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Full Length Research Paper

Selection of alfalfa (*Medicago sativa* L.) cultivars for salt stress tolerance using germination indices

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To select the most tolerant alfalfa genotypes to salinity stress, an experiment was performed in a factorial scheme having a completely randomized design (CRD) with three replications. The cultivar and salinity stress factors comprised 20 cultivars and four levels of salinity stress (control, 75, 150 and 225 mM) with NaCl, respectively. Results indicate that a significant decrease was observed in all traits except for mean germination time in stress conditions. The Bami Garmsiri cultivar showed the highest germination percentage, germination rate, radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour traits in salinity conditions. Results of the cluster analysis using the data for all measured traits and salinity levels clustered cultivars into two groups. Cultivars KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri as well as KFA_3, KFA_6, KFA_7, KFA_9, KFA_{17}, KFA_8, KFA_{13}, KFA_{10}, KFA_{14} and KFA_{15} were placed in the first and second clusters, respectively. Cultivars in the first cluster were found to be tolerant, while those in the second cluster were sensitive to salt. Bami Garmsiri was the most tolerant cultivar to salt. However, all the cultivars were used in large field trials to confirm their tolerance to salt waiting creation of new hybrid varieties.

Key words: Cluster analysis, Nacl stress factorial scheme, completely randomized design, analysis, new hybrid varieties, Iran.

INTRODUCTION

Medicago sativa L. is a perennial, forage and diploid legume of the Fabaceae family (http://en.wikipedia.org/wiki/Alfalfa, on the 18th February 2012). Salinity of soil and rarity of water resources is a serious threat to human lives and worldwide peace (Amer, 2010). Soil salinity may affect the germination of seeds either by creating low osmotic potential to the seeds preventing water uptake or through the toxic effects of Na^+ and Cl^- ions on germinating seed (Khajeh-Hosseini et al., 2003; Atak et al., 2006; Kaya et al., 2006; Golbashy et al., 2010). Likewise, the rarity of water triggers conflicts among waterside countries.

Seed sowing is generally considered as a stage that is both critical and sensitive in the life cycle of plants. Seeds are frequently exposed to unfavorable environmental conditions that may compromise the establishment of seedling (Figueiredo-e-Albuquerque and Carvalho, 2003).

Alfalfa is moderately tolerant to salinity (Al-Khatib et al., 1992; Rumbaugh and Pendery, 1990) but there are high morphological variations between and within cultivars. Such morphological variations noted in center of diversity among the germplasms led to the selection of the most tolerant cultivars to salinity.

It has also been reported that under saline conditions, germination ability of seeds differs from crop to crop and even a significant variation is observed amongst the different varieties of the same crop (Asana and Kale, 1965; Maas and Hoffman, 1977). Water stress acts by

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decreasing the percentage and rate of germination and seedling growth (Rahimi et al., 2006; Bhardwaj et al., 2010; Hamidi and Safarnejad, 2010).

Several investigations of seed germination under salinity stress have indicated that seeds of most species achieve their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Monirifar, 2008; Bhardwaj et al., 2010; Yarnia, 2011; Torabi et al., 2011). Monirifar (2008) studying Syah-Roud, Gara-Yonjeh, Hasht-Roud, Khor-Khor and Bash-Kand cultivars indicated that the response was significantly different among cultivars and within the NaCl levels. The NaCl effect was significant in reducing all measured traits of all the five ecotypes. Bash-Kand had recorded the highest yield reduction percentage, whereas Syah-Roud and Gara-Yonjeh cultivars had the lowest one, in general, Syah-Roud and Gara-Yonjeh expressed the highest tolerant to salinity.

Sodium chloride (NaCl) salinity is one of the major environmental factors that limit plant growth, productivity and distribution (Wang et al., 2003).

Many tools are now available to study relationships among cultivars, including various types of molecular markers; however, morphological characterization is the first step in the description and classification of germplasm (Smith and Smith, 1989). The availability of genetic variation, both at intra or inter-specific level, is a prerequisite for the success of breeding programs (Ashraf et al., 1987; Maas 1986). Genetic variability for salt tolerance was reported in alfalfa (Mckimmie and Dobrenz, 1991; Yarnia et al., 2001; Peel et al., 2004; Torabi et al., 2011). Alfalfa is being increasingly cultivated in Iran; therefore, it is important to develop new alfalfa hybrids with a high genetic capacity to tolerate salt stress. The first important step in breeding new varieties with high salt tolerance is to lay out useful and substantial genetic variation in tolerance to salinity stress.

The tolerance level to salinity of cultivars KFA_1, KFA_2, KFA_3, KFA_4, KFA_5, KFA_6, KFA_7, KFA_8, KFA_9, KFA_{10}, KFA_{11}, KFA_{12}, KFA_{13}, KFA_{14}, KFA_{15}, KFA_{16}, KFA_{17}, Bami Garmsiri, Nikshahri Garmsiri and Yazdii Garmsiri is still not observed; this is a problem that needs urgent consideration. Among the cultivars, some might be tolerant and so they adapt to saline soils from Iran, though this is as an assumption. The objective of this research was to select the most tolerant genotypes to salinity stress in further hybridization purposes.

MATERIALS AND METHODS

Plant material and culture conditions

Twenty alfalfa cultivars (KFA_1, KFA_2, KFA_3, KFA_4, KFA_5, KFA_6, KFA_7, KFA_8, KFA_9, KFA_{10}, KFA_{11}, KFA_{12}, KFA_{13}, KFA_{14}, KFA_{15}, KFA_{16}, KFA_{17}, Bami Garmsiri, Nikshahri Garmsiri and Yazdii Garmsiri) were provided by the Department of Plant and Seed Research Institute of Karaj, Iran. These cultivars were used in four levels of salinity treatment (distilled water as control, 75, 150 and 225 mM) of sodium chloride (NaCl) solution. This experiment was carried out at Seed Laboratory, Islamic Azad University - Shoushtar Branch in Iran in 2011.

At each level of stress, 25 seeds of each genotype were selected and sterilized in sodium hypochlorite (1%) and then washed in distilled water twice. The seeds of cultivars were germinated in Petri-dishes (11 cm) on two layers of filter paper (90 mm) in an incubator (40% relative humidity) maintained at 25°C.

Experimental design and measured variables

20 x 4 factorial scheme having a completely randomized design with three replications was used. In all, 240 treatments were tested. Two factors comprising cultivar and salinity with 20 (KFA_1, KFA_2, KFA_3, KFA_4, KFA_5, KFA_6, KFA_7, KFA_8, KFA_9, KFA_{10}, KFA_{11}, KFA_{12}, KFA_{13}, KFA_{14}, KFA_{15}, KFA_{16}, KFA_{17}, Bami Garmsiri, Nikshahri Garmsiri and Yazdii Garmsiri) and 4 levels (control, 75, 150 and 225 mM) of sodium chloride (NaCl) for each were used, respectively. Treatment consisted of one cultivar associated with one level of salinity laid out in one Petri-dish. Daily, germination rate was calculated and the filter papers were replaced. NaCl soluble was added to the treatment. Seeds were considered germinated when the emergent radicle reached 2 mm length. After 10 days, germination percentage was calculated by ISTA (1996) standard method. On the 10th day, the germination percentage (Formula 1), mean germination time (MGT) (Ellis and Robert, 1981), germination rate (Formula 2), radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour (Formula 3) were calculated.

Formula 1: $$GP = \frac{SNG}{SN0} \times 100$$

GP is the germination percentage, SNG is the number of germinated seeds, and SNO is the number of experimental seeds with viability (Scott et al., 1984).

Formula 2: $$GR = \frac{\sum N}{\sum (n \times g)}$$

Where, GR is the germination rate; n is the number of germinated seed on gth day and g is the number of total germinated seeds (Ellis and Robert, 1981).

Formula 3: Seed vigour = germination percentage × seedling length

Statistical analyses

For statistical analysis, the data of germinating percentage were transformed to arcsin $\sqrt{\frac{X}{100}}$. Analyses were done using the Minitab software. Differences among means were determined by Duncan's Multiple Range Tests (DMRT) at 1% probability level.

Cluster analysis is a convenient method for organizing a large data set so information can be retrieved more efficiently and it can be easily understood without the need for complicated mathematical techniques. All investigated traits were subjected to hierarchical cluster analysis using the procedure of Ward's minimum variance method as a clustering algorithm. Ward's minimum method is a hierarchical clustering procedure in which similarity is used to join clusters and is calculated as the sum of squares between the two clusters summed over all variables (Hair et al., 1998). It minimizes them within cluster sums of squares

Table 1. Cultivar and salinity level effects and their interaction on the recorded germination indices.

Source of variance	Df	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)	Radicle length/Plumule length ratio	Seed vigour
Salinity levels	3	674.367**	0.090 ns	0.002*	1.050**	0.324**	2.467**	0.064 ns	3.697**
Cultivar	19	7328.086**	77.670**	0.0852**	123.907**	70.729**	363.695**	6.137**	364.200**
Salinity levels×Cultivar	57	133.549**	0.086 ns	0.001ns	0.410 ns	0.193 ns	0.690 ns	0.055 ns	0.952 ns
Error	160	66.367	0.067	0.001	0.397	0.150	0.795	0.063	0.801
CV%	-	9.7	9.4	8.1	24.3	16.1	17.9	24.2	20.3

ns and **, non significant and significant at 1% probability level respectively.

Table 2. Classification of means simple effect of salinity stress levels on germination and seedling growth.

Salinity stress (mM)	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)	Radicle length/Plumule length ratio	Seed vigour
0	92.033a	2.15917 c	0.464167 a	3.9232 a	3.73117 a	7.2657 a	1.05457c	669.90 a
75	90.467a	2.07650 c	0.480500 a	3.5983 a	2.81950 b	6.8057 b	1.27950a	616.53 b
150	86.067b	2.36217 b	0.424500 b	2.0030 c	1.78850 c	3.8082 c	1.12983 b	330.60 c
225	68.000c	4.47610 a	0.221356 c	0.8391 d	1.28190 d	2.0693d	0.66362 d	144.98 d
Percentage of decrease	-26	-	-54	-79	-66	-72	-54	-78

Means with similar letter(s) in each trait is not significantly different at 1% probability level according to Duncan's Multiple Range Test.

across all partitions.

RESULTS AND DISCUSSION

Results show that, there were significant differences between salinity stress levels except for mean germination time and radicle length/ plumule length ratio. For cultivars, there were significant differences for all traits which demonstrated existence of high diversity among cultivars studied for salinity tolerance. Also, analysis of variance showed that interaction effects were significant only for germination percentage (Table 1).

Germination percentage and germination rate

It was observed that in all cultivars, there was a decrease in germination percentage because increment salinity stress and maximum germination percentage was delayed. In this experiment, different cultivars had different response to the salinity stress. Among the alfalfa cultivars, Bami Garmsiri had the highest germination percentage and germination rate, while KFA_{15} had the lowest germination percentage and germination rate (Table 3). However, maximum reduction in germination percentage and germination rate was observed at the highest level at 225 mM of NaCl level (Table 2). Results show that germination percentage (26%) and germination rate (54%) decreased with a decrease in osmotic potential, while the maximum germination percentage and germination rate were obtained at the control treatment and 75 mM of NaCl (Table 2). Similar results were also reported by Rahimi et al. (2006), Bhardwaj et al. (2010) and Hamidi and Safarnejad (2010) on alfalfa at drought stress condition. Some studies showed that stress can contribute to improve germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), enhancement of ATPase activity, RNA and acid

Table 3. Classification of means simple effect of cultivar on germination and seedling growth.

Cultivar	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)	Radicle length/Plumule length ratio	Seed vigour
KFA_1	86.833 a-g	2.8233 ab	0.40000 abc	2.5908 abc	2.4358 ab	4.9433 a-e	1.0242 a	445.67 bcd
KFA_2	85.000 a-g	2.9175 a	0.39167 abc	2.9558 abc	2.3842 ab	5.3400a-e	1.2150 a	589.42 abcd
KFA_3	77.091 g	2.7082 ab	0.39727 abc	2.4773 abc	2.3764 ab	4.3945 a-e	1.0309 a	391.45 de
KFA_4	78.000fg	2.6950 ab	0.40250 abc	2.8167 abc	2.5075ab	5.4425 abcd	1.1017a	446.67 bcd
KFA_5	94.000 ab	2.8017 ab	0.39250 abc	2.7417 abc	2.6708 a	5.4100 abcd	1.0075 a	510.17 abc
KFA_6	80.333efg	2.8092 ab	0.39667 abc	2.2800 bc	2.3892 ab	4.5858 cde	0.9342 a	385.83 de
KFA_7	86.8333 a-g	2.8708 a	0.39583 abc	2.4108 abc	2.2592 ab	4.5867 cde	1.0633 a	413.00 cde
KFA_8	88.667 a-e	2.7100 ab	0.40250 abc	2.4758 abc	2.3225 ab	4.7983 a-e	1.0383 a	445.92 bcd
KFA_9	84.667 b-g	2.8225 ab	0.38833 cb	2.4333 abc	2.2400 ab	4.6733 bcde	1.0417 a	410.67 cde
KFA_{10}	92.667 abc	2.7840 ab	0.40000abc	2.1958 c	2.1550 b	4.2675 e	1.0442 a	405.75 cde
KFA_{11}	87.000 a-g	2.7058 ab	0.41167 ab	3.1417 a	2.6150 ab	5.7567 ab	1.1317a	531.42 ab
KFA_{12}	83.667 c-g	2.7508 ab	0.39750 abc	2.7125 abc	2.5667 ab	5.2792 a-e	1.0317a	455.17 bcd
KFA_{13}	83.167 c-g	2.7992 ab	0.38750 bc	2.5650 abc	2.3292 ab	4.8942 a-e	1.0833a	442.00 bcd
KFA_{14}	88.000 a-f	2.7042 ab	0.39667 abc	2.1608 c	2.2067 ab	4.3675 de	0.9317a	404.83 cde
KFA_{15}	61.3333 h	2.8708 a	0.36833 c	2.3055 bc	2.3618 ab	4.6673 bcde	0.9936a	315.45 e
KFA_{16}	91.000 abcd	2.6683 ab	0.41250 ab	2.5683 abc	2.3067 ab	4.8750 a-e	1.0567a	460.00 abcd
KFA_{17}	82.333 defg	2.7667 ab	0.39083 abc	2.5383abc	2.2575 ab	4.7958 a-e	1.0658a	397.67 cde
Bami Garmsiri	95.000 a	2.5283 b	0.43167 a	3.1475 a	2.6817 a	5.8167 a	1.0908a	564.08 a
Nikshahri Garmsiri	77.500 g	2.7208 ab	0.40000 abc	3.0658 ab	2.6158 ab	5.7017 abc	1.1150a	480.92 abcd
Yazdii Garmsiri	81.000 defg	2.7650 ab	0.40333 abc	2.4950 abc	2.6050 ab	5.1000 a-e	0.8975a	448.75 bcd

Means with similar letter(s) in each trait are not significantly different at 1% probability level according to Duncan's Multiple Range Test.

phosphathase synthesis (Fu et al., 1988), also by increase of amylases, proteases or lipases activity (Ashraf and Foolad, 2005).

Mean germination time

Among the alfalfa cultivars, KFA_2, KFA_7 and KFA_{15} had the highest mean germination time and Bami Garmsiri had the lowest mean germination time (Table 3). Mean germination time was delayed by decreasing water potential (Table 2). The maximum mean germination time was obtained at 225 mM of NaCl (Table 2). The results are in agreement with Rahimi et al. (2006) and Hamidi and Safarnejad (2010) on alfalfa.

Radicle, plumule, seedling length and radicle length/plumule length ratio

The radicle length provides an important clue to the response of plants to salinity stress. A special reduction in the radicle length, the plumule length and the seedling length of all cultivars of alfalfa was observed because of salt stress. Among the alfalfa cultivars, KFA_{11} and Bami Garmsiri had the longest radicle length, KFA_5 and Bami Garmsiri had the longest plumule length and Bami Garmsiri had the longest seedling length, but between cultivars, there was no significant difference for radicle length/plumule length ratio (Table 3). Results of this study show that radicle length (79%), plumule length (66%) and seedling length (72%) decreased with increasing salinity levels in all cultivars. The most effective level in reducing

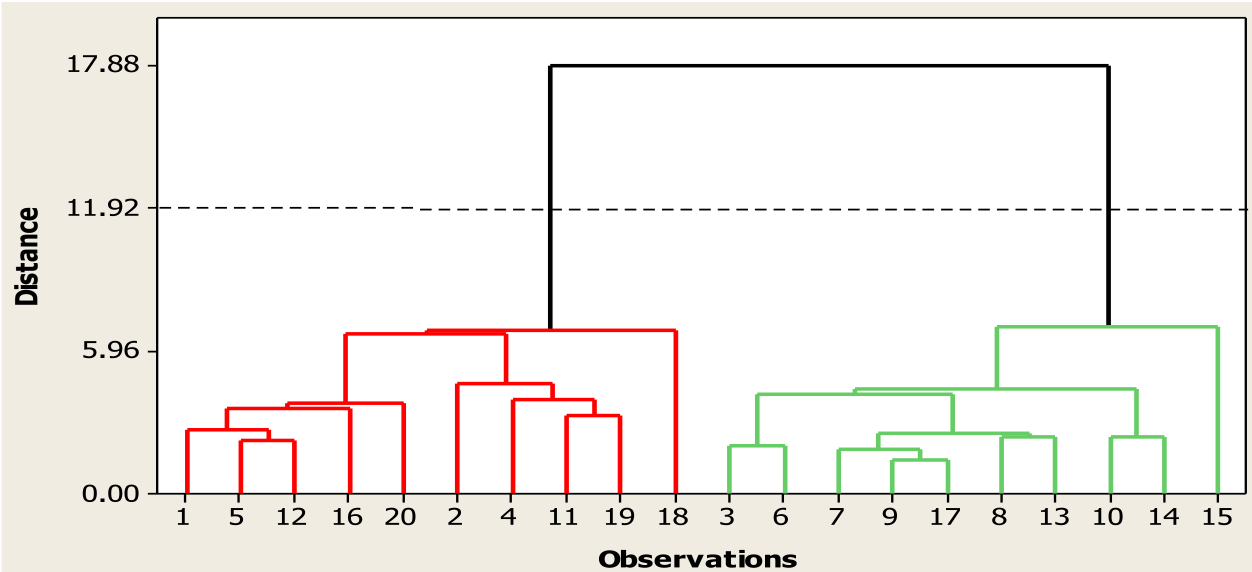

Figure 1. Cluster analysis of alfalfa cultivars under different levels of salinity stress using Ward's minimum variance method. 1, KFA_1; 2, KFA_2; 3, KFA_3; 4, KFA_4; 5, KFA_5; 6, KFA_6; 7, KFA_7; 8, KFA_8; 9, KFA_9; 10, KFA_{10}; 11, KFA_{11}; 12, KFA_{12}; 13, KFA_{13}; 14, KFA_{14}; 15, KFA_{15}; 16, KFA_{16}; 17, KFA_{17}; 18, Bami Garmsiri; 19, Nikshahri Garmsiri; 20, Yazdii Garmsiri.

these attributes was 225 mM of NaCl (Table 2). The best level of NaCl concentration in radicle length was the control treatment and 75 mM of NaCl, and for plumule length and seedling length, it was the control treatment and for radicle length/Plumule length ratio, it was 75 mM NaCl (Table 2). Water stress acts by decreasing the percentage rate of germination and seedling growth (Rahimi et al., 2006; Bhardwaj et al., 2010; Hamidi and Safarnejad, 2010). Bhardwaj et al. (2010) and Monirifar (2008) reported that with increasing salinity concentration, radicle, plumule and seedling length decreases.

Seed vigour

Among the cultivars, Bami Garmsiri was affected the least by salinity stress because it gave the lowest reduction rate for seed vigour (Table 3). Seed vigour (78%) decreased with increased concentration of NaCl solution. The best level in seed vigour was the control treatment (Table 2). Of all the cultivars, Bami Garmsiri produced the highest seed vigour at all salt regimes while KFA_{15} cultivar produced the lowest seed vigour at all salt regimes. Hamidi and Safarnejad (2010) studied alfalfa cultivars in drought stress conditions and reported that seed vigour decreased with a decrease in osmotic potential.

Classification of alfalfa cultivars

Results of cluster analysis (Ward's minimum variance method) arranged cultivars at interval 11.92 into two groups (Figure 1). The results of the first cluster which included ten of the cultivars (KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri) for the following traits: germination percentage, germination rate, radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour, were higher than the total mean, while for the mean germination time trait, they were lower than the total mean (Table 4). The results of the second cluster which included ten cultivars (KFA_3, KFA_6, KFA_7, KFA_9, KFA_{17}, KFA_8, KFA_{13}, KFA_{10}, KFA_{14} and KFA_{15}) for all desirable traits: germination percentage, germination rate, radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour, were lower than the total mean, while for the undesirable trait mean germination time, they were higher than the total mean. Therefore, cultivars KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri in the first cluster are tolerant to salinity stress and cultivars KFA_3, KFA_6, KFA_7, KFA_9, KFA_{17}, KFA_8, KFA_{13}, KFA_{10}, KFA_{14} and KFA_{15} in second cluster are sensitive to salinity stress. Yarnia et al. (2001) reported that Golestan (20313) and Fao (2526) were the most tolerant and sensitive respectively to salinity stress. Lines Hamadani Ahar and Sistan and Baluchestan lines were selected as semisensitive and semitolerant respectively. Torabi et al. (2011) reported that the ecotypes Bami, Galehbani, Nik-Shahri, Rehnani and Gharegozloo were superior to others in terms of salt tolerance at germination stage. Rezaei et al. (2010) assessed genetic diversity in alfalfa ecotypes from central and eastern regions of Iran using SSR markers and reported that

Table 4. Means and deviation precentage from total mean for alfalfa difference traits of groups in cluster analysis in salinity condition.

Traits	Cluster 1	Cluster 2	Total mean
	KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri	KFA_3, KFA_6, KFA_7, KFA_9, KFA_{17}, KFA_8, KFA_{13}, KFA_{10}, KFA_{14} and KFA_{15}	
Germination percentage	85.87, +2.06	82.49, -1.96	84.14
Mean germination time (day)	2.74, -1.08	2.80, +1.08	2.77
Germination rate (number in day)	0.40, +1.58	0.39, -1.41	0.398
Radicle length (cm)	2.76, +6.4	2.42, -6.56	2.59
Plumule length (cm)	2.51, +4.38	2.3, -4.37	2.405
Seedling length (cm)	5.37, +7.55	4.6, -7.82	4.99
Radicle/Plumule length ratio	1.07, +3.61	0.99, -3.88	1.03
Seed vigour	493.2, +11.97	387.8, -14.01	440.50

despite the similarities of genetic structures between the ecotypes from two regions, high variation was observedamong individual plants possibly due to the high allogamy 90%), insects activity (particularly bees) and pollen transmission among ecotypes.

Conclusion

In this study, we tested the hypothesis assuming the existence of some cultivars tolerant to salinity. We observed that there exist some cultivars tolerant to saline stress. Indeed, KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri expressed a good tolerance to salt stress. Afterward, the most important result was presented among others.

Salt stress adversely affected the germination percentage, germination rate, mean germination time, radicle length, plumule length, seedling length and seed vigour of 20 cultivars of alfalfa, and a significant variation in salt tolerance was observed among all the cultivars. In conclusion, the germination and early seedling growth stages of the investigated cultivars showed different responses to salt stress. Furthermore, germination failure due to NaCl resulted from an osmotic barrier induced by NaCl. Redmann (1974) found that the osmotic effect of

NaCl on germination of alfalfa was more important than the toxic effect. However, seedling growth was more sensitive to salt stress than was germination; this is because germination percentage (26%) and seedling length (72%) decreased. Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to the ability of seeds to undergo the best germination under unfavorable conditions, hence evaluation of salinity tolerant genotypes is important at the primary growth stage.

Ranking of the genotypes was done using the data of all measured traits at all levels of salt. Results show that the cultivars KFA_1, KFA_5, KFA_{12}, KFA_{16}, Yazdii Garmsiri, KFA_2, KFA_4, KFA_{11}, Nikshahri Garmsiri and Bami Garmsiri were in the first cluster and for the traits of germination percentage, germination rate, radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour results were higher than the total mean. For mean germination time, trait was lower than the total mean. Therefore, cultivars of the first cluster are tolerant to salinity stress. The second cluster included cultivars KFA_3, KFA_6, KFA_7, KFA_9, KFA_{17}, KFA_8, KFA_{13}, KFA_{10}, KFA_{14} and KFA_{15} and for all desirable traits; germination percentage, germination rate, radicle length, plumule length, seedling length, radicle length/plumule length ratio and seed vigour results were lower than the total mean; for undesirable trait, mean germination time was higher than the total mean. Therefore, cultivars of the second cluster are sensitive to salinity stress. In this research, Bami Garmsiri cultivar had the highest germination percentage, radicle length, plumule length, seedling length and seed vigour traits, and therefore was found to be the most tolerant to salt stress. These results can be related to some earlier studies in which cultivars identified as salt tolerant at the earlier growth stages showed tolerance when tested at the later growth stages. Although, a considerable magnitude of variation for salt tolerance was observed in the 20 cultivars of alfalfa while screening them at germination stages, further studies need to be carried out to assess whether the genotypes marked as salt tolerant at the initial growth stages maintain their degree of salt tolerance when tested as adult plants.

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