Sea coast of Iran. *Seed Sci. Technol.* 29, 653–662.
34. Ungar, I. A., 1996. Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). *Amer. J. Bot.*, 83, 604–607.
35. Williams, W.A. (1967). Seedling growth of hypogeal legume, *Vicia dasycarpa*, in relation to seed weight. *Crop Science*, 7, 163-165.
36. Woodell, S. R. J., 1985. Salinity and seed germination patterns in coastal halophytes. *Vegetatio.* 61, 223–229.
37. Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* 53, 247–273.

12/15/2012