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Variation among Iranian alfalfa genotypes for absolute growth rates and salt stress tolerance indices

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Abstract

In many parts of the world, one of the most important restricting factors in cropproduction is salinity stress. Screening and selecting tolerant genotypes based on morphological characteristics is one of the primary actions toward achieving salt tolerant varieties. In the present study, 20 genotypes of alfalfa, mostly from Iran, were evaluated in two separate experimentsin Agriculture Research Center of Safi Abad, Dezful, SW Iran, in 2014-2015. However highly significant variations were observed among genotypes in the both experiments, the results obtained from the first experiment (carried out within growth chamber) were more or less different from those obtained from the second one (carried out in a heated greenhouse) regarding to categorizing the genotypes as sensitive or tolerant. The results obtained from the growth chamber study illustrated that Nikshahri was the most tolerant genotype and Harpinger and Diablo-verde were the most sensitive ones. While in greenhouse experiment, the genotype Yazdi showed the highest tolerance and Bami showed the highest sensitivity to salt under moderate salinity stress environment. A similar trend was also found under severe salt stress conditions. Correlation analysis indicated highly significant relationships among the tolerance indices. In addition, principle component analysis revealed that the dimensions of data could be reduced to two components with explaining approximately the 99 percent of total variations among the genotypes.

Key Words: Alfalfa, Germination, Salt stress, Stress indices, Variation

Introduction

One of the most important factors limiting plant growth, productivity and distribution is sodium chloride (NaCl) salinity (Wang *et al.*, 2003). Salty land comprises almost 10% the total of the Iranian arable farmland and approximately 23% of all the cultivated lands (Seifi *et al.*, 2010). Conventional techniques of selection and breeding have been used to improve salt tolerance in crop plants (Ashraf, 2002). The agronomical parameters which have been considered for salt tolerance screening includes yield, plant height, survival rate, leaf injury, reduction in leaf area and reduction in relative growth rate (Ashraf and Harris, 2004). Various biochemical processes of plants are also adversely affected by the salt content of the soil. The magnitude of salt stress, however, impose various effects on plant species relevant to the type of stress and levels of salinity (Bhardwaj *et al.*, 2011). Literature is very diverse about ability of alfalfa genotypes to cope with salinity. Shanon (1984) has demonstrated that alfalfa tolerate salinity in the range between 2 dS/m (equal to 20 mMNaCl) and 16 dS/m (160 mMNaCl). High morphological dissimilarities are noted among the alfalfa germplasm regarding to salt tolerance (Soltani *et al.*, 2012). The first step to describe and classify germplasm is evaluating them for morphological characterization (Smith and Smith, 1989). The most sensitive stage to salt stress isgermination stage (Patanea *et al.,* 2009). Many factors which have a share in reduction of the production of alfalfa under salt stress noticeably, affect alfalfa population growth, root and dry weight and number and length of the main stem (Noble *et al.*, 1984).Aminpour and Aqaei (1997) also demonstrated that salinity in germination phase reduces germination speed and percentage and the length of radicle and plumule. In addition, Kant and

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Silverbush (1994) reported that salinity stress causes greater decrease in shoot growthcompared to root. Furthermore, other researchers have also reported reduction in the root and shoot weights as a result of salinity (Roger, 1998).In order to enhance salinity tolerance in alfalfa, it is crucial to find sufficient variation and to design suitable screening techniques that are reliable for the recognition of tolerant genotypes. In this regard, several schemes have been proposed for screening and selection (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez, 1992; Pecetti and Gorham, 1997). Some researchers proposed that selection should be carried out under favorable environments,with an opinion that high yield potential is anticipated to sustain high yields in saline conditions (Van-Ginkel *et al.*, 1998 and Betran *et al.*, 2003). In addition to absolute growth and development parameters, several indices obtained from purely statistical/mathematical relationship between stress and non-stress environments have been also offered in order to discriminate between tolerant and sensitive genotypes (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez,1992; Clarke *et al.*, 1984; Huang, 2000; Mohammadi *et al.*, 2010; Nouri *et al.*, 2011). Among them, stress susceptibility index (SSI) was suggested by Fischer and Maurer (1978), stress tolerance (TOL) and mean productivity index (MP) were described by Rosielle and Hamblin (1981) and tolerance index (STI) was proposed by Fernandez (1992). Some researchers (Basafa and Taherian, 2010; Khodarahmpor, 2013) have used principal component analysis (PCA) to analyze all the indices collectively. Due to alfalfa longevity and its ability to improve the characteristics of the land, it is cultivated in the vast area of the world (Jiang *et al.*, 2006). Alfalfa is also the most commonly produced forage crop in Iran (Babakhani *et al.*, 2011). Therefore, variation for salt tolerance in alfalfa landraces and wild specie could be explored in breeding programs aimed to overcome this phenomenon when salt stress exists. Iran is the centre of origin for alfalfa(Falahati *et al.*, 2007) and many alfalfa species are grown in the nature or are cultivated as landraces or local varieties throughout the country. The presence of different genotypes of alfalfa in Iran offers a valuable source for screening and identifying the tolerant types regarding to environmental stresses such as salinity nonetheless, variation among Iranian alfalfa genotypes for salt stress has not been identified adequately implying the need for more investigations. The main objective of this study was to determine variations for salt stress and some related characters among some Iranian landraces and wild species and also to identity tolerant and susceptible genotypes based on absolute growth parameters and calculated indices.

Material and methods

Genetic Materials

In order to evaluate growth rate and forage production of Iranian alfalfa genotypes under salt stress conditions, 13 alfalfa genotypes originated from Iran were included in the current study. In addition, 7 genotypes from overseas were also included in the experiment in order to provide an opportunity for comparisons between Iranian local genotypes and those originated from other parts of the world. All the genotypes received from Seed and Plant Improvement Institute (SPII), Karaj, Iran. The descriptions of these genotypes are presented in Table 1.

Table 1.Description of genotypes used in the study experiment-1

No	Genotype name	Locality	Country
1	Yazdi	Yazd	Iran
2	Nikshahri	Nikshahr	Iran
3	Bami	Bam	Iran
4	Rahnani	Isfahan	Iran
5	Gomi	Gom	Iran
6	Mesa-Sirsa	-	United states
7	Hamedani	Hamedan	Iran
8	Ramandi	Qazvin	Iran
9	Sahandava	East azerbaijan	Iran
10	Siriver	-	Australia
11	Harpinger	-	Iran
12	KF15	Hamedan	Iran
13	Kodi	-	United states
14	Defi	-	France
15	Kaiseri	Kaiseri	Turkey
16	Baghdadi	Dezful	Iran
17	Dastgerd	Dastgerd	Iran
18	Gargologh	Khoy	Iran
19	Melissa	-	France
20	Diablo verde	-	United states

- : **unknown locality**

This experiment was conducted to evaluate the genotypes for germination rate and related characters. Germination characteristics were evaluated in a growth chamber setting at 60% ± 3% air humidity, 16h/8h day/night photoperiod and an ambient temperature 25±2 °C. In this study, a completely randomized design (CRD) with four replications was carried out. Salinity levels of 75, 150 and 225 mMNaCl were used along with a control treatment. A modified version of the North American Alfalfa Improvement Conference (NAAIC) protocol was applied in conducting germination tests (Rumbaugh, 1991). So, germinating seeds were observed in petri dishes every day for one week and the indices of germination were controlled by counting the germinated seeds. Regarding germination process in alfalfa, the seeds were considered to have germinated when the radicles were visibly protruded from the seed coat by at least 2 mm. After germination of seeds, following salt tolerance indices were measured for each genotype:

CVG: Coefficient of velocity of germination is an index for speed and acceleration of seed germination (Maguire, 1962). This index was calculated using following formula:

$$CVG = \frac{G_1 + G_2 + G_3 + ... + G_n}{(1 \times G_1) + (2 \times G_2) + (3 \times G_3) + ... + (n \times G_n)}$$

(seed/day)

In which, G_1 to G_n are number of germinated seeds from the first to the last day of observations.

MTG: Means time to germination which is an index for germination rate (Ellis and Roberts, 1981) was calculated using following equation. In this equation, n, d and Σn are the number seeds germinated per day, number of days from the starting of the experiment and total germinated seeds, respectively.

$$MTG = \frac{\Sigma(nd)}{\Sigma n} \quad \text{(day-1)}$$

MDG: Mean daily germination that is a display from daily germination rate (Scott, 1984) was used as

$$\text{MDG} = \frac{\text{FGP}}{\text{d}}$$

In the above formula, FGP is the final germination percent and d is the period of experiment.

SLVI: Seedling length vigor index (Abbasian and Moemeni, 2013) was calculated as SLVI= FGP × (mean shoot length + mean root length).

DGS: Daily germination speed was calculated as:

$$\text{DGS} = \frac{1}{\text{MDG}}$$ (Huntr *et al.*, 1984)

IC (50): In order to recognize tolerant and sensitive genotypes for salt stress at germination stage, in addition to salt indices, percent of germination values were also used to estimate an IC(50) values. IC (50) is inhibitory concentrations of salt or osmotic potential necessary to inhibit the germination of 50% of viable seeds. To estimate IC (50) values, regression equations were performed between percentage of germination and four salt concentrations for each genotype. Among different types of regression, 2nd order polynomial equation had the highest R^2 value and therefore was selected as an indicator for the recognition of tolerant and sensitive genotypes. Recently, Scasta et al (2012) have also deduced that the regression method that adequately explains the relationship between mean germination and salt concentrations is 2nd order polynomial equation.

Experiment 2

In addition to germination related characters, the genetic materials were also evaluated for some other characters related to forage production within a greenhouse with the average temperature of 30°C/20°C day/night, the average relative humidity 60% and 16h/8h day/night photoperiod. Four sterilized seeds of each 20 genotypes were sown in individual 3cm×18cm cylindrical containers containing 15g of perlite and peat and thinned to two plants per container after seedling appearance. The pots were irrigated with 0.25X Hoagland's standard solution. A completely randomized design (CRD) with three replications with a split-plot arrangement was applied with NaCl levels as main plots and genotypes as subplots. Three salt levels were respectively obtained by adding 0, 50 and 100 mMNaCl to 0.25X Hoagland's standard solution from 14th days after onset of the experiment. Plants were grown for 50 days under the above conditions and then all vegetative parts of plants were harvested, dried and used for calculating salt tolerance indices. For each genotype, many salt tolerance indices were measured as follow:

Tolerance index was measured using the following formula (Rosielle and Hamblin, 1981):

$$TOL=Yp - Ys.$$

Mean productivity of the two conditions was also calculated as proposed by Rosielle and Hamblin (1981). In addition, stress tolerance index and geometric mean productivity proposed by Fernandez (1992) was calculated as (Ys). (Yp)/ $(\hat{Y}p)^2$and $\sqrt{Yp}.Ys$, respectively. Finally, two other stress susceptibility index were calculated as proposed by (Fischer and Maurer, 1978) using following equations:

$$SSI = 1- (Ys/ Yp) /SI$$

$$SI=1-(\hat{Y}s/ \hat{Y}p)$$

In all above equations, Yp and Ys are forage dry matter under salt stress and non stress condition, respectively.

Statistical analyses

Variance analyses of the data, obtained from the both experiments, were done using the SAS software. Differences among the means were recognized by Duncan's Multiple Range Tests (DMRT) at 5% probability level. Correlation analysis, principle component analysis and regression between forage dry matter and salinity level were all performed using MINITAB software.

Results and Discussion

Experiment-1

The results obtained from Exp. 1 showed significant differences among genotypes at different stress levels (Table 3) and over all treatments (Table 4). According to the results presented in Table 2 the interaction effects between salinity and cultivars were also significantly different for all the traits. These highly significant variations proved the fact that the studied genotypes are promising for being involved in any breeding programs aimed at improving salinity stress in alfalfa. Moreover, the existence of significant differences between interaction effects revealed that cultivars had different responses to the salinity stress. These differences are partly due to the fact that salinity effects are different at different stages of growth and development in alfalfa. In this regard, Table 3 demonstrates the results of comparisons among effects of salinity levels on the germination related characters. As can be seen from the table, the minimum germination percentage was obtained at 225 mM of NaCl. As it was expected, the maximum germination percentage was obtained at the control treatment. These results showed that germination percentage decreased by 66% in 225 mM of NaCl treatment compared with control treatment. Other germination traits were also declined due to salt stress treatments. Similar to germination percentage, radicle length, plumule length and shoot dry weights were also reduced by 96%, 86%, 48%, respectively at 225 mM of NaCl stress indicating that this treatment is more effective than other treatments. The effectiveness of salt treatments on germination related characters has been also reported by other researchers.Soltani et al (2012), Monirifar (2008) and Bhardwaj et al (2011) reported that increases in salinity concentration can cause decreases in radicle, plumule and seedling length in alfalfa. In the current experiment, the highest and lowest reductions happened for radicle length and shoot dry weight, respectively. This result shows that radicle length provides an important sign to the response of plants to salinity stress. Similar results were also reported by Soltani et al (2012).

The studied genotypes showed considerable amounts of reduction in germination related traits when exposed to salt stress treatments.Among the cultivars, Mesa-Sirsa had the highest germination percentage(89%), radicle length (30mm), plumule length (4.96mm), mean daily germination (12.77) and seedling length vigor index (34.14) while Diablo verde had the lowest germination percentage (35%), lowest radical (10.8mm) and plumule (2.86 mm) lengths, lowest coefficient of velocity of germination (0.145), lowest mean daily germination (5.051) and lowest seedling length vigor index (8.61) (see Table 4). These extreme differences in the performance of the above genotypes imply that both of them could be used in breeding programs in order to investigate the inheritance mode of the characters they are different for. In addition to these two genotypes, Harpinger had the highest mean germination time and Nikshahri and Yazdi cultivars had the lowest amount of this character (Table 4). Mean germination time was delayed by increasing the levels of salinity treatments (Table 3).This phenomenon is partly due to decreasing water potential which happens by increasing salinity

Table 2.Analysis of variance for studied traits in Exp1

Source of variation	Df	Germination (%)	Radicle length (mm)	Plumule length (mm)	Shoot dry weight (mg)	MGT	CVG	MDG	DGS	SLVI
Salinity	3	53927.2**	32425**	419.4**	12.538**	18.23**	0.107**	1100.7**	0.198**	34577**
Cultivar	19	4837.2**	758**	15.8**	2.001**	4.53**	0.025**	98.7**	0.029**	1009**
Salinity *Cultivar	57	759.9**	221**	2.4**	0.437**	0.773**	0.007**	15.5**	0.012**	249**
Error	240	72.3	10	0.30	0.032	0.061	0.001	1.475	0.0003	17
CV%		13.31	15.29	14.89	12.49	5.33	11.33	13.31	26.93	20.16

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

Table 3. The effects of salinity levels on the traits studied in Exp 1

Salinity stress (mM)	Germination (%)	Radicle length (mm)	Plumule length (mm)	Shoot dry weight (mg)	MGT (day)	CVG	MDG	DGS	SLVI
0	89a	45.12a	6.138a	1.632a	4.243d	0.237a	12.74a	0.079d	46.45a
75	78b	29.04b	4.773b	1.700a	4.388c	0.229a	11.11b	0.090c	27.62b
150	59c	6.952c	2.954c	1.533b	4.3636b	0.211b	8.38c	0.119b	6.50c
225	30d	1.695d	0.8584d	0.842c	5.320a	0.156c	4.26d	0.235a	1.09d
Percentage of decrease	-66	-96	-86	-48	-	-34	-67	-	-98

Means with similar letter(s) in each column is not significantly different at 5% of probability level

levels making difficulties for seeds to absorb water quickly. From the point of view of farmers, germination percentage is a very important characters providing better canopy in the farm when it is at suitable rate. Variations among genotypes were found for this trait. In addition, a highly significant interaction was also observed between genotype and salinity levels for germination rate. Because of theimportanceof this character, the mean comparisons between different genotypes at different salinity levels are presented in table 5, separately.As can be seen from the table, Mesa-Sirsa had the highest germination percentage at 75mM of NaCl treatment (98.75%) whileNikshahrihad the highest percentage of germination (80%) at 225 mMNaCl. Therefore, these two genotypes are promising for enhancing the character. On the other side, Diablo verdehad the lowest germination percentage (50%) at 75mM and did not germinate at all at 150 mMNaClindicating highly susceptibility of this genotype to salt stress. IC (50) was used to determine tolerant and sensitive genotypes for salt stress at germination stage. Thus, IC (50) values were calculated for all genotypes using a 2^{nd} order polynomial equation as described in the section of material and methods. These equations are presented in Table 6. As the table indicates, the tolerant cultivar (Nikshahri) showed resiliency to increase in salinity (with IC50=472 mMNaCl) while susceptible cultivars Diablo verde and Harpinger (with IC50 = 62 and 41 mMNaCl, respectively) showed an inability to tolerate increased salinity levels at the germination stage. The ability of a cultivar to germinate and establish under highly saline conditions is clearly quantified in this experiment and the selection of the appropriate cultivar is a critical decision.

Table 4. Comparison between means of genotypes obtained over all treatments

Genotypes	Germination (%)	Radicle length (mm)	Plumule length (mm)	Shoot dry weight (mg)	MGT (day)	CVG	MDG	DGS	SLVI
Yazdi	71.88ef	29.52ab	4.844a	1.875b	4.119j	0.2437a	10.27ef	0.10ef	29.96b
Nikshahri	88.75ab	26.91c	4.634a	1.810b	4.115j	0.2444a	12.68ab	0.08h	29.36bc
Bami	74.06def	24.38de	4.001b	1.591cd	4.187ij	0.2400ab	10.58def	0.09gh	24.75de
Rahnani	75.63de	27.31bc	4.995a	1.822b	4.229hij	0.2356ab	10.80cde	0.09gh	27.67bcd
Gomi	75.63de	16.93ij	3.921b	1.660c	4.192ij	0.2381ab	10.81de	0.09gh	18.44gh
Mesa-Sirsa	89.38a	30.01a	4.962a	1.525cde	4.278hij	0.2356ab	12.77a	0.08h	34.14a
Hamedani	82.50bc	27.49bc	3.881b	1.463de	4.254hij	0.2356ab	11.78bc	0.08gh	27.33bcd
Ramandi	56.00hi	19.95gh	2.946cd	1.313fgh	4.676f	0.2138bc	8.00hi	0.13e	17.24h
Sahandava	73.75def	26.13cd	3.758b	1.395efg	4.274hij	0.2338ab	10.53def	0.09gh	24.56de
Siriver	79.50cd	20.94fg	3.263cd	1.315fgh	4.431gh	0.2262abc	11.36cd	0.09gh	22.16ef
Harpinger	28.19m	3.406m	.928e	.625l	6.104a	0.0963h	4.03m	0.25a	2.96k
KF15	69.06ef	26.07cd	4.069b	1.437ef	4.559fg	0.2212abc	9.87ef	0.10ef	26.41cd
Kodi	59.69h	17.82hi	2.837d	1.123jk	4.871e	0.2013cd	8.53hi	0.12d	17.29h
Defi	49.88ij	16.26ij	2.891cd	1.236hij	5.134cd	0.1663efg	7.13ij	0.14c	15.13h
Kaiseri	42.25k	15.01jk	3.132cd	1.156ijk	5.251c	0.1619fg	6.04k	0.17c	11.94i
Baghdadi	67.19fg	21.04fg	4.953a	2.066a	4.383ghi	0.2300ab	9.60fg	0.10ef	21.47efg
Dastgerd	61.56gh	22.50ef	4.180b	1.810b	4.476g	0.2244abc	8.80gh	0.11e	21.24fg
Gargologh	47.81k	18.12hi	3.329c	1.024k	5.016de	0.1794def	6.83jk	0.15c	15.76h
Melissa	49.36j	13.40k	3.231cd	1.271ghi	4.935e	0.1887de	7.05ij	0.14d	11.91i
Diablo verde	35.34l	10.82l	2.868d	1.020k	5.452b	0.1456g	5.05l	0.20b	8.61j

Means with similar letter(s) in each column is not significantly at 5% of probability level

Table 5.Mean comparisons of different cultivar in salinity levels for germination rate

Genotypes	75 mM	150 mM	225 mM
Yazdi	81.25h	76.25g	35.00i
Nikshahri	88.75e	90.00b	80.00a
Bami	78.75j	71.25h	51.25f
Rahnani	83.75g	68.75j	60.00d
Gomi	90.00d	78.00f	43.50g
Mesa-Sirsa	98.75a	96.25a	63.75c
Hamedani	86.25f	85.00d	70.00b
Ramandi	75.25l	43.75m	22.75k
Sahandava	80.00i	70.00i	58.75e
Siriver	96.50b	88.50c	42.00h
Harpinger	35.75q	0.00s	0.00q
KF15	91.25c	78.75e	16.25l
Kodi	83.75g	60.00l	5.00o
Defi	78.00k	31.50p	0.00q
Kaiseri	60.13o	26.00q	0.00q
Baghdadi	86.25f	68.75j	25.00j
Dastgerd	78.75j	63.75k	15.00m
Gargologh	65.00n	35.00o	1.25p
Melissa	67.50m	35.44n	6.75n
Diablo verde	50.38p	6.50r	0.00q

Means with similar letter(s) in each column is not significantly different at 5% of probability level

Experiment 2

In order to specify the most tolerant genotypes of alfalfa to salt stress, indices such as STI, SSI, GMP, MP, and TOL were studied under moderate and severe salinity conditions. In addition, correlation analysis was conducted between dry forage performance and tolerance indices in the both conditions. These results are presented in Tables 7 and 8. Under the both conditions, GMP, MP, STI indicated positive and significant correlation with forage dry matters. Considering these positive correlations, the above mentioned indices could be chosen as the best indices in selecting tolerant varieties. These results were in line with the results obtained by Basafa and Taherian (2010). Generally, the indices with high and positive correlations with dry matters are introduced as the best indices because these indices can help distinguishing between high performance genotypes in both stress and optimum conditions(Fernandez, 1992). It is worth mentioning that the correlation between SSI index and dry matters was significant only in stress conditions; therefore, the SSI index is applicable in analyzing the correlations under stress conditions only and not related with dry matter in normal conditions. Applying stress indices as selection criteria can result in improvement of selected genotypes for salinity stress. In the current study, genotypes with higher MP, GMP, STI indices may be considered as the most tolerant genotypes. In this regard, the amounts of stress indices under moderate and severe salinity stress conditions for the 20 genotypes are presented in Tables 9 and 10, respectively. According to the results presented in Table 9, Harpinger, Yazdi, Melissa, and KF15 are considered as tolerant genotypes in moderate stress conditions. The values of stress indices for 20 studied genotypes in severe stress conditions are given in table 9. Based on GMP, MP, STI indices Rahnani, Melissa, Yazdi and Gomi genotypes were known as the most tolerant genotypes to severe salinity conditions. These results indicated that the response of genotypes differ due to severity of stress. A comparison between the two conditions of stress indicated that the genotypes Yazdi and Melissa have had a suitable response in both conditions. While the genotypes Harpinger and Kf15, which had high amounts of tolerance indices under moderate stress conditions, were not able to express their ability under severe salinity conditions. Principal component analysis was also performed aimed to provide two principal components explaining the variation among genotypes. This analysis was able to do so and results are presented in Tables 11 and 12. Principal component analysis showed that the variations between the data, in the both conditions, are justified by two components. The 99% of total variations in moderate salinity stress and 98.5% of that in severe salinity stress were explained by the two first components. The data shown in Tables 11 and 12 also revealed that PCA1 have a bigger share in total variations in moderate and severe stress conditions compared with PCA2 (Fig 1 and Fig 2). A simple correlation analysis between the two first components and tolerance indices are also presented in Tables 11 and 12 for moderate and severe salinity conditions, respectively. As can be seen from the Tables, there are positive high associations between STI, GMP, MP, and PCA1

Table 6. IC (50) of alfalfa genotypes, 2nd order polynomial equation and R^2 values

Genotypes	2nd polynomial equation	R^2	IC(50)
Yazdi	$y = -1200x^2 + 23.33x + 92.75$	0.949	199
Nikshahri	$y = -133.3x^2 - 32.66x + 95.05$	0.862	472
Bami	$y = -166.6x^2 - 147.5x + 93.93$	0.977	235
Rahnani	$y = -111.1x^2 - 115x + 90.75$	0.980	279
Gomi	$y = -1488x^2 + 129x + 90.42$	0.995	214
Mesa-Sirsa	$y = -1444x^2 + 181.6x + 97.37$	0.957	255
Hamedani	$y = -555.5x^2 + 48.33x + 88$	0.947	309
Ramandi	$y = -622.2x^2 - 140x + 84$	0.973	147
Sahandava	$y = -222.2x^2 - 73.33x + 86.37$	0.999	272
Siriver	$y = -2311x^2 + 313.3x + 89.75$	0.983	215
Harpinger	$y = 1833x^2 - 768.1x + 78.51$	0.988	41
KF15	$y = -2833x^2 + 325.8x + 88.18$	0.982	187
Kodi	$y = -2166x^2 + 115.8x + 89.31$	0.997	164
Defi	$y = -866.6x^2 - 227x + 92.47$	0.976	126
Kaiseri	$y = -144.4x^2 - 344.5x + 83.85$	0.995	95
Baghdadi	$y = -1833x^2 + 134.1x + 88.18$	0.997	185
Dastgerd	$y = -1722x^2 + 72.5x + 87.31$	0.987	170
Gargologh	$y = -388.8x^2 - 307.5x + 90.06$	0.999	114
Melissa	$y = -375x^2 - 282.3x + 88.50$	0.997	118
Diablo verde	$y = 1227x^2 - 672.7x + 86.85$	0.976	62

In all above equations, y and x are percentage of germination and salt concentrations (mMNaCl), respectively.

Table 7. Correlation coefficients among studied characters under moderate stress conditions (Exp 2)

			Stress indices				
Stress indices	Yp	Ys	MP	GMP	SSI	STI	TOL
Yp	1						
Ys	0.81**	1					
MP	0.96**	0.94**	1				
GMP	0.94**	0.96**	0.99**	1			
SSI	0.05ns	-0.53*	-0.22ns	-0.28ns	1		
STI	0.94**	0.92**	0.98**	0.98**	-0.23ns	1	
TOL	0.51*	-0.09ns	0.25ns	0.19ns	0.86**	0.24ns	1

Table 8. Correlation coefficients among studied characters under severe stress conditions (Exp2)

			Stress indices				
Stress indices	Yp	Ys	MP	GMP	SSI	STI	TOL
Yp	1						
Ys	0.46	1					
MP	0.86**	0.84**	1				
GMP	0.71**	0.95**	0.96**	1			
SSI	0.18 ns	-0.75**	-0.32 ns	-0.52*	1		
STI	0.67**	0.94**	0.93**	0.98**	-0.53*	1	
TOL	0.55*	-0.48*	0.06	-0.19 ns	0.88**	-0.22 ns	1

Table 9 Value of salt tolerance indices, Yp and Ys in moderate stress (Exp2)

	YpYs		Stress indices				
Genotypes	**gr/plant**	**gr/plant**	**MP**	**GMP**	**SSI**	**STI**	**TOL**
Yazdi	0.63	0.60	0.62	0.62	0.24	1.36	0.03
Nikshahri	0.45	0.43	0.44	0.44	0.25	0.70	0.02
Bami	0.51	0.34	0.42	0.41	1.70	0.61	0.17
Rahnani	0.56	0.55	0.55	0.55	0.12	1.09	0.01
Gomi	0.62	0.46	0.54	0.53	1.26	1.01	0.16
Mesa-Sirsa	0.41	0.34	0.38	0.38	0.85	0.50	0.07
Hamedani	0.38	0.22	0.30	0.28	2.13	0.29	0.16
Ramandi	0.22	0.15	0.19	0.18	1.69	0.12	0.08
Sahandava	0.46	0.22	0.34	0.32	2.59	0.37	0.24
Siriver	0.16	0.13	0.14	0.14	0.79	0.07	0.02
Harpinger	0.89	0.48	0.69	0.65	2.30	1.52	0.41
KF15	0.70	0.58	0.64	0.64	0.83	1.45	0.12
Kodi	0.41	0.40	0.41	0.40	0.12	0.58	0.01
Defi	0.61	0.59	0.60	0.60	0.15	1.28	0.02
Kaiseri	0.44	0.40	0.42	0.42	0.37	0.63	0.03
Baghdadi	0.55	0.49	0.52	0.52	0.50	0.97	0.06
Dastgerd	0.59	0.58	0.58	0.58	0.08	1.21	0.01
Gargologh	0.53	0.50	0.52	0.51	0.35	0.94	0.04
Melissa	0.89	0.65	0.77	0.76	1.33	2.05	0.24
Diablo verde	0.52	0.31	0.42	0.40	2.07	0.57	0.22

Table 10. Value of salt tolerance indices, Yp and Ys in severe stress conditions (Exp 2)

	YpYs		Stress indices				
Genotypes	**gr/plant**	**gr/plant**	**MP**	**GMP**	**SSI**	**STI**	**TOL**
Yazdi	0.63	0.60	0.62	0.62	0.13	1.35	0.03
Nikshahri	0.45	0.34	0.40	0.39	0.59	0.55	0.11
Bami	0.51	0.29	0.40	0.38	1.02	0.53	0.22
Rahnani	0.56	0.54	0.55	0.55	0.08	1.08	0.02
Gomi	0.62	0.45	0.53	0.53	0.63	0.99	0.16
Mesa-Sirsa	0.41	0.27	0.34	0.33	0.85	0.39	0.15
Hamedani	0.38	0.12	0.25	0.21	1.61	0.16	0.25
Ramandi	0.22	0.15	0.19	0.18	0.82	0.12	0.08
Sahandava	0.46	0.13	0.30	0.25	1.70	0.22	0.33
Siriver	0.16	0.13	0.14	0.14	0.43	0.07	0.03
Harpinger	0.89	0.13	0.51	0.34	2.04	0.41	0.76
KF15	0.70	0.27	0.49	0.43	1.47	0.67	0.43
Kodi	0.41	0.18	0.29	0.27	1.34	0.26	0.23
Defi	0.61	0.17	0.39	0.32	1.74	0.36	0.44
Kaiseri	0.44	0.30	0.37	0.36	0.75	0.46	0.14
Baghdadi	0.55	0.40	0.47	0.47	0.66	0.78	0.15
Dastgerd	0.59	0.42	0.50	0.50	0.67	0.88	0.17
Gargologh	0.53	0.49	0.51	0.51	0.21	0.92	0.05
Melissa	0.89	0.64	0.76	0.75	0.68	2.01	0.25
Diablo verde	0.52	0.11	0.32	0.24	1.87	0.21	0.41

Table 11. The eigen values, variance and coefficients of two principle components obtained from data under 50 mM of NaCl stress (Exp 2)

Component	Eigen value	Variance%	Cumulative variance	Stress indices						
				YP	YS	MP	GMP	SSI	STI	TOL
1	4.885	0.700	0.700	0.43	0.43	0.45	0.45	-0.12	0.45	0.10
2	2.047	0.290	0.990	0.22	-0.21	0.02	-0.02	0.67	0.01	0.68

Table 12. The eigen values, variance and coefficients of two principle components obtained from data in 100 mM of NaCl stress (Exp 2)

Component	Eigen value	Variance%	Cumulative variance	Stress indices						
				YP	YS	MP	GMP	SSI	STI	TOL
1	4.642	0.663	0.633	0.30	0.45	0.44	0.46	-0.28	0.46	-0.13
2	2.254	0.322	0.985	0.51	-0.15	0.22	0.06	0.51	0.04	0.64

Fig 1. Graphical biplot for 20 alfalfa genotypes and 5 salt tolerance indices on the basis of first and second principle components under moderate stress conditions.

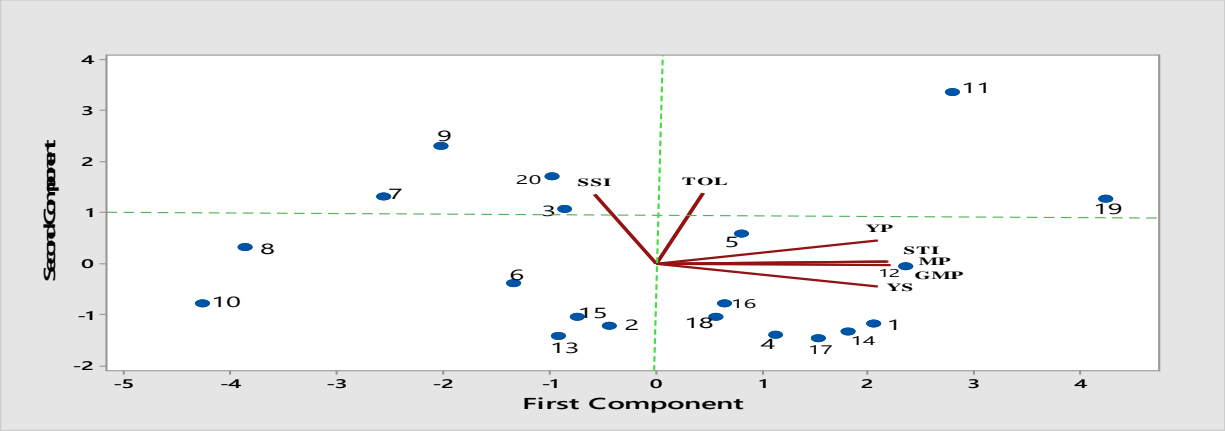
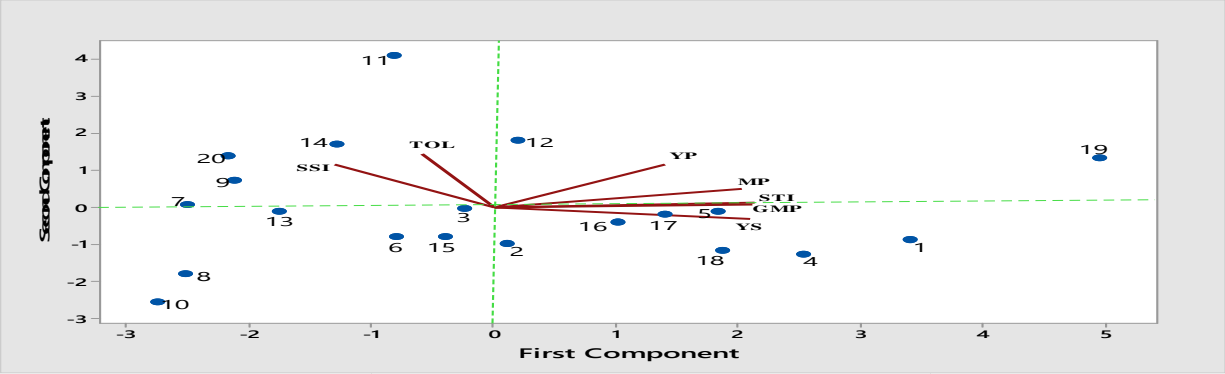


Fig 2. Graphical biplot for 20 alfalfa genotypes and 5 salt tolerance indices on the basis of first and second principle components under severe stress conditions.



1-Yazdi 2-Nikshahri 3-Bami 4-Rahnani 5-Gomi 6-Mesa-Sirsa 7-Hamedani 8-Ramandi 9-Sahandava 10-Siriver 11-Harpinger 12-KF15 13-Kodi 14-Defi 15-Kaiseri 16-Baghdadi 17-Dastgerd 18-Gargolog 19-Melissa 20-Diablo verde

and negative associations between SSI and this component in both moderate and severe salinity stress conditions. Thus, the PCA1 can be named as a factor indicating the potential performance of salinity stress tolerance. In fact, this factor distinguishes the genotypes potentials for high performance under salinity stress. In contrast to PCA1, the second factor had a negative association with the performance of dry forage in salinity stress conditions. Thus PCA2 had a high and positive association with SSI and TOL indices in moderate and severe condition. As a result PCA2 can be considered as a factor which indicates the sensitivity of genotypes to salinity stress. Since the two principal components achieved by PCA analysis, biplot graphs were obtained based on PCA1 and PCA2 in order to classify the studied genotypes into tolerant and susceptible to salinity stress. Graph 1 locates the studied genotypes along with the salinity stress indices axes in moderate conditions. According to graph 1 Dastgerd, Defi, Yazdi and KF15 genotypes are chosen as the tolerant genotypes in moderate salinity stress conditions due to the high PCA1 and low PCA2. In addition, Defi, Yazdi, Dastgerd genotypes are next to Ys. This shows that their tolerance is because of their high performance in stress conditions. Meanwhile, KF15 is between Ys and Yp which shows that this genotype has a moderate performance in both stress and non-stress conditions. On the other side, Hamedani, Diablo verde, bami, Sahand Ava are all considered as sensitive cultivars due to low PCA1 and high PCA2 and also proximity to SSI axis. Gomi genotype located between two significant salinity stress tolerance and salinity sensitivity indices and displayed a suitable tolerance to salinity, though the obtained results from SSI and TOL indices show that this genotype does not perform properly. In addition this genotype is close to Yp which show that high tolerance of this genotype relates to its high performance in non-stress conditions. Graph 2 indicates the position of the studied genotypes within the axes ofbiplot diagram. As can be seen from the Graph, genotypes Yazdi and Rahnani are the most tolerant genotypes because of high PCA1 and low PCA2. Additionally, Dastgerd and Gomi genotypes displayed a moderate tolerance due to proximity to the main salinity stress indices such as GMP, MP, STI. Proximity of these genotypes to Ys axis reveals that salinity tolerance of these genotypes is due to their high performance in severe salinity stress conditions. Graph 2 also indicated that genotypes Defi, Harpinger and Shand Ava are the most sensitive genotypes. In biplot graphs, obtuse angle between axes shows a negative and severe relationship, right angle shows an almost no relationship and acute angle shows a positive relationship between indices (Yan and Rajcan, 2002). Thus, taking into account the angles among GMP, MP and STI indices in two moderate and severe levels of salinity stress, it can be deduced that there is a positive and significant correlation between forage dry matter and these indices.

Taken collectivelythe current study revealed that significant variations exist among Iranian genotypes for salinity stress tolerance under both severe and moderate stress conditions. In addition, the results obtained from the first experiment (carried out within growth chamber) were more or less different from those obtained from the second one (carried out in a heated greenhouse) implying that considering real and suitable characters as screening criteria is crucial. This also implies that considering different stages of growth for evaluating alfalfa genotypes regarding to their responses to salinity may be more suitable than one single stage of growth. Principal component analysis was also able to reduce the dimensions of data into two important components. This analysis showed the variations between the data, in the both conditions, are justified by two components. So that, the 99% of total variations in moderate salinity stress and 98.5% of that in severe salinity stress were explained by the two first components.A comparison between the two conditions of stress indicated that the genotypes Yazdi and Melissa have had a suitable response in both conditions. While the genotypes Harpinger and Kf15, which had high amounts of tolerance indices under moderate stress conditions, were not able to express their ability under severe salinity conditions. In general, using different characters in early and mature stages of growth for screening tolerant genotypes indicated that among the 20 genotypes evaluated in the current study, genotype Yazdi is seemed to be the most tolerant genotype under the severe and moderate salinity stress conditions.

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