



## Evaluation of salinity tolerance of different clover species at germination and seedling stages

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### Abstract

In order to study the effects of salinity stress on growth indices of three clover species including, *Trifolium resupinatum*, *T. alexandrinum*, and *T. incarnatum*, an experiment was conducted as factorial based on a completely randomized design arrangement with three replications. Factors included seven levels of salinity stress (0, 41, 82, 123, 164, 205, and 246 mM) and three clover species. Different performance was observed for germination and seedling parameters among the species. In addition, results indicated significant differences among the treatments in all traits and showed that the effects of salinity on all traits were the same at low-stress levels (0 and 41 mM), but differed at higher stress levels. The experimental results revealed that with an increase in salinity level, the greater reduction was observed in vigor index, seedling length, and root and shoot fresh and dry weight while MGT and GU increased. The highest levels of vigor index and root and shoot fresh and dry weight was related to *T. resupinatum* and *T. incarnatum*, respectively, while MGT and GU in *T. resupinatum* were higher than those of the other two species. In addition, *T. resupinatum* seedling length was higher than the other two species.

**Keywords:** clover; salinity; germination; seedling parameters

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### Introduction

The genus *Trifolium* contains about 300 species of plants in the leguminous family. Clover or trefoil is one of the most important forage crops in agriculture that grow in varied environments throughout the world, which differ markedly in their stature, perennially, architecture and pigmentation (Abberton and Marshall, 2005; Price et al., 1987).

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Agriculture has been influenced by different abiotic stresses such as temperature, drought, and salinity which reduce roughly half the yield of crops. Water stress that is caused by salinity and drought is a problem in agriculture in the world. Salinity is one of the most important environmental stress that influences nearly half of the irrigated lands and 20% of the worlds cultivated lands (Fallahi et al., 2013; Khayatnezhad et al., 2010). Salinity is also one of the most important factors in crop yield reduction in the

world, particularly in arid and semi-arid regions (Dadkhah and Grrifiths, 2010).

Germination is one of the most critical phases during plant growth cycle when they are particularly sensitive to environmental stresses such as excess salt (Khan et al., 2002; Pujol et al., 2000). The adaptation of plants during seed germination with salinity stress is a key factor in assessing their performance under this type of stress because seedling establishment influences the plant vigor during other growth phases of their life cycle (Anaya et al., 2015). Salt stress is responsible for both inhibition or delayed seed germination and seedling growth. On the other hand, soil salinity influences seed germination through the production of external osmotic potential which prevents water absorption and Na<sup>+</sup> and Cl<sup>-</sup> ions' toxic effects (Turhan and Ayaz, 2004).

Regarding the high proportions of lands under salinity all over the world and increasing the world population combined with the reduction of water resources, research on plants resistant to unfavorable environmental conditions is necessary (Fallahi et al., 2009). Exposure to NaCl can be very effective in order to simulate salinity conditions to evaluate the tolerance of plants to this stress, because this substance is used to reduce the potential of water and prevent germination under osmotic stress conditions (Dodd and Donovan, 1999; Hohl and Schopfer, 1991; Sidari et al., 2008). In many forage plants, germination and early seedling growth are the most sensitive stages of their growth to environmental stresses (Kaya et al., 2006). The adverse effects of water shortage on germination and seedling growth has been well reported in different crops (Khodarahmpour, 2011; Mostafavi et al., 2011). Increased salinity is reported to cause a significant reduction in germinated seeds number, root and shoot and seedling length of *Trifolium pratense* L. (Niste et al., 2015). Investigation of the effects of salt stress on germination of 28 red clovers (*Trifolium pratense* L.) populations, showed that with increasing salinity levels, growth parameters of all cultivars including root and shoot length and germination percentage decreased while mean germination time increased (Asci, 2011). Investigation of the effect of salinity on germination and early seedling

growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum* showed that with increasing salinity levels, germination percentage, shoot length, and root length of both crops significantly decreased (Tsegay and Gebreslassie, 2014).

The current research was conducted to study the effects of salinity stress on germination indices and growth characteristics of three clover species, including *Trifolium resupinatum*, *T. alexandrinum* and *T. incarnatum*.

## Material and Methods

In order to study the effects of salinity stress on germination and seedling growth of three clover species including, *Trifolium resupinatum*, *T. alexandrinum*, and *T. incarnatum*, an experiment was conducted as factorial based on a completely randomized design arrangement, with three replications in Faculty of Agriculture, Lorestan University, Khorramabad, Iran. Factors included seven levels of salinity stress (0, 41, 82, 123, 164, 205, and 246 mM) and three clover species. The first factor was the clover species with three levels, including *T. resupinatum*, *T. alexandrinum*, and *T. incarnatum*. The second factor was the salinity stress (NaCl) with seven levels. The seeds were obtained from PAKAN BAZR Company. Salinity stress levels were established with NaCl according to molecular weights of NaCl. Before the beginning of the experiment, seed viability was tested by standard germination test (ISTA, 2016), which was 100%. Seeds were sterilized in sodium hypochlorite 10% for 3 minutes and then carefully washed three times with distilled water. Sterile disposable Petri dishes with a diameter of 10 cm were used, in which 27 seeds were placed per dish on pieces of filter paper (Whatman No. 2), and then 5 ml of the desired treatment solution was added to each petri dish. Petri dishes were then sealed in a small plastic bag before they were placed in a germinator set at 20 °C and relative humidity of 75% and 1000 lx (12/12 h light-dark period). Seeds were considered germinated when the radicle had extended for at least 2 mm. Petri dishes remained in the germinator until no changes appeared in germination for three days. After the end of the test (about 14 days), 10 seedlings were randomly chosen from each petri dish and their germination

Table 1  
Analysis of variance for traits of three clover species under salinity conditions

SOV	df	Mean square							
		GU	MGT	Vigor index	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight	Seedling length
Salinity (A)	6	275467.6**	43.17**	5.408**	0.0149**	0.0002**	0.685**	0.004**	7860**
Species (B)	2	19434.3**	4.488 <sup>ns</sup>	0.4430**	0.00049**	0.000006**	0.451**	0.0003**	979.46**
A × B	12	63504.79**	11.197 <sup>ns</sup>	0.2031**	0.00008**	0.000004 <sup>ns</sup>	0.089**	0.0001**	110.80**
Residual	42	11902.49	72.804	0.0157	0.000001	0.00001	0.0021	0.00005	3.622
CV (%)		18	27.30	4.71	5.66	14.29	3.16	8.78	4.04

\*\* Significant at 1% probability level and ns: Non-significant.

indices, including the fresh and dry weight of root and shoot and seedling length were measured. The average time of germination and vigor index (VI) was calculated according to equations 1 and 2.

The average time of germination =  $GMT = \frac{\sum(n_i \times d_i)}{N}$  (1)

where  $n_i$  is the number of germinated seeds at the end of each day ( $d_i$ ) and  $N$  is the total number of germinated seeds at the end of the experiment (Manjkhola et al., 2003).

The vigor index (VI) was calculated according to Abdul-Baki and Anderson (1973) as:

VI = mean length of seedling (mm) × percentage germination / 100 (2)

GU was calculated using a linear estimation of cumulative germination curve using Germin software (Soltani and Maddah, 2010).

### Statistical Analysis

Germination data were arcsin transformed to ensure homogeneity of variance. Data were subjected to analysis of variance and regression analysis by (SAS 9.2) software. The differences between the means were compared by the least significant difference (LSD) test ( $p \leq 0.05$ ). Charts were plotted using Excel software.

### Results

#### Uniformity of germination (GU)

The effects of salinity, species, and the interactions of salinity with species on GU were significant (Table 1). GU increased significantly with increasing salinity levels. At control, the highest level of GU was related to *T. alexandrinum* (19.70 hour). At 246 mM, the highest and lowest levels of GU were related to *T. alexandrinum* and *T. resupinatum* (250.36 and 202.83 hour, respectively). GU of *T. resupinatum* and *T. incarnatum* at lowest stress level was 19.20 hour, which was lower than *T. alexandrinum*. GU of all three species at 41 mM, did not significantly differ from the control, but increased at lower osmotic potentials (Table 2).

#### Mean germination time (MGT)

Salinity significantly affected the vigor index, but there was no significant effect of species or its interaction with salinity (Table 1). For all three species, MGT increased with increasing salinity and did not significantly differ from the control at 41 mM. At control, MGTs of all three species were lower than the other treatments and

Table 2  
Effects of different salinity (NaCl) stress levels on seedling traits of three clover species

Species	Salinity	GU	MGT	Vigor index	Root fresh Weight	Root dry weight	Shoot fresh weight	Shoot dry weight	Seedling length
	(mM)	hour	day		(gr)	(gr)	(gr)	(gr)	(mm)
<i>T. resupinatum</i>	0	19.20g	3.60abc	0.89a	0.030cd	0.0050cd	0.16i	0.020de	88.60a
	41	19.20g	4.45abc	0.85ab	0.028d	0.0050cd	0.16i	0.019ef	85.06ab
	82	19.20g	5.40a	0.81c	0.025e	0.0040e	0.16i	0.018f	81.73c
	123	60.93f	5.70a	0.63f	0.013k	0.0036e	0.11j	0.011g	64.50f
	164	67.31ef	5.74a	0.32j	0.007i	0.0023f	0.09k	0.0066i	36.26i
	205	137.67cd	5.74a	0.13m	0.005jk	0.001g	0.07l	0.004j	24.36k
	246	202.83b	5.74a	*	*	*	*	*	*
<i>T. alexandrinum</i>	0	19.70g	3.05bc	0.69e	0.039b	0.0063a	0.338c	0.023bc	69.28e
	41	20.20g	3.76abc	0.66ef	0.038b	0.0060ab	0.335cd	0.021cd	67.16ef
	82	76.14ef	4.32abc	0.44h	0.0306c	0.0050cd	0.32de	0.018f	52.93g
	123	125.38d	4.58abc	0.22k	0.016g	0.0040e	0.29f	0.009h	40.26h
	164	129.28d	5.25a	0.12mn	0.008i	0.0023f	0.24g	0.006i	29.16j
	205	215.59d	5.70a	0.06o	0.004k	0.001g	0.21h	0.004j	23.28k
	246	250.36a	5.74a	*	*	*	*	*	*
<i>T. incarnatum</i>	0	19.20g	2.70c	0.75d	0.054a	0.0065a	0.44a	0.029a	76.65d
	41	19.97g	3.60abc	0.73d	0.053a	0.0060ab	0.43a	0.027a	73.76d
	82	73.39ef	4.46abc	0.60g	0.030c	0.0053bc	0.42b	0.024b	69.46e
	123	91.52e	4.89ab	0.37i	0.019f	0.0043de	0.34c	0.018f	51.36g
	164	158.26c	5.33a	0.19l	0.014k	0.0026f	0.31e	0.011g	31.65j
	205	210.39b	5.70a	0.09no	0.006ij	0.0013g	0.23g	0.0056ij	21.50k
	246	216d	5.74a	*	*	*	*	*	*

Means followed by the same letter within a column for each species are not significantly different ( $p \leq 0.05$ ) according to LSD multiple range test.

\* Lack of seedling formation and no shoot development was observed at this osmotic potential.

the lowest level was related to *T. incarnatum*. The highest level of MGT was observed at 246 mM, which was 5.74 hour in all three species (Table 2).

### Regression analysis

According to regression analysis and coefficient of polynomial regression equation in each species, it was determined that the effect of salinity on GU and GMT was significant for all three species and the highest amount of GU and MGT were related to *T. resupinatum* (Fig. 1).

### Vigor index

The effects of osmotic potentials, species, and their interactions on vigor index were significant (Table 1). For all three species, the vigor index decreased with increasing salinity, with the vigor index of the control higher than all other treatments, including the least stressful of 41 mM. However, the vigor index reduction of *T. resupinatum* at the lowest stress level was slower than that of the other two species. At control, the highest and lowest levels of vigor index were related to *T. resupinatum* and *T. alexandrinum* (0.89 and 0.69), respectively). At 246 mM, none of

the species did form seedlings (Table 2). Among all three species, the minimum value of the vigor index was related to *T. alexandrinum* (Figure 2).

### Root fresh weight

The effects of osmotic potentials, species, and their interactions on root fresh weight were significant (Table 1). Root fresh weight decreased significantly with increasing salinity. Root fresh weight of *T. resupinatum* and *T. alexandrinum* showed an immediate decrease at 123 mM onward while decrease in root fresh weight of *T. incarnatum* was observed at 82 mM or more. At control, the highest and lowest levels were related to *T. incarnatum* and *T. resupinatum*, 0.054 and 0.030 g, respectively.

### Root dry weight

The effects of salinity and species on root dry weight were significant, but there was no significant effect of interactions between these variables (Table 1). For all three species, root dry weight increased with increasing salinity and 41 mM treatment was not significantly different in its effect compared with the control. At control, for all three species, the effect was higher than other treatments and the highest and lowest levels recorded were related to *T. incarnatum* and *T. resupinatum*, 0.0065 and 0.0050 g, respectively. However, root dry weight significantly decreased at 123 mM and higher salinity levels for *T. incarnatum*. For *T. resupinatum* and *T. alexandrinum*, a significant decline was observed at treatments containing 82 mM and more (Table 2).

### Shoot fresh weight

Effects of osmotic potentials, species, and their interactions on shoot fresh weight were significant (Table 1). For all three species, shoot fresh weight decreased with increasing salinity, and it was higher in control than all other treatments with the highest and lowest levels being related to *T. incarnatum* and *T. resupinatum*, 0.44 and 0.16 g, respectively. Shoot fresh weight reduction of *T. resupinatum* at the lowest stress

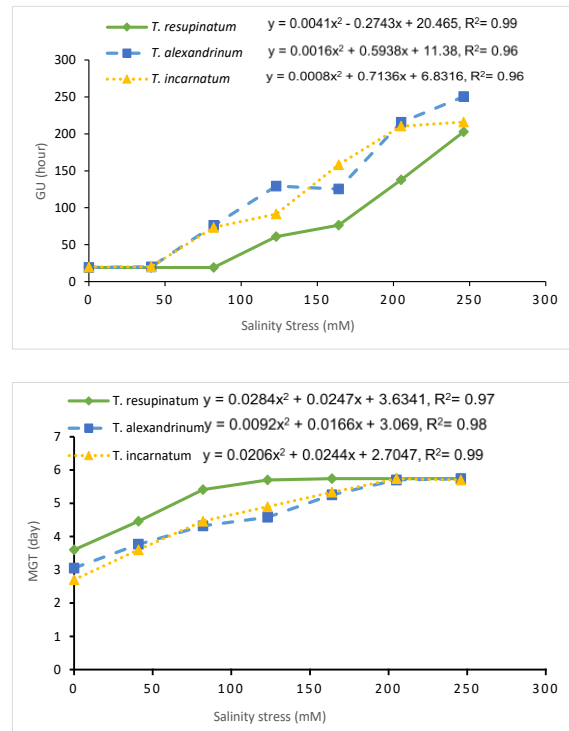


Fig. I. Regression diagrams comparison of GU and MGT, averaged over three clover species under salinity potentials ( $R^2$  at 95% confidence interval).

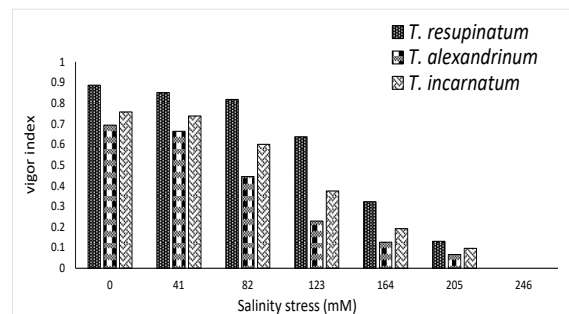


Fig. II. Effects of salinity stress on vigor index of three clover species

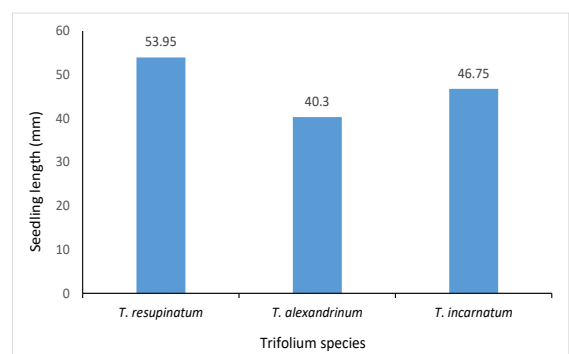


Fig. III. Difference of species based on seedling length under salinity stress

level was faster than that of the other two species (Table 2).

### Shoot dry weight

The effects of osmotic potentials, species, and their interactions on shoot dry weight were significant (Table 1). For all three species, shoot dry weight at 41 mM did not significantly differ from the control and decreased with increasing salinity. Also, it was higher in control than all other treatments, with the highest and lowest levels being related to *T. incarnatum* and *T. resupinatum*, 0.029 and 0.020 g, respectively.

### Seedling length

The effects of salinity, species, and the interactions of effects of salinity with species on seedling length were significant. Shoot length decreased significantly with increasing salinity (Tables 1 and 2). At control, seedling length of *T. resupinatum* was 88.60 mm while for *T. alexandrinum* and *T. incarnatum*, seedling lengths were 69.28 and 76.65 mm, respectively. All three species produced no seedlings at 246 mM (Table 2). Overall, seedling length of *T. resupinatum* was higher than that of the other two species (Fig. III).

### Discussion

All germination parameters were influenced by salinity stress. Exposure to saline conditions clearly resulted in a reduced germination in three clover species and at higher salinities, mean germination time (MGT) and uniformity of germination (GU) increased. In fact, the ability of seeds to germinate at lower levels indicated that the water potential was not able to provide an osmotic impediment to germination; however, the reduced water potential did result in an increase in GU and MGT while other growth parameters including seedling length, root and shoot fresh and dry weight, and vigor index all decreased with increasing stress levels. (Khajeh-Hosseini et al., 2003) reported that MGT of six soybean cultivars increased with increasing salinity levels of NaCl. At low stress levels of salinity the main effect of NaCl was reduction in germination due to the reduced water potential and slower rate of imbibition. At a higher salinity, increasing mean germination time and decreasing

germination ability of seeds can be due to the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  and toxic effects of NaCl.

In this study, with increasing salinity, seedling length and root and shoot fresh and dry weights decreased (Table 2). This may be due to a reduction in water absorption or accumulation of toxic NaCl ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) in cell vacuoles (Esfandiari and Javadi, 2014; Huang et al., 2006). Due to the accumulation of these toxic ions in cell vacuoles, the plant requires the preparation of soluble materials such as proline, glycine, betaine, sucrose, and sorbitol which accumulate in the cytoplasm and maintain the water potentials in equilibrium. The production of these substances is associated with energy; therefore, the energy used in the process reduces the growth of plant organs such as roots and stems. On the other hand, biosynthesis of these biomolecules is an energy-consuming process which could inhibit growth of plant organs such as roots and shoots length due to the deficiency of energy under stress (Nilsen and Orcutt, 1996; Peñuelas et al., 1997). Also, our results clearly indicate that decline in fresh and dry weight of root and stem in response to salinity is a consequence of the decline in weight of mobilized seed reserve (Soltani et al., 2006). Salt concentrations significantly decreased the seedling length of red clover and the highest length was reported at (0 mM) of NaCl solutions (Mandić et al., 2014). Investigation of the effects of germination and early seedling stages of (*Vigna unguiculata* L.) showed that with increasing salinity a significant reduction was observed in radicle and plumule length and plumule and radicle fresh and dry weight (Ilori, 2017). Oujii et al. (2015) reported that the length of root and shoot and their fresh and dry weight in *Lens culinaris* L. decreased with increase in the concentration of salt.

Because of the toxic effects of NaCl and reduction in water absorption, the vigor index decreased with increasing levels of stress in all three species. Seed vigor is the ability of the seed to germinate and means rapid and uniform emergence and development of normal seedlings under a wide range of field conditions. Also, vigor index determines the longevity of seed without adverse consequences (ISTA, 2009). The vigor index is a function of germination percentage and seedling length and has a direct relation with

them. In fact, vigor index represents the percentage and potential of seed germination. Therefore, if the seeds under stress have lower germination or seedling length, they will have a lower vigor index (Azad and Tobeh, 1994). In this research, it was found that with decreasing seedling growth and seed germination, vigor index also decreased. At 246 mM, seeds germinated but did not form seedlings. It can be concluded that the seedling development stage of the three clover species was more sensitive to salinity than the germination stage. Since the vigor index incorporates seedling length measurements, seedling length and vigor index of *T. alexandrinum* were lower than the other two species and lower its vigor index compared to the two other species reflected species differences (Table 2).

In the study of the effects of salinity on germination and seedling growth of green gram varieties, it was determined with an increase in salinity levels, the greater reduction was observed for all the parameters. Germination percentage, shoot and root length, seed vigor, and salt tolerance index reduced in all varieties (Prakash, 2017).

In this study, germination percentage of *T. alexandrinum* was lower than the other two species while no differences were obtained at low osmotic stress, inhibitory effects of salinity on germination and its dependent parameters (GU and MGT) differed at 82 mM and more. These differences could be due to the osmotic potentials at which germination was possible under salinity stress. Different responses of clover species under salinity in germination and seedling growth was according to the change in NaCl levels. Therefore, NaCl has direct harmful effects on species under study. Consideration of the inhibitory effects of NaCl on germination and seedling growth of these species is very important in the selection of resistant species for cultivation in unfavorable conditions.

## References

**Abberton, M. T and A. H. Marshall.** 2005. 'Progress in breeding perennial clovers for temperate agriculture'. *The Journal of Agricultural Science*, 143: 117-135.

**Abdul-Baki, A.A and J. D. Anderson.** 1973. 'Vigor determination in soybean seed by multiple criteria'. *Crop science*, 13: 630-633.

**Anaya, F., R. Fghire, S. Wahbi and K. Loutfi.** 2015. 'Influence of salicylic acid on seed germination of *Vicia faba* L. under salt stress'. *Journal of the Saudi Society of Agricultural Sciences*.

**Asci, O. O.** 2011. 'Salt tolerance in red clover (*Trifolium pratense* L.) seedlings'. *African Journal of Biotechnology*, 10: 8774-8781.

**Azad, F and A. Tobeh.** 1994. 'Relationship between grain yield and dry matter production of green and some other characteristics in laboratory and greenhouse cultivation'. *The 6<sup>th</sup> Iranian Congress of crop production and plant breeding*, 3-6 Sep, University of Mazandaran, Babolsar, Iran, pp: 233.

**Dadkhah, A and H. Grrifiths.** 2010. 'The effect of salinity on growth, inorganic ions and dry matter partitioning in sugar beet cultivars'. *Journal of Agricultural Science and Technology*, 8: 199-210.

**Dodd, G. L and L.A. Donovan.** 1999. 'Water potential and ionic effects on germination and seedling growth of two cold desert shrubs'. *American Journal of Botany*, 86 (8): 1146-1153.

**Esfandiari, E and A. Javadi.** 2014. 'Role of scavenging enzymes and hydrogen peroxide and glutathione S-transferase in mitigating the salinity effects on wheat'. *Journal of Plant Biology*, (2008-8264), 6(20):1-17.

**Fallahi, J., M. T. Ebadi and R. Ghorbani.** 2009. 'The effects of salinity and drought stresses on germination and seedling growth of clary (*Salvia sclarea*)'. *Environmental Stresses in Crop Sciences*.

**Fallahi, J., P. Rezvani Moghaddam, M. Nassiri Mahallati, M.A. Behdani, M.A. Shajari and M.B. Amiri.** 2013. 'Influence of seed nitrogen content and biofertilizer priming on wheat germination in salinity stress conditions'. *Archives of Agronomy and Soil Science*, 59: 791-801.

**Hohl, M and P. Schopfer.** 1991. 'Water relations of growing maize coleoptiles comparison between mannitol and polyethylene glycol

- 6000 as external osmotic for adjusting turgor pressure'. *Plant Physiology*, 95 (3): 716-722.
- Huang, Y., G. Zhang, F. Wu, J. Chen and M. Zhou.** 2006. 'Differences in Physiological Traits Among Salt-Stressed Barley Genotypes'. *Communications in soil science and plant analysis*, 37 (3-4): 557-570.
- Ilori, O.** 2017. 'NaCl salinity-induced changes on the germination and growth of *Vigna unguiculata* (L.) Walp'. *Seed*, 4(8): 4381-4387.
- ISTA.** 2009. 'International rules for seed testing'. *International Seed Testing Association Bassersdorf, Switzerland*.
- ISTA.** 2016. 'Ista certificates. International rules for seed testing'. *Association (ISTA), Zurichstr. 50, CH-8303 Bassersdorf, Switzerland*, i: 1-14.
- Kaya, M. D., G. Okçu, M. Atak, Y. Çıkılı and Ö. Kolsarıcı.** 2006. 'Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.)'. *European journal of agronomy*, 24: 291-295.
- Khajeh-Hosseini, M., A. Powell and I. Bingham.** 2003. 'The interaction between salinity stress and seed vigor during germination of soybean seeds'. *Seed Science and technology*, 31: 715-725.
- Khan, M., B. Gul and D. Weber.** 2002. 'Seed germination in relation to salinity and temperature in *Sarcobatus vermiculatus*'. *Biologia Plantarum*, 45: 133-135.
- Khayatnezhad, M., R. Gholamin, S. Jamaatie-Somarin and R. Zabihi-Mahmoodabad.** 2010. 'Effects of peg stress on corn cultivars (*Zea mays* L.) at germination stage'. *World Appl. Sci. J*, 11: 504-506.
- Khodarahmpour, Z.** 2011. 'Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrids'. *African Journal of Biotechnology*, 10: 18222-18227.
- Mandić, V., V. Krnjaja, Z. Bijelić, Z. Tomić, A. Simić, M. D. Ružić and A. Stanojković.** 2014. 'Genetic variability of red clover seedlings in relation to salt stress'. *Biotechnology in Animal Husbandry*, 30: 529-538.
- Manjkhola, S., U. Dhar and R. Rawal.** 2003. 'Treatments to improve seed germination of *Arnebia benthamii*: an endangered medicinal herb of high altitude Himalaya'. *Seed science and technology*, 31: 571-577.
- Mostafavi, K., H. S. Geive, M. Dadresan and M. Zarabi.** 2011. 'Effects of drought stress on germination indices of corn hybrids (*Zea mays* L.)'. *International Journal of AgriScience*, 1: 10-18.
- Nilsen, E and D. Orcutt.** 1996. '*The physiology of plants under stress: abiotic factors*'. (John Wiley & Sons, Inc., New York).
- Niste, M., R. Vidican, V. Stoian, I. Berindean, A. Criste, R. Miclea and R. Pop.** 2015. 'The Effect of salinity stress on seed germination of red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) varieties'. *Bulletin USAMV series Agriculture*, 72: 2.
- Ouji, A., S. El-Bok, M. Mouelhi, M. B. Younes and M. Kharrat.** 2015. 'Effect of salinity stress on germination of five Tunisian lentil (*Lens Culinaris* L.) genotypes'. *European Scientific Journal*, 11 (21): 63-75.
- Peñuelas, J., R. Isla, I. Filella and J. L. Araus.** 1997. 'Visible and near-infrared reflectance assessment of salinity effects on barley'. *Crop Science*, 37: 198-202.
- Prakash, M.** 2017. 'Effect of salinity on germination and seedling growth of green gram varieties'. *International Journal of Plant Sciences (Muzaffarnagar)*, 12: 79-84.
- Price, K., I. Johnson, G. Fenwick and M. Malinow.** 1987. 'The chemistry and biological significance of saponins in foods and feedingstuffs'. *Critical Reviews in Food Science & Nutrition*, 26: 27-135.
- Pujol, J. A., J. F. CALVO and L. Ramirez-Diaz.** 2000. 'Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain'. *Annals of Botany*, 85: 279-286.
- Sidari, M., C. Mallamaci and A. Muscolo.** 2008. 'Drought, salinity and heat differently affect seed germination of *Pinus pinea*'. *Journal of forest research*, 13: 326-330.
- Soltani, A., M. Gholipoor and E. Zeinali.** 2006. 'Seed reserve utilization and seedling growth of wheat as affected by drought and salinity'. *Environmental and Experimental Botany*, 55: 195-200.



**Soltani, A** and **V. Maddah**. 2010. 'Simple applied programs for education and research in agronomy'. ISSA press.

**Tsegay, B. A** and **B. Gebreslassie**. 2014. 'The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*'. *African Journal of Plant Science*, 8: 225-231.

**Turhan, H** and **C. Ayaz**. 2004. 'Effect of salinity on seedling emergence and growth of sunflower (*Helianthus annuus* L.) cultivars'. *Int. J. Agric. Biol*, 6: 149-152.