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Short Communication

Screening of alfalfa (*Medicago sativa* L.) ecotypes from Azarbayjan region for salinity stress tolerance

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Abstract

Salinity of soil is one of the most important limiting factors for crop production. An experiment was conducted to screen alfalfa ecotypes (landraces) for tolerance to salinity (EC=4.59 dS m^{-1}) stress during 2018 and 2019. Twenty plants of each experimental unit were removed and divided into leaves and stem. Fresh and dry forage yield, number of nodes of stems and leaf area were measured. Analysis of variance revealed significant differences among the ecotypes for all the studied traits under salinity stress. The traits studied showed high genetic variability among the landrace for different traits. Comparisons of mean for fresh and dry forage yield indicated that ecotype, Qarayonjeh was significantly different than other ecotypes. Results also showed that Qarayonjeh had higher tolerance to salinity stress, whereas, Bahraman and Khajeh and Leghlan were selected as semi- tolerant ecotypes for salinity stress. The findings may be useful for farmers as well as in breeding suitable genotypes of alfalfa for salinity stress tolerance.

Key words: Alfalfa, ecotype, forage yield, salinity tolerance, screening

The growth, development and yield of crop plants is affected by various environmental stresses. Among which, abiotic stresses such as drought and salinity are the major environmental constraints to crop production in the world (Tani et al. 2018). Nearly 20% of the world's cultivated area and nearly half of world's irrigated lands are affected by salinity (Gupta and Hang 2014). Salinity of soil and rarity of water resources is a serious threat to human lives. High salt stress generally inhibits growth and development of different traits of plants (Munns and Tester 2008) contributing towards yield. Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in many countries around the world. It is used for grazing, hey and silage as well as a green manure and cover crop and also utilized as medicine by the human beings. It is a perennial flowering plant belonging to Fabaceae family that is planted on the slops and remains in field for four to six years. Alfalfa or lucerne (*Medicago sativa*) is comparatively more tolerant, and halophytes such as salt bush (*Atriplex* spp.) continue to grow well at salinity level greater than that of seawater. Screening of the germplasm and identification of tolerant genotypes would remain to be to the most effective and quick approach of developing genotype tolerant to salinity stress (Rekha et al. 2021). However, the success of breeding program depends on the genetic variability available to breeders. Therefore, the objectives of this study were to screen alfalfa landraces (ecotypes) of Azarbayjan region of Iran for salinity stress tolerance.

Ten landraces of alfalfa ecotypes from Azarbayjan region were screened for salinity stress tolerance at Khageh Station of the Agriculture and Natural Research Center of East Azarbayjan, located near Tabriz during 2018 and 2019. The average of ten soil samples of this land had electrical conductivity of EC=4.59 dS

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m^{-1}. The saline soils are characterized as > 4dS/m equivalent to 40 mM NaCl. The material was planted in randomized completely block design with three replications in plots of 3 rows in 4m length. Plants harvested in four turn (each year two cuts) and forage yield and its components were measured. For sampling twenty plants of each experimental unit were removed and divided into leaves and stems. Then fresh and dry weight, number of stems and leaves, number of node of stems and leaf area were measured. All of the traits were measured per plant and mean of two cuts calculated for each year. After analysis of variance, comparison of means for studied traits was done by least significant difference (LSD) method at 5% probability level. Software such as SAS and SPSS were used for statistical calculations.

The results of analysis of variance showed significant differences at 1% probability level among ecotypes for all the studied traits indicating sufficient amount of genetic variability for salinity stress tolerance (Table 1). Comparison of means of all the studied traits (Table 2) indicated that ecotypes, Qarayonjeh, Khajeh, Leghlan and Bahraman had highest number of stem per plant, Bahraman, Nir, Leghlan, Qarayonjeh, Seivan and Satlo had highest number of nodes on stem and whereas Qarayonjeh and Bahraman with significantly difference than other ecotypes had highest leaf area. Djilianov et al. (2003) reported that leaf area was the most sensitive growth parameter in response to high salinity stress in alfalfa. Comparison of mean for fresh and dry forage yield indicated Qarayonjeh with higher mean value was significantly different than other ecotypes. Significant differences for forage yield of alfalfa under salinity stress have been reported earlier by Petcu et al. (2007) and Soltani et al. (2012). There are many genotypes fall within the same non-significant group indicating that they are equally good in producing number of nodes/stem. Dry forage is very important for the period when green fodder is not available to the live stocks (Table 2). Differences in response of the landrace population of alfalfa to salt stress were observed. Al-Lawati et al. (2016) reported fresh weight of forage

Table 1. Analysis of variance for studied traits of Azarbayjan alfalfa ecotypes

Source of variation	Degree of freedom	Mean of squares					
		No. of stems	No. of leaves	No. of nodes on stem	Leaf area	Fresh forage yield	Dry forage yield
Replication	2	14.420**	182.853^{ns}	0.534 ^{ns}	157.306 ^{ns}	484.086**	39.697**
Ecotypes	9	11.706**	1301.931**	2.560**	3155.108**	97.984**	12.755**
Error	18	2.389	0.536	0.536	304.591	14.388	1.387
Coefficient of variation	14.860	9.472	9.472	12.718	12.283	11.335	

*, ** : significant difference at 5 and 1% probability levels respectively
ns: non significant difference

Table 2. Comparisons of mean for studied traits of Azarbayjan alfalfa ecotypes

Ecotypes	Number of stems	Number of leaves	Number of nodes on stem	Leaf area (cm^2)	Fresh forage yield (gr/plant)	Dry forage yield (gr/plant)
Leghlan	12.023	102.627	8.523	147.83	27.083	11.063
Seivan	9.420	77.900	7.963	115.68	26.940	8.040
Satlo	8.983	77.207	7.920	117.20	30.390	9.903
Bahraman	11.210	107.347	8.986	183.10	34.047	11.020
Nir	9.737	80.767	8.286	122.31	26.727	9.170
Khajeh	13.427	106.617	7.943	158.25	36.453	12.773
DizajSafarali	8.777	74.377	6.253	117.43	27.967	9.313
Joshin	8.533	70.083	6.950	108.05	27.517	8.866
Alhord	8.457	71.873	6.306	105.97	27.967	8.996
Qarayonjeh	13.460	132.483	8.190	196.38	44.103	14.756
LSD 5%	2.651	15.891	1.256	29.938	6.507	2.020

yield was significantly different at 5% probability level among five alfalfa ecotypes and indicated high variability among the salinity treatments. Also dry weight of forage yield at all treatments of salinity (50,100, 200 and 400 mM) was significantly different at 1% probability level. Emam et al. (2009) showed low dry matter of forage yield at high salinity level treatments. Alfalfa has a large diversity of varieties with different tolerance levels to salinity stress conditions. The complex effects of salinity stress on the metabolism and physiological traits of plants include osmotic balance, alternation in enzyme activity, ion homeostasis, signal transduction and gene expression (Li et al. 2014). In the present study, different ecotypes had shown different response to salinity stress. Monirifar et al. (2004) had also reported a variable response among alfalfa cultivars in Azarbayjan at different salinity levels. Also Rezaie et al. (2010) reported genetic diversity in alfalfa ecotypes from central and eastern regions of Iran. It has been reported by several workers that soil salinity leads to adverse effects on many agronomical traits, growth, development and productivity of alfalfa ecotypes (Jiang et al. 2014), however, in present investigation genotype, Qarayonjeh showed a high degree of tolerance to salinity stress. The genotypes, Bahraman, Khajeh and Leghlan were selected as semi-tolerant ecotypes. Polycross and synthetic varieties are some of the breeding approaches, which may result in selection of salinity tolerant genotypes. The success of breeding program depends on the occurrence of the genetic variation in the population. The identification of the candidate landrace ecotypes would provide valuable information about the genetic mechanisms involved in salt tolerance response, and it would also supply important resources to the breeding programs in order to breed salt tolerant crop plants (Hernandez 2019).

Authors' contribution

Conceptualization of research (AM, MK, HM); Designing of the experiments (MK, GN); Contribution of experimental materials (HM, GN); Execution of field/lab experiments and data collection (AM, HM); Analysis of data and interpretation (AM, MK); Preparation of manuscript (AM, MK).

Declaration

The authors declare no conflict of interest.

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