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Germination and Seedling Growth in Grasspea (*Lathyrus sativus*) Cultivars under Salinity Conditions

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Abstract: In four grasspea varieties include ardabil, sharekord, mashhad and zanjan, the effects of different salinity concentrations on seed germination percent, proline concentration, malondialdehyde (MDA), germination index, radicle and hypocotyl length and weight were studied. Result showed that salinity had significant effects on seed germination percentage and germination index. The most and least of germination percentage were observed in 6 and 18 dS m^{-1}, respectively. Salinity had significantly effect on radicle and hypocotyl length, dry and fresh weight, MDA and proline concentration of seedlings. Salinity had not effect on dry weight of seedling. Increasing salinity reduced radicle and hypocotyl length, dry and fresh weight of seedlings and enhanced proline and malondialdehyde in them. Sharkord cultivar had the most germination percentage at 18 dS m^{-1} sodium chloride. Sharkord and ardabil varieties were the most tolerance and sensitive varieties to salinity stress, respectively.

Key words: Germination, grasspea (*Lathyrus sativus*), malondialdehyde, proline, salinity stress

INTRODUCTION

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri *et al*., 2001). Crop establishment depends on an interaction between seedbed environment and seed quality (Khajeh-Hosseini *et al*., 2003). Salinity has also been identified as the major seedbed factor influencing establishment in arid and semi-arid regions (Almansouri *et al*., 2001). It imposes serious environmental problems that affect grassland cover and the availability of animal feed in this regions (El-Kharbotly *et al*., 2003). Othman (2005) reported that seed germination can be initiated by water imbibitions and any shortage in water supply will let seed under stress. Reduction in osmotic potential in salt stressed plants can be a result of inorganic ion (Na^+, Cl^- and K^+) and compatible organic solute (soluble carbohydrates, amino acids, proline, betaines, etc) accumulations (Hasegawa *et al*., 2000). The specific ions likely to be most abundant and to cause the greatest problems are sodium (Na^+) and chloride (Cl^-). Total germination of many species may be more affected by low osmotic potential than by specific ion effects. Redmann (1974) found that the osmotic effect of NaCl on germination of alfalfa was more important than the toxic effect. Variation in salt levels may restricted seed germination and in some cases resulting in the death of seeds (Radic *et al*., 2005).

Radical growth may be strongly inhibited by specific ions. Cowpea seeds germination was significantly reduced when electrical conductivity in sand culture exceeded 1.2 dS m^{-1} (West and Francoise, 1982), decreasing 30% when exposed to 1.56 Sm^{-1} (Murillo-Amador and Troyo-Diéguez, 2000). Salt concentrations higher than 50 mol m^{-3} NaCl affect the germination of cowpea seeds (Bábara *et al*., 2005).

Although some crops are moderately tolerant of saline conditions, many crops are adversely affected by even low levels of salt. Paliwal and Maliwal (1980) reported the seed of mung bean (*Vigna radiata* Wilczek.) could tolerate 6 dS m^{-1} salinity equivalent to 53 mM NaCl comparable to sorghum [*Sorghum bicolor* (L.) Moench.] (6 dS m^{-1}) and pearl millet [*Pennisetum americanum* (L.) Leeke] (9 dS m^{-1}).

Salinity adversely affects the metabolism of plants and causes important modifications in gene expression in plants. Such modifications may lead to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear or disappear after salt treatment. Tolerant plants adjust osmotically by the synthesis of highly water soluble compatible osmotica (e.g., glycinebetaine, free proline and low molecular weight sugars) and maintain turgor. Among these, free proline ameliorates salt-induced oxidative damage to membranes (Jain *et al*., 2001). In organisms

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ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery (Sairam *et al*., 2004). Proline, sugars and sucrose help osmotic adjustment during stress and protect native structure of macromolecules and membranes during extreme dehydration (Hoekstra *et al*., 2001). Salt causes oxidative stress and can generate reactive oxygen species that damage membranes and the macromolecules. MDA is an end product of lipid peroxidation and has been used as a marker for free radical generation and membrane damage under abiotic stress condition (Parvanova *et al*., 2004).

Grasspea (*Lathyrus sativus*) is a prostrate quick maturing legume grown as a food for man and both food and forage for domesticated animals. It is extremely drought tolerant, capable of yielding well even under the most adverse conditions and well adapted to cool season (winter) production in warm temperate and sub-tropical areas (Mediterranean basin, Bangladesh, India and Pakistan) (Cocks *et al*., 2000). Grasspea cultures also have the ability to adapt to saline, alkaline, clay or otherwise poor soils and are hardy and easy to cultivate (Berger *et al*., 1999). Salinity is one of the major limiting factors in crop production in arid and semiarid regions of the world. It is necessary to understand the response of grasspea to salinity stress if cultivation in saline areas is considered. Little information on the effect of salinity stress on grasspea seed germination and seedling establishment is available.

MATERIALS AND METHODS

The experimental was carried out at Faculty of Agriculture, Tarbit Modarres University, Tehran, Iran, in 2005. Three replications of 50 seeds of the four grasspeas (*Lathyrus sativus* L.) cultivars including Zanjan, Sharkord, Ardabil and Mashhad were submitted to germination test in germinator at 25°C, according to a factorial with the layout of completely randomized design. These seeds germinated in germitest papers imbibed in distilled water (H_2O) or in sodium chloride (NaCl) solutions in a proportion of 2,5 times the weight of the paper. The NaCl concentrations were 0, 65, 130 and 197 mM, which electrical conductivity (0, 6, 12 and 18 dS m^{-1}) was measured with a conductivimeter. The evaluations were carried out according to the Rules for Seed Analysis after 10 days. The data were accessed for percentage of normal seedlings. After germination evaluations, 10 normal seedlings were oven dried at 65°C, during 72 h and weighted, determining the dry matter weight. Fresh weight was also accessed before drying the seedlings. After 10 days in a 25°C germinator the hypocotyls length (HL) and radical length (RL) were measured.

Total protein content in the seedlings cotyledons were obtained from 10 days germinated seedlings. The proteins were obtained according to Kejeldal method, which amunium ions were measured with electrode of Kejeltec Auto 1030 Analyzer. The method used for total proline in seedlings was Bates *et al*. (1973). Samples proline concentration (μg μmol^{-1}) was determined by spectrophotometery and fixed net proline concentrations in standard curve as control in 520 nm wave length.

MDA content were measured using a thiobarbutyric acid reaction following the method of De Vos ***et al.*** (1991). The absorbance of samples were recorded at 532 nm and corrected for 600 nm. MDA level was expressed using extinction coefficient of 155 mM^{-1} cm^{-1}.

The experimental data were statistically analyzed for variance using the SAS system (SAS Institute Inc. 1991). When analysis of variance showed significant treatment effects, Duncan's Multiple Range Test was applied to compare the means at $p<0.05$.

RESULTS AND DISCUSSION

There were differences ($p\leq 0.01$) among cultivars and salinity concentration levels for germination percent-age, but cultivar * salinity interaction did not affect germination rate. Germination percentage did not change up to 6 dS m^{-1} but 12 dS m^{-1} salinity concentration decreased this trait. Seed germination rate reduced 60% in 18 dS m^{-1} treatment as compared to control (Table 2). Sharkord and zanjan had the most germination percentage with 95 and 86%, respectively. The lowest germination percentage was belonged to mashhad and ardabil with 71 and 74%, respectively (Table 2). The highest and lowest germination percent was for sharkord cultivars at 6 dS m^{-1} (100%) indicated salt tolerance of this cultivar to NaCl. Ardabil cultivar at 18 dS m^{-1} had the lowest germination percentage, indicated salt sensitiveness of this cultivar to NaCl.

Seed germination in barely seed was 100% for control and 0 at 2.5% salinity treatments. Barely can tolerate salinity up to 2%. Germination percentage and rate reduced among different pea cultivars when salinity concentration increased from 0 to 20 mmos cm^{-1} (Shoeoan and Garo, 1985). Salinity may also affect the germination of seeds by creating an external osmotic potential that prevents water uptake or due to the toxic effects of Na^+ and Cl^- ions on the germinating seed

Table 1: Variance analysis of seed germination traits for four grasspea varieties under salinity stress

		Mean square							
SOV	df	Germination percentage	Radical length (RL)	Hypocotyl length (HL)	Seedling Fresh weight	Seedling dry weight	Radical dry weight	hypocotyl dry weight	Protein percentage (%)
Cultivar	3	985.51**	1.15**	2.27**	0.67**	0.0002**	0.00004**	0.00006**	19.91^{ns}
Salinity	3	2716.88**	9.74**	15.96**	1.04**	0.0015**	0.0003**	0.0006**	69.23**
Cultivar*salinity	9	141.77^{ns}	0.20**	0.98**	0.03^{ns}	0.00006**	0.00001*	0.00003**	13.79^{ns}
error	32	65.66	0.043	0.054	0.089	0.000009	0.000004	0.000002	10.22
c.v.		10.06	13.14	18.37	16.20	17.74	21.10	18.41	10.37

Table 1: Continue

		Mean square				
SOV	df	Germination index	Germination rate coefficient	Seedling vigour index	Malondialdehyde (MDA)	Proline rate
cultivar	3	0.641**	2105.71**	2.38**	1.71**	1.73^{ns}
Salinity	3	0.054^{ns}	2016.15**	15.53**	0.68**	64.47**
Cultivar*salinity	9	.067*	77.07^{ns}	0.52**	0.09^{ns}	0.85^{ns}
error	32	0.29	78.09	0.065	0.07	1.14
c.v.		13.06	13.87	17.05	14.24	21.76

* Significant at 5%, ** Significant at 1% and ^{ns} not Significant

Table 2: Mean values of seed germination traits for four grasspea varieties under salinity stress.

Salinity (dS m^{-1})	Germination percentage	Seedling fresh weight (g)	Protein percentage (%)	Germination rate coefficient	Malondialdehyde (MDA) (μ mol g^{-1})	Proline rate (μ mol g^{-1})
0	93.67a	2.06a	32.04ab	77.44a	1.53c	0.023d
6	92.67a	2.07a	30.09cb	71.40a	1.83b	0.040c
12	69.00b	1.77b	33.36a	56.40b	1.96ab	0.054b
18	34.66c	1.45c	27.85c	49.46b	2.09a	0.078a
Cultivars						
Sharkord	90.00a	1.99a	31.94a	72.12a	1.43d	4.80a
Zanjan	86.33a	1.64b	31.86a	74.48a	1.67c	5.36a
Mashhad	71.33b	1.63b	29.30a	62.77b	2.03b	5.02a
Ardabil	74.33b	2.09a	30.23a	45.31c	2.29a	4.46a

Means with similar letter(s) in each trait are not significantly different at 5% probability level according to Duncan's Multiple Range Test

Table 3: Mean values of seed germination traits for four grasspea varieties under salinity stress

Cultivar	Salinity (dS m^{-1})	Germination index	Seedling vigour index	Seedling dry weight (g)	Radical dry weight (g)	Hypocotyl dry weight (g)	Radical length (RL) (cm)	Hypocotyl length (HL) (cm)
Mashhad	0	1.29bcde	1.76de	0.019de	0.008def	0.010ed	2.10edc	2.08bc
	6	1.21cdef	2.15cd	0.026bc	0.012bc	0.013c	2.30dc	2.34b
	12	1.13def	0.85f	0.013fg	0.008efg	0.005f	1.26f	0.69e
	18	0.89f	0.19hi	0.004h	0.003gi	0.0001hg	0.46hi	0.20f
Ardabil	0	1.65a	2.13cd	0.023cd	0.012bcd	0.01cd	2.00ed	1.29d
	6	1.72a	1.76de	0.020de	0.011bcde	0.009e	1.83e	1.00de
	12	1.61ab	0.60fgh	0.009g	0.007fgh	0.003fg	0.76hg	0.29f
	18	1.60ab	0.04i	0.0006h	0.001j	0.000h	0.23i	0.000f
Zanjan	0	1.05ef	2.99b	0.031b	0.013bc	0.01b	2.66b	3.45a
	6	1.08ef	2.49c	0.025c	0.014abc	0.01cd	2.43bc	2.28b
	12	1.41abcd	0.36hi	0.004h	0.004hji	0.000h	1.00fg	0.07f
	18	1.21cdef	0.21hi	0.003h	0.003ji	0.000h	0.50hi	0.03f
Sharkord	0	1.09def	3.88a	0.039a	0.017a	0.02a	3.06a	3.50a
	6	1.21cdef	2.46c	0.026bc	0.015ab	0.01cd	2.13edc	1.86c
	12	1.46abc	1.47e	0.015ef	0.011cde	0.004f	1.86e	0.98de
	18	1.32bcde	0.69fg	0.009g	0.005hgi	0.004f	0.76hg	0.22f

Means with similar letter(s) in each trait are not significantly different at 5% probability level according to Duncan's Multiple Range Test

(Redmann, 1974; Khajeh-Hosseini *et al.*, 2003). Many researches emphasize that composition containing chloride (Cl^-) have the most effect on germination reduction (Sharma *et al.*, 2004). Salinity had negative effect on germination percent of pea seedling. The maximum and minimum germination percentage was obtained at highest level of salinity (12.2 dS m^{-1}) and control, respectively (Esechie *et al.*, 2002). In our research the most and least germination percent observed at control and the highest salinity level, respectively. Salts affect the functions of the membrane and cell walls. NaCl affects the permeability of the plasma membranes and increases influx of external ions and efflux of cytosolic solutes (Allen *et al.*, 1995) in plant cells. NaCl also causes hardening of the cell wall and a decrease in water conductance of the plasma membrane. These effects on the functions of the cell membranes and the cell walls may affect the water potential of the cytosol and cellular extensibility and thus, may affect seed germination and seedling growth. Germination reduction under salinity condition may be due to this fact that dormancy increases in crop seeds under salinity stress.

Germination rate coefficient had the same trend as germination percentage (Table 1). Germination Index was affected by cultivars ($p \leq 0.01$) and cultivar * salinity interaction ($p \leq 0.05$). Salinity had not effect on this trait (Table1). The maximum and minimum germination indices were belonged to ardabil and mashhad cultivars, respectively (Table 3). Salinity may affect germination through an osmotic component that compromises water uptake and through an ionic component linked to accumulation of Na and Cl. Thus, saline condition reduces the ability of plants to absorb water, causing rapid reductions in growth rate and induce many metabolic changes, similar to those caused by water stress.

Salinity, cultivar and those interactions were significant on seedling vigour index ($p \leq 0.01$) (Table 1). The highest and lowest seedling vigour indices were belonged to sharkord cultivar at control treatment and ardabil cultivar at 18 dS m^{-1}, respectively. This result is showed that sharkord cultivar is more resistance to salinity than other varieties at germination stage. Ardabil cultivar showed the most sensitivity to salinity (Table 3). In all varieties, salinity decreased seedling vigour index. Germination and seedling growth are reduced in saline soils with varying responses for species and cultivars (Hampson and Simpson, 1990).

Radical length means difference was significant among salinity levels and varieties ($p \leq 0.01$). The maximum radical length was observed in sharkord cultivar at control treatment (3.06 cm) and the minimum was belonged to ardabil cultivar at 18 dS m^{-1} (0.23 cm) (Table 3). This result is indicated that sharkord and ardabil varieties are resisting and sensitive to salinity, respectively. Radical growth may be strongly inhibited by specific ions. Bewley and Black (1994) suggested that the inhibition of the radicle under water stress was due to a reduction in the turgor of the radicle cells.

The effect of salinity, cultivar and those interactions were significant on hypocotyl length ($p \leq 0.01$) (Table 1). Hypocotyl length reduced with increasing salinity from 0 to 18 ds m^{-1}. Sharkord and zanjan varieties had the most hypocotyl length (3.5 and 3.45, respectively) at control treatment and ardabil cultivar produced the least it at 18 dS m^{-1}. Hypocotyl of all varieties had not growth at salinity level higher than 12 dS m^{-1} (Table 3). Salt stress inhibited the growth of hypocotyls more than radicals in all grasspea varieties. Salt stress resulted in growth reduction of both hypocotyls and radicles. Radical and hypocotyl lengths reduced 20 and 45% at 6 dS m^{-1} salinity relative to control treatment, respectively. Hence hypocotyl elongation was more sensitive to salt treatments than radicle elongation, particularly at higher salt concentration. Similar observations have been reported in barley (Huang and Redmann, 1995), pigeon pea (*Cajanus Cajan*) (Subbarao *et al*., 1991) and tepary bean (*Phaseeolus acutifolius* A. Gray) (Goertz and coons, 1991). Sensitiveness of germination to salinity was less than hypocotyl and radical length. This implies that although a certain percentage of germination can be achieved under salinity stress, successful emergence and establishment may not be achieved due to weak hypocotyls elongation. Forcella *et al*. (2000) observed that the elongation of the coleoptile is governed by soil water potential. Sharkord and zanjan genotypes, with longer hypocotyls under salinity stress, may have better tolerance to salt during the germination stage. The salt tolerant varieties develop an osmotic adjustment mechanism that allows cell enlargement and plant growth at low water violability caused by the presence of ions especially Na^+ and Cl^-. Present results are in accord with the observations of Zaiter and Mahfouz (1993) who noted a decrease in shoot length under salinity stress. Cultivar selection for better salt tolerance during the germination stage should include hypocotyl elongation as a parameter in addition to the rate and percentage of germination.

There were significant differences ($p \leq 0.01$) among cultivars and salinity levels for seedling fresh weight, but their interaction was not significant (Table 1). Increasing salt concentrations severely affected seedling fresh weight. Progressive decrease was observed in this trait when NaCl level was higher than 6 dS m^{-1}. Control treatment and 18 dS m^{-1} salinity produced the most (2.06) and the least (1.45) seedling fresh weight, respectively (Table 2). Sharkord and ardabil varieties had the same seedling fresh weight, Although they were resistance and sensitive varieties in aspect of other measured traits, respectively. It seems that reduction in seedling fresh weight is due to decreasing water uptake by seedlings. This result is similar to the previous report of Sharma *et al*. (2004), which showed decreasing seedling fresh weight under 20 mmos cm^{-1} salinity. Salinity also reduced shoot and root fresh weight and leaf number of canola (Jamil *et al*., 2005). Salt decrease the osmotic water potential, creating a water stress in seedlings. The loss of water from the cells, one of the initial events of water deficit, may affect turgor and bring about changes in size and membrane properties.

The main effects of cultivar, salt concentration and cultivar*salinity interaction were significant for seedling dry weight ($p \leq 0.01$) (Table 1). Salinity reduced the mentioned trait in all varieties (Table 3). The greatest seedling dry weight was belonged to sharkord cultivar (0.039) while zanjan and mashhad varieties were ranked after that (Table 3). Ardabil at 18 dS m^{-1} had the lowest seedling dry weight. Thus ardabil is more sensitive to salinity than other varieties at seedling stage. Salinity stress caused reduction in fresh and dry shoot and root of bean (Ashraf and Rasul, 1988).

Cultivar, salinity stress and cultivar*salinity interaction had significant effect on root and hypocotyl dry weight ($p \leq 0.001$) (Table 1). Salinity increment reduced radicle and hypocotyl dry weight (Table 3). These traits were higher in sharkord cultivar than other cultivars. Significant reduction in radicle dry weight was observed in salinity stress higher than 6 dS m^{-1}. 18 dS m^{-1} salinity reduced radicle dry weight up to 75% in compared to control. Hypocotyl dry weight was reduced by the first level of salinity concentration (6 dS m^{-1}). This trait reduced up to 80% in 18 dS m^{-1} salinity stress in compared to control treatment. Hence, hypocotyl elongation was more sensitive to salt treatments than radicle elongation, particularly at higher salt concentration. Salt stress resulted in growth reduction of both hypocotyls and radicals, but hypocotyls were more sensitive since a little growth was observed at high concentration of NaCl.

The most radicle and hypocotyl dry weight was belonged to sharkored cultivar at control treatment. Ardabil cultivar produced the least those mentioned traits at 18 dS m^{-1} (Table 3). Radicle and hypocotyl dry weight of wheat and triticale were reduced by salinity (Shalaby *et al*., 1993).

There were differences ($p<0.01$) among salinity levels for seedling protein percent. Varieties had the same seedling protein percent. There was not also a cultivar * salinity interaction for this trait (Table 1). The highest seedling protein percent was obtained at 12 dS m^{-1} salinity level with an average protein 33.36% that had not difference with control treatment. The lowest seedling protein percent was belonged to 18 dS m^{-1} salinity (Table 2). This reduction is related to decreasing amino acid percent inner seeds. Salinity stress increased protein and carbohydrates in bean plants (Ashraf and Rasul, 1988). According to Dell'aquila and Spada (1993) salinity may activate or stimulate the genesis of some proteins during germination and these salt stress proteins have been associated with a protective function in wheat embryos. This may have been the reason for enhanced germination at low salt concentrations. Proteins conduct water molecules across membranes, implicating that gating of water channels could have an impact on intercompartmental movement of water in terms of salt tolerance, such aquaporins could be a mechanism for maintaining Osmotic homeostasis and turgor in the cells of salt stressed plants. This result was also confirmed by bean experiment at salt stress (Lopez *et al*., 2004).

Salinity affected on proline content ($p \leq 0.01$). There were not differences among cultivars for proline content. There was not also an interaction between salinity and cultivar for this trait (Table 1). The highest proline content was obtained at the highest salinity stress with an average proline of 0.078 μmol g^{-1}. Control treatment produced the lowest proline (Table 2). While proline concentration increase in tolerance cell to salinity, near to 75% of amino acid in seed is used for production proline, whereas 15% of it in sensitive cell is changed to proline (Sayed and Kirkwood, 1992). The amino acid proline accumulates in plants subjected to salt or osmotic stress. Proline is one of the compatible solutes that accumulate many folds in stressed plants. Its accumulation is dependent on the expression of both Δ-proline-5-carboxylate synthase and proline dehydrogenase (Coruzzi and Last, 2000). Proline transport is also up regulated under salt stress and screening for such a response maybe a way to find stress tolerant breeding stock in citrus (Mademba-Sy *et al*., 2003) and barley (Pakniyat *et al*., 2003).

There were differences ($p \leq 0.01$) among cultivars and salinity levels for malondialdhyde (MDA) concentration. Cultivar*salinity interaction was not significant on this trait (Table 1). The most MDA was produced at the highest salinity level and control had the lowest MDA. Malondialdehyde contents increased in NaCl-treated seedlings of all the cultivars. However, highest MDA content was noted in cv. ardabil and the least in sharkord (Table 2). A considerable increase in accumulation of MDA contents recorded in ardabil cultivar as compared to other cultivars indicated salt sensitiveness of this cultivar to NaCl. Accumulation of MDA was reported in a number of salt - sensitive plants (Gehlot *et al*., 2003).

Salinity increasing exerts complex effects on the plant as a result of ionic, osmotic and physiological complexity of the organized plant. This fact makes it difficult to find ways to increase salt tolerance to large degrees. However, it does give hope that salt tolerance can be increased by finding the factor that is most limited by salt stress during growth and development. Germination and seedling development is very important for early establishment of plants under stress conditions. Selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seedling establishment. In this research, germination and seedling survival were reduced by increasing salt concentrations. There was 34.66% germination percent at 18 dS m^{-1} NaCl as compared to 93.67% at control treatment. Hypocotyl and radicle lengths as well as seedling dry mass were reduced by increasing salt stress. Ardabil cultivar was the most sensitive genotype, with the lowest germination percent, seedling hypocotyl and radicle length, dry weight and seedling vigour index, while sharkord was the most tolerant with the highest traits that mentioned above.

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