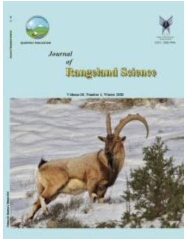
Contents available at ISC and SID

Journal homepage: www.rangeland.ir



Research and Full Length Article:

Effect of Drought Stress on Seedling Morpho-physiological Traits of Alfalfa (*Medicago sativa*) Populations grown in Glasshouse

Mehrnaz Riasat[A]*, Ali Ashraf Jafari[B], Armin Saed-Mouchehsi[C]
[A] Department of Natural Resources, Fars Agricultural and Natural Resources Research and Education Centre; Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran *(Corresponding author), riasat.mehrnaz@yahoo.com
[B] Research Institute of Forests and Rangelands; Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
[C] Department of Agronomy and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran

Received on: 30/12/2018
Accepted on: 27/07/2019

Abstract. In order to study the effect of drought stress on seedling morpho-physiological traits of alfalfa *(Medicago sativa*), the seeds of ten populations were collected in their natural habitats from different parts of the Fars province, Iran in 2013. A factorial experiment was conducted using 10 alfalfa populations and four water stress treatments of 100, 75, 50, and 25% Field Capacity (FC) based on a completely randomized design with three replications in the glasshouse of agriculture and natural resources research center of Fars province, Iran. Data collected for shoot and root fresh and dry weight, root and stem length, Root/Stem length ratio (RS) and free proline content. Data were subjected to analysis of variance, cluster analysis, principle component analysis. The main effect of population and water stress treatments were significant for all of traits ($P<0.01$) and population by water stress interaction effects were significant only for proline and root dry weight ($P<0.01$). The results showed high variation among populations under different water stress treatments. The means comparison among populations for all the water stress treatments showed that population of Kamal Abad Neiriz had high seedling growth coupled with long root length and was considered as drought tolerance to water stress that could be used to improve new varieties. The higher heritabilities were obtained for stem length (h^2=0.52), root length (h^2=0.48.8) and proline content (h^2=0.47.7). According to cluster analysis, the four populations of Deh Kohneh, Dozkord, Kamal Abad, and Komhar that were placed into cluster 2 had higher mean values for many traits than other populations/clusters. This high variation could be used for breeding programs and making synthetic populations for improving alfalfa productivity.

Keywords: Alfalfa, Drought stress, Local populations, Genetic variation, Proline

Introduction

The major environmental constrain for the productivity and stability of plants is water stress (Araus *et al.,* 2002). Drought stress directly or indirectly affects other stresses. Plants resistant to drought stress are usually, at least partially, resistant to the other environmental stresses (Saeedi Goraghani *et al.,* 2013). So, providing experiments and information regarding crops grown under water shortage conditions in arid and semiarid climates are essential for optimum irrigation management strategies and water conservation. In terms of irrigation and water stress, alfalfa (*Medicago sativa* L.) has a high water requirement compared to other crops (Blad and Rosenberg, 1976). Several studies have shown that soil water deficits resulted in reduced alfalfa growth and yields (Brown and Tanner, 1983; Grimes *et al.,* 1992). Alfalfa stem density (Saeed and El-Nadi, 1997), stem height (Bolger and Matches, 1990), and leaf size decreased when soil water deficits developed. A number of reports have shown a linear relationship between Dry Matter (DM) production and water use for alfalfa (Aranjuelo *et al.*, 2013; Gindel, 2013; Signorelli *et al.*, 2013). Alfalfa has a wide-range distribution and is thus expected to show differing levels of drought tolerance (Erice *et al.*, 2010). Kang *et al.* (2011) carried out a research on two varieties of alfalfa to consider the effect of drought stress. They showed that at a qualitative level, molecular, biochemical, and physiological responses to drought stress were similar in the two varieties, indicating that they employ the same strategies to cope with drought.

Because of the uniformity of the crops that are planted and used directly or indirectly in human's consumptions, genetic erosion is practically happening in these crops (Marco *et al.,* 2015). On the other side, variability is the base for plant adaptation to different harsh conditions and situations. Therefore, finding natural sources for plant variability or making synthetic variations, using different methods are essential for saving crops and improving crop's productivity and quality (Bonawitz and Chapple, 2013).

Natural genetic resources are the foundations for breeding approaches under both normal and water stress conditions (Noorka *et al.,* 2013). Moreover, with regard to the importance of alfalfa as a forage crop, it indirectly contributes to human consumption through meat and milk production. In addition, the influence of drought and water stress on the productivity of the crop, considering its genetic variability and breeding plans for this crop via finding and producing synthetic populations are essential (Lizhen *et al.,* 2015).

Proline is one of the most common compatible osmolytes in drought stress of plants. Proline is gradually increased with drought stress, it seems that with the increase in drought stress, the plant cell initially began to store glucose, and then with more intense tension, the proline is stored in the cell membrane and in other words, proline accumulation in cell membranes is an emergency mechanism for drought tolerance. The drought stress in two ways increases proline contents in the plant; a) by increasing proline synthesis enzymes activities and b) decreasing proline degradation enzymes activity (Heuer, 1994; Ingram and Bartles, 1996).

Taking these facts to the consideration, collection and evaluation of natural plant genetic resources to provide high yielding populations and comparing their morphological and physiological characteristics is in high priority for improved breeding varieties (Rauf *et al.,* 2016). The current study was carried out to compare different native populations of alfalfa in order to initially screen for drought tolerance and find out proper populations for future breeding work under drought stress condition.

Materials and Methods

Plant materials

Ten populations of alfalfa (*Medicago sativa*) were collected from different parts of the Fars province, Iran during the middle of growth season of 2013. Collection was made in two stages. First, in each pasture, different samples comprised of whole plants (root, stem, leaves and flowers) were collected and for each site, herbarium specimen were provide, and then were transmitted to herbarium lab of the agriculture and natural resources researches center of Fars province where samples were completely dried. The standard sampling procedure to discriminate different populations was based on the herbarium of Iranica in a plant systemic lab. These samples were collected so that they could be clear representative of the rain fed perennial alfalfa of the Fars province. The altitude of the studied areas was between 1600 to 2500 m. Different characteristics of these areas comprised of latitude, longitude; altitude, average precipitation, and average temperature are presented in Table 1. In the next period in maturity stages when the flowers and their grains were completely matured; by returning to the same points of the noticed areas, seed samples were collected for further operations.

Table 1. Characteristics of the places where the samples of populations were collected

Populations local names	Populations origin	Longitude	latitude	Altitude (m)	Annual Precipitation (mm)	Average Temperature (^{0}C)
Hasan Abad	Dasht Arjhan	53º14'14"	29º37'09"	2000	356	19
Mian Jangal	Fasa	53º23'50"	29º09'46"	1700	249	19.5
Kheir	Estahban	54º15'12"	29º07'13"	1600	233	17.5
Kamal Abad	Neiriz	54º14'24"	29º06'36"	1650	190	17
Sarchahan	Bavanat Abadeh	53º53'96"	29º55'33"	1950	232	13.5
Simakan	Bavanat Abadeh	53º15'36"	30º15'00"	2500	311	12
Bazbacheh	Eqlid	52º24'05"	30º54'18"	2300	355	11
Dozkord	Eqlid	51º58'54"	30º43'26"	2400	629	10
Komhar	Sepidan	51º52'51"	30º27'21"	2400	758	11.9
Deh Kohneh	Sepidan	51º49'33"	30º"20'02"	2150	630	12

Experimental design

The experiment was conducted in the glasshouse of agriculture and natural resources researches center of Fars province in 14-10 hour light and dark, and 20 to 25°C minimum and maximum temperature, respectively. The soil samples for the pots were mixed at ratio of 1:1:1 for sand, garden soil, and compost. After mixing of these components, the final mixture of the soil samples was oven dried and sanitized under 150°C for 24 h. The pots with size of 20 cm height and 10 cm diameter were washed using ethanol (95% pure) and then with distilled water. Four holes were forged in the bottom of the pots to provide a proper water drain. After that, the pots were filled with soil mixtures which had been prepared earlier and were weighed to a proper weight for all pots. Since 10 populations of alfalfa (Factor A) and four water stress treatment (100, 75, 50, and 25% FC) (Factor B) were repeated three times in this experiment, the final number of pots was 120 (3×10×4). Seeds were sanitized using sodium hypochlorite (10%) and then in Benomil fungicide (2g/l) for 30 s and immediately were washed with distilled water and dried. In each pot, six seeds were sown in 1 cm depth of the soil. Along with seeds emergence, each pot was thinned to only one seedling. In the 3-4 leaves number stage of seedlings, the drought stress was applied using weighing method by measuring pots for each treatment.

Data collection

At the end of growing stage, the plants were harvested separately and transferred to the lab for measuring the morphological traits. Aerial fresh and dry weight of shoot (stem + leaves) and root were weighed while the root and stem length were measured using a ruler as cm. For measuring the dry weight, the samples were dried in an oven with 70°C temperature for 48 h. Before harvesting, fresh and newly expanded leaves were cut for assessing free proline content (µg/g). For assessing free proline content, 0.5 g fresh leaves samples were used using spectrophotometry at 625 nm wave length (Bates *et al.,* 1973).

Statistical Analysis

Factorial experiment with two factors based on a Completely Randomized Design (CRD) in three replications was applied. First factor of the experiment was alfalfa collected populations while the second factor was four different levels of water stress providing for plants containing 100, 75, 50 and 25% field capacity (FC).

Statistical Analysis System (SAS) software v. 9.4 (GLM procedure) was used for data analysis and means comparisons were made based on Least Significant Difference (LSD) at 5% significant level. Genetic parameters were estimated using VARCOMP procedure of SAS software. Broad sense heritability, genotypic and phenotypic coefficient of variation were calculated according to following formula:

Heritability $= h^2 = \frac{Vg}{Vp}$

Genotypic Coefficient of Variation $= GCV = \frac{\sigma_g}{\bar{x}} \times 100$

Phenotypic Coefficient of Variation $= PCV = \frac{\sigma_p}{\bar{x}} \times 100$

Where:

V_g= Genotypic Variance and V_p = Phenotypic Variance

σ_g= Square Root of Genotypic Variance and σ_p = Square Root Phenotypic Variance.

Data were also multivariate analyzed for principle component analysis and cluster analysis. The genetic distance and population classification were determined using cluster analysis by Ward (1963) method. All statistical analyses were conducted by MINITAB ver16 software

Results

Analysis of variance and Mean comparison

Results of analysis of variance (ANOVA) for measured traits according to factorial experiment are presented in Table 2. Effect of population and stress levels were significant for all measured traits while the interaction effect was significant for root dry weight and proline content. Since for many traits, the population by stress interaction was not significant. So, the means comparisons for these traits were made only for main effect separately (population and stress) (Tables 3 and 4). For root fresh weight and shoot fresh and dry weight, the highest values were obtained in Kamal Abad population. In contrast, the lowest values of these traits were found for Kheir and Simakan populations (Table 3).

For root length, Kamal Abad and Komhar populations had the highest values and Hasan Abad had the lowest root length (Table 3). For stem length, the highest and lowest values were obtained in Kamal Abad and Sarchahan, respectively (Table 3). The highest root stem/length ratios (RS) were obtained in Sarchahan and Simakan populations. In contrast, the lowest values of RS were obtained in Hasan Abad and Bazbacheh populations (Table 3).

The means comparison in relation to water stress treatments (as main effects) showed that the highest and lowest root fresh weight was recorded for slight stress (75% field capacity (FC)) and severe water stress (25% FC), respectively. For shoot fresh and dry weight, the highest and lowest values were obtained in control (100% FC) and severe water stress (25% FC) (Table 4). The highest root length was observed in control and slight stress regime. The highest stem length was obtained in control. For both traits, the lowest root and stem lengths were observed in severe water stress (Table 4). For RS ratio, the highest values was found in severe water stress treatment, while, the lowest values was found in control and 75% FC treatments (Table 4).

As shown in Table 2, there were significant population by stress interaction effects for root dry weight and proline concentration. In such a case when we have significant interaction effects, we cannot interpret the main effects without considering the interactions. Therefore, the two way population by stress interaction Tables were provided for root dry weight and proline content (Tables 5 and 6). For both root dry weight and proline content, the responses of population were the similar. For example, the mean values of Deh Kohneh were high in control. Its value sharply decreased in severe water stress (25% FC) and dropped as low to moderated class than the other populations. In contrast, the population of Simakan that had the lowest values root dry weight and proline content, their values were stable by increasing drought stress and were ranked as high class in severe water stress (25% FC) (Tables 5 and 6).

Table 2. Analysis of variance according to factorial experiment for measured traits

Source	DF	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	Root length	Stem length	Root shoot length ratio	Proline content
Population	9	4433.92**	33675.09**	27.83**	900.64**	8.36*	19.42**	0.36**	1.18**
Stress	3	41899.1**	1122566**	629.9**	33840.2**	73.04**	1817.62**	23.10**	3.51**
Interaction	27	1533.9^{ns}	9814.6^{ns}	21.81**	307.29^{ns}	4.08^{ns}	4.68^{ns}	0.14^{ns}	0.18**
Error	80	1246.09	8068.79	11.24	233.21	3.39	5.83	0.08	0.01
CV%		45.16	26.59	27.56	23.8	12.59	15.48	21.56	13.02

**, *, and "ns" are indicating significant level of 0.01, 0.05, and not significant, respectively

Table 3. Mean comparison between the populations for seedling traits of the alfalfa

Populations local names	Populations origin	Root fresh weight (g/p)	Shoot fresh weight (g/p)	Shoot dry weight (g/p)	Root length (cm)	Stem length (cm)	Root Shoot length ratio
Bazbacheh	Eqlid	63.58 bc	320.25 bcd	59.64 bcd	13.54 bc	15.58 bcd	1.08 c
Deh Kohneh	Sepidan	89.75 b	363.00 bc	68.28 abc	14.42 abc	15.75 abc	1.20 bc
Dozkord	Eqlid	80.44 b	375.36 b	72.46 ab	14.13 abc	16.33 ab	1.24 bc
Hasan Abad	Dasht Arjhan	68.58 bc	345.58 bcd	75.50 a	12.96 c	16.50 ab	1.07 c
Kamal Abad	Neiriz	119.4 a	453.25 a	76.50 a	15.38 a	17.63 a	1.12 c
Komhar	Sepidan	87.73 b	356.14 bcd	64.75 bcd	15.42 a	15.58 bcd	1.38 ab
Kheir	Estahban	46.08 c	276.08 d	56.75 cd	14.15 abc	15.70 bcd	1.30 bc
Mian angal	Fasa	76.67 bc	301.08 bcd	58.25 cd	14.88 ab	14.46 bcd	1.37 ab
Sarchahan	Abadeh	70.67 bc	301.58 bcd	57.58 cd	14.75 ab	13.42 d	1.55 a
Simakan	Abadeh	78.83 b	285.75 cd	51.75 d	15.38 a	13.88 cd	1.52 a

Means of column followed by same letters has no significant differences based on LSD method

Table 4. Means comparison between drought stress levels on seedling traits of the alfalfa

Drought stress	Root fresh weight (g/p)	Shoot fresh weight (g/p)	Shoot dry weight (g/p)	Root length (cm)	Stem length (cm)	Root Shoot length ratio
Control	83.13 b	510.17 a	95.56 a	15.9 a	22.7 a	0.71 c
%75 FC	111.4 a	457.7 b	84.05 b	15.62 a	20.02 b	0.79 c
%50 FC	93.23 b	304.53 c	56.97 c	13.93 b	14.17 c	1.05 b
%25 FC	24.93 c	78.83 d	20.01 d	12.54 c	5.05 d	2.58 a

Means of column followed by same letters has no significant differences based on LSD method

Table 5. Means comparisons between populations for **root dry matter weight** (g/p) in different levels of drought stress (population by drought stress Interaction effect) of the alfalfa

Populations names	origin	Control	%75 FC	%50 FC	%25 FC	Main effect
Bazbacheh	Eqlid	13.00bc	14.33ab	13.63ab	4.33b	11.33 bc
Deh Kohneh	Sepidan	19.43a	14.67ab	12.67ab	5.00b	12.94 b
Dozkord	Eqlid	9.67bc	17.00a	13.00ab	6.00ab	11.42 bc
Hasan Abad	Dasht Arjhan	11.67bc	19.00a	18.00a	7.33a	14.00 ab
Kamal Abad	Neiriz	18.67a	19.33a	17.00ab	4.67b	14.92 a
Komhar	Sepidan	18.67a	14.00ab	11.33ab	5.67b	12.42 b
Kheir	Estahban	11.33bc	12.00b	10.33ab	4.67b	9.58c
Mian angal	Fasa	9.00c	18.67a	15.33ab	5.33b	12.08 b
Sarchahan	Bavanat Abadeh	14.67ab	15.00ab	9.67b	4.67b	11.00 bc
Simakan	Bavanat Abadeh	10.33bc	17.67a	12.00ab	8.00a	12.00 b
Total Mean		13.64 B	16.17 A	13.30 B	5.57 C	

Means of column followed by same lowercase letters are not significantly differences based on LSD method
Means of the last row followed by same uppercase letters are not significantly differences based on LSD method

Table 6. Means comparisons between populations for **Proline content** (μg/g) in different levels of drought stress (population by drought stress Interaction effect) of the alfalfa

Populations names	origin	Control	%75 FC	%50 FC	%25 FC	Main effect
Bazbacheh	Eqlid	0.18 de	0.21 d	0.28 g	0.36 e	0.26 f
Deh Kohneh	Sepidan	0.49 a	1.22 a	1.32 b	1.41 b	1.11 a
Dozkord	Eqlid	0.22 cd	1.13 a	1.61 a	1.26 b	1.05 ab
Hasan Abad	Dasht Arjhan	0.06 ef	0.22 d	0.22 g	0.55 d	0.26 f
Kamal Abad	Neiriz	0.27 bcd	0.35 cd	0.50 f	0.43 de	0.39 e
Komhar	Sepidan	0.04 f	0.53 bc	1.33 b	0.75 c	0.66 d
Kheir	Estahban	0.07 ef	0.68 b	1.06 d	0.90 c	0.68 d
Mian angal	Fasa	0.26 bcd	0.63 b	1.19 c	0.82 c	0.73 cd
Sarchahan	Bavanat Abadeh	0.33 bc	0.75 b	1.33 b	1.68 a	1.02 b
Simakan	Bavanat Abadeh	0.37 b	0.74 b	0.76 e	1.27 b	0.79 c
Total Mean		0.23 C	0.65 B	0.96 A	0.94 A	

Means of column followed by same lowercase letters are not significantly differences based on LSD method
Means of the last row followed by same uppercase letters are not significantly differences based on LSD method

Estimation of genotypic parameters

To consider the potential of breeding among these selected populations, the genotypic parameter specially heritability should be surveyed. The results of descriptive statistics and genotypic parameters are presented in Table 7. The highest heritability among all traits was appraised for stem length (52%) and root length (48.88%), and proline content (47.52%) had been indicated to have moderate heritability than other traits. Minimum heritability rate was recorded for root dry weight (23.03%). The highest phenotypic coefficient of variance (GCV) was obtained for shoot fresh weight (84.58%), free proline content (77.5%) and stem length (70.61%). Unlike the phenotypic coefficient of variance, the highest genotypic coefficient of variance (PCV) was obtained for shoot dry weight (49.92%). The lowest phenotypic and genotypic coefficients of variation were obtained for root dry weight (28.78%).

Table 7. Genotypic parameters related to measured traits for both normal and stress conditions together and for all four water treatments and all populations

Parameter	Proline Content %	Root Fresh weight g/p	Shoot Fresh weight g/p	Root dry weight g/p	Shoot dry weight g/p	Root Length cm	Stem Length cm
Mean	0.69	78.18	337.81	12.17	64.15	14.5	0.69
Std Dev	0.31	19.22	52.98	1.52	8.66	0.83	0.31
Maximum	1.11	119.42	453.25	14.92	76.5	15.42	1.11
Minimum	0.26	46.08	276.08	9.58	51.75	12.96	0.26
h^2 (%)	47.52	27.55	40.84	23.03	39.15	48.88	52.65
PCV (%)	77.5	45.25	84.58	28.78	54.89	49.99	70.61
GCV (%)	42.32	34.57	40.61	24.27	49.92	40.03	44.98

Std Dev: Standard Deviation. h^2= Broad Sense Heritability

Principle component analysis

Results of PCA analysis (Table 8) showed that two components with eigen values higher than one explained 83% of total variations. The amount of variance explained by each component reflects its importance in explaining the total variance of the traits under study. The first two components account for 56%, and 27% of variations (Table 8). In the first component, seedling growth indices as shoot fresh and dry weight, root dry weight and stem length had positive eigen vectors coefficients. In the second component, root fresh weight, root length, RS ratio and proline content had higher eigen vectors and trends of these traits were in the same direction. So, this component was named as drought resistance component (Table 8). The results of this study indicated that selection of variables for the second components (PCA2) could enable breeders to release the desirable increment in drought resistance alfalfa.

Table 8. Result of principle components analysis and total variance explained for each component in 10 populations of alfalfa

Variable	PC1	PC2
Shoot fresh weight	**0.43**	-0.22
Shoot dry weight	**0.44**	0.03
Stem length	**0.43**	0.14
Root dry weight	**0.39**	-0.20
Root fresh weight	0.32	**-0.49**
Root length	-0.07	**-0.62**
Root/Stem length ratio (RS)	-0.37	**-0.38**
Proline content	-0.21	**-0.35**
Eigen value	4.49	2.18
% of variance	0.56	0.27
Cumulative % of variance	0.56	0.83

The bold and underlined data had higher Eigen values in the relevant factors

Cluster analysis

According to denderogram of cluster analysis, (Fig. 1), the 10 populations of *alfalfa* were divided into three groups by genetic distance of 5.56. According to the obtained results, cluster 1 had 3 populations as Bazbacheh, Hassan Abad and Kheir. Cluster 2 with four populations as Deh Kohneh, Dozkord, Kamal Abad and Komhar, and finally cluster 3 had three populations as Mianangal, Sarchahan and Simakan, respectively (Fig 1).

Also, means comparison based on LSD method was carried out on all measured traits for comparing the two distinguished clusters (Table 9). The results of this comparison showed that cluster 2 had higher values for all of traits except RS ratio. The cluster 1 had lower values for all traits except stem length. The cluster 3 had higher values for RS ratio, root length and proline contents and ranked as mid for other traits.

Based on the first two components scores, we scattered the populations in biplot (Fig. 2). The first component was considered as seedling growth indices since it had higher positive eigen vectors coefficients for shoot fresh and dry weight, root dry weight and stem length, so populations in the right hand side of Fig. 2 (cluster 2) had higher seedling growth. In the second components, we found significant negative components for root fresh weight, root length, RS ratio and proline content in the second component. So, populations in lower part of Fig. 2 (clusters 2 and 3) had negative values of these traits indicating more resistance to drought stress. There was a good agreement between the results obtained from cluster and PCA analyses in scatter diagram representation of 10 populations based on the first and second components.

Table 9. Means comparison between three distinguished clusters for measured traits

cluster	Root fresh Weight (g/p)	Shoot fresh weight (g/p)	Root dry weight (g/p)	Shoot dry weight (g/p)	Root Length (cm)	Stem length (cm)	Root Shoot Length ratio (RS)	Proline (µg/g)
Cluster 1	59.41 c	313.97 b	11.64 b	63.96 b	13.55 b	15.93 a	1.15 b	0.40 b
Cluster 2	94.33 a	386.94 a	12.93 a	70.50 a	14.84 a	16.32 a	1.24 b	0.80 a
Cluster 3	75.39 b	296.14 c	11.69 b	55.86 c	15.00 a	13.92 b	1.48 a	0.85 a

Means of column with the same letter are not significantly different based on LSD method at 5%.

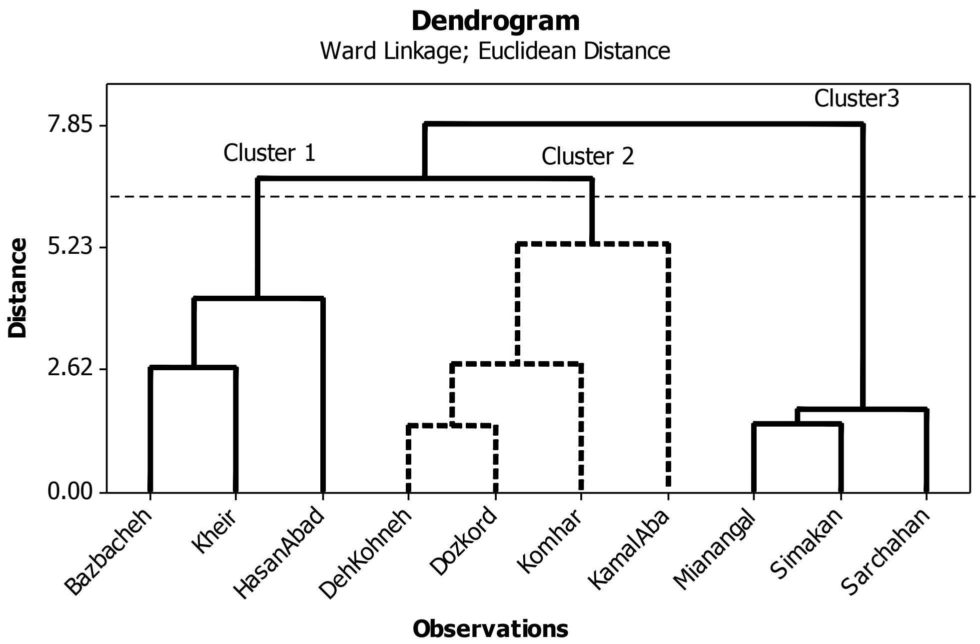


Fig. 1. Dendrogram of 10 populations of *alfalfa* using ward cluster analysis method

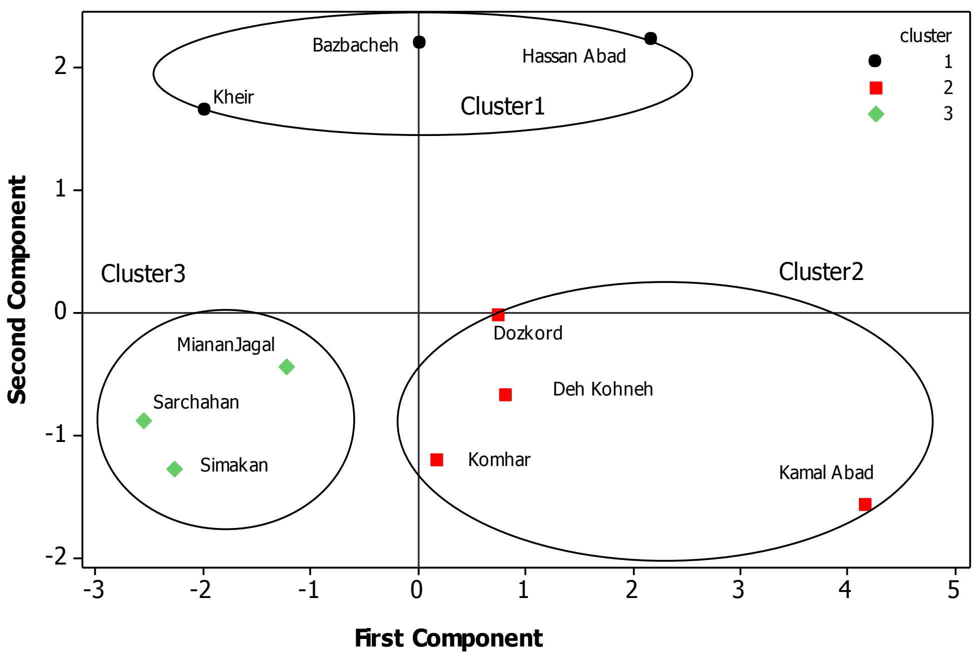


Fig. 2. Biplot Scatter plot of 10 populations of *alfalfa* for the first two components

Discussion

Results of comparing different populations regarding measured traits in this study indicated that the population collected from Kamal Abad Neiriz region had the highest mean values for all traits except for free proline content. So, this population could be more surveyed as the potential population for breeding and screening work under both normal irrigation and water stress conditions, which is normally occurred in this region. The proper resistance of this population could be due to harsh and somehow drought condition environment of Neiriz in Fars province of Iran which makes this population to be adopted to this environment; hence, more research is needed on this population in order to distinguish its resistance genes and its physiological response to drought stress and harsh environment. Both Kamal Abad and Hasan Abad populations showed high values for measured traits which makes them proper for more breeding work in line with the aforementioned population under different environments and locations. Bazbacheh, Simakan, and Kheir populations showed the lowest mean values for the most traits in this study.

Cluster analysis which is a method to distinguish separated groups of some objects was used for clustering the populations. The cluster analysis resulted in three clusters in which the result was corroborated by principal component analysis. This result is indicating that the variation among populations in regard to all of their measured traits was high and it could be used for breeding approach and making higher synthetic variation. Both Kamal Abad and Hasan Abad populations showed higher values for most of the traits that had some similarity with each other in cluster dendrogram but they were grouped in different clusters. The highest similarity was observed in populations in cluster2 which contained Deh Kohneh, Dozkord, Kamal Abad and Komhar while these populations had the highest distance and dissimilarity with Kamal Abad population. The distance between these populations could easily lead to a heterosis in the next generation of the mating between these groups and could be applied in breeding purposes.

Plant productivity is a polygenic trait and greatly influenced by environmental condition. The breeders have used different traits and components as selection criteria to improve plants productivity by indirect selection (Singh *et al.*, 1999). Selection efficiency depends on the magnitude of its heritability and genetic variation for a character (Mirarab *et al.*, 2011). Information on the nature and extent of genetic variability and degree of transmission of traits is a high priority factor in enhancing selection efficiency (Peratoner *et al.*, 2016). Genetic parameters such as genotypic coefficient of variability, heritability and/or genetic advance can provide precise estimates for genetic variation in quantitative traits and are used by breeders for many years to understand the genetic and environmental effects on different traits (Nawaz *et al.*, 2016). Since the final productivity of a plant is an outcome indicating relationships of several traits, it is imperative to identify effective traits under different environmental conditions (Vosough *et al.*, 2015). In the current study, the genetic parameters have been estimated using expectation of MS values of the ANOVA Tables and could be applied for predicting the work that is needed to improve the productivity of these or other populations of alfalfa. The moderate heritability among traits were firstly obtained for stem length, then root length and finally in proline content. Stem length is a trait that is directly related to plant productivity and as a result of this experiment, it could be used as selection criteria for improving the productivity of alfalfa in the next generation. Root length is a very important attribute in alfalfa and also all other plants under environmental stresses and specially under water deficit condition. In the present experiment, this trait showed to be an effective trait as result of its high heritability and also coefficient of variation in alfalfa to take it as selection criteria. Free proline content as a free amino acid in the plants and other living organisms has some special and influential rolls which make it a very crucial factor under stressful conditions. Proline is gradually increased with drought stress, it seems that with the increase in drought stress, the plant cell initially began to store glucose, and then with more intense tension, the proline is stored in the cell membrane, and in other words, proline accumulation in cell membranes is an emergency mechanism for drought tolerance. The drought stress increasing proline content by a) increasing proline synthesis enzymes activities and b) decreasing proline degradation enzymes activity (Heuer, 1994; Ingram and Bartles, 1996). High heritability of this trait is indicating that screening for higher productivity populations in alfalfa could be indirectly based on the content of this free amino acid. The result of phenotypic and genotypic coefficient of variations showed that high variability is presented among the surveyed population and also, they indicate that the genetic and environmental conditions are both important in plant productivity and also all other traits of the plants. Means comparison and cluster analysis in addition to genetic parameters estimated in alfalfa populations indicated high variation among them under different water treatments. Similar results have been reported by Jafari *et al.* (2012) for alfalfa (*Medicago sativa* L.) populations under dry land farming system in Hamadan, Iran.

Conclusion

This variation could be used for improved breeding varieties and making synthetic populations for improving the alfalfa productivity. Most of the traits showed moderate heritability indicating the potential of screening and breeding program among these populations that have been used in the current study. On the other side, means comparison among populations for all water stress treatments showed that Kamal Abad Neiriz population had high productivity with high adaptability and resistance to stress conditions that could be more surveyed for future work.

References

Aranjuelo, I., Tcherkez, G., Molero, G., Gilard, F., Avice, J. C. and Nogués, S., 2013. Concerted changes in N and C primary metabolism in alfalfa (Medicago sativa) under water restriction. Journal of experimental botany, 64(4): 885-897.

Araus, J., Slafer, G., Reynolds, M. and Royo, C., 2002. Plant breeding and drought in C3 cereals: what should we breed for? Annals of Botany 89(7): 925-940.

Bates, L., Waldren, R. and Teare, I., 1973. Rapid determination of free proline for water-stress studies. Plant and soil 39(1): 205-207.

Blad, B. L. and Rosenberg N. J., 1976. Measurement of crop temperature by leaf thermocouple, infrared thermometry and remotely sensed thermal imagery. Agronomy Journal 68(4): 635-641.

Bolger, T. and Matches, A., 1990. Water-use efficiency and yield of sainfoin and alfalfa. Crop Science 30(1): 143-148.

Bonawitz, N. D. and Chapple, C., 2013. Can genetic engineering of lignin deposition be accomplished without an unacceptable yield penalty? Current opinion in biotechnology 24(2): 336-343.

Brown, P. W. and Tanner, C., 1983. Alfalfa stem and leaf growth during water stress. Agronomy Journal 75(5): 799-805.

Erice G,. Louahlia, S. Irigoyen, J. J. Sanchez-Diaz, M. 2010. Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. Journal of plant physiology 167(2):114-120.

Gindel, I., 2013. A new ecophysiological approach to forest-water relationships in arid climates. Springer Science & Business Media.

Grimes, D. W., Wiley, P. L. and Sheesley, W. R., 1992. Alfalfa yield and plant water relations with variable irrigation. Crop Science 32(6): 1381-1387.

Jafari, A. A., Lak, M. and Assadian, G., 2012. Variability and Correlation between the Seed Yield and its Component in Alfalfa (*Medicago sativa* L.) Populations under Dry Land Farming System, Hamadan, Iran. Journal of Rangeland Science 2(3): 567-576.

Heuer B. 1994. Osmoregulatory role of proline in water-and salt–stressed plants: M. Pessarakli (Ed), Handbook of Plant and Crop Stress. Marcel Dekker pub, New York, 481 PP

Ingram J, Bartles D. 1996. The molecular basis of dehydration tolerance in plants. Ann, Rev, Plant Physiol, Plant Mol. Biol, 47: 377-403

Kang, Y., Han, Y., Torres-Jerez, I., Wang, M., Tang, Y., Monteros, M., and Udvardi, M. 2011. System responses to long-term drought and re-watering of two contrasting alfalfa varieties. The Plant Journal 68(5), 871-889.

Lizhen, N., Hongkui, L., Jie, S., Jing Y. and Yongzhi, L., 2015. A Study on Cold Tolerance Transgenic Alfalfa (Medicago sativa L.) with the AmDHN Gene. Molecular Breeding of Forage and Turf, Springer**.** 173-181 pp.

Marco, F., Bitrián, M., Carrasco, P., Rajam, M. V., Alcázar, R. and Tiburcio, A. F., 2015. Genetic engineering strategies for abiotic stress tolerance in plants. Plant Biology and Biotechnology, Springer**.** 579-609 pp.

Nawaz, H., Yasmeen, A., Anjum M. A. and Hussain, N., 2016. Exogenous Application of Growth Enhancers Mitigate Water Stress in Wheat by Antioxidant Elevation. Frontiers in plant science 7: 597

Mirarab, M., Ahmadikhah, A., and Pahlavani, M. H. 2011. Study on combining ability, heterosis and genetic parameters of yield traits in rice. African Journal of Biotechnology 10(59): 12512-12519.

Noorka, I. R., Tabassum S. and Afzal M., 2013. Detection of genotypic variation in response to water stress at seedling stage in escalating selection intensity for rapid evaluation of drought tolerance in wheat breeding. Pakistan Journal of Botany 45(1): 99-104.

Peratoner, G., Seling, S., Klotz, C., Florian, C., Figl, U. and Schmitt, A., 2016. Variation of agronomic and qualitative traits and local adaptation of mountain landraces of winter rye (Secale cereale L.) from Val Venosta/Vinschgau (South Tyrol). Genetic Resources and Crop Evolution 63(2): 261-273.

Rauf, S., Al-Khayri, J. M., Zaharieva, M., Monneveux, P. and Khalil, F., 2016. Breeding Strategies to Enhance Drought Tolerance in Crops. Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits, Springer. 397-445 pp.

Saeed, I. and El-Nadi, A., 1997. Irrigation effects on the growth, yield, and water use efficiency of alfalfa. Irrigation Science 17(2): 63-68.

Saeedi Goraghani, H. R., Alah Heidary, G. and Solaimani Sardo, M., 2013. Effects of Salinity and Drought Stresses on Seed Germination and Seedling Growth of Desert Wheatgrass *Agropyron desertorum*. Journal of Rangeland Science 4(1): 14-22.

Signorelli, S., Corpas, F. J., Borsani, O., Barroso, J. B. and Monza, J., 2013. Water stress induces a differential and spatially distributed nitro-oxidative stress response in roots and leaves of Lotus japonicus. Plant Science 201: 137-146.

Singh, S., Horn, S., Chowdhury, S. and Yu, F., 1999. Theory & Methods: Calibration of the estimators of variance. Australian & New Zealand Journal of Statistics 41(2): 199-212.

Vosough, A., Ghouchani, R. and Saed-Moucheshi, A., 2015. Genotypic Variation and Heritability of Antioxidant related Traits in Wheat Landraces of Iran. Biological Forum 7 (2): 43-47.

Ward, J. H., 1963. Hierarchical grouping to optimize an objective function, Journal of the American Statistical Association, 58:236-244

اثرات سطوح مختلف تنش خشکی بر پاسخ مورفو فیزیولوژیکی جمعیت‌های یونجه (*Medicago sativa*) در گلخانه

مهرناز ریاست[الف*]، علی اشرف جعفری[ب]، آرمین ساعد موچشی[ج]

[الف]استادیار مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی فارس؛ سازمان تحقیقات، آموزش و ترویج کشاورزی؛ شیراز، ایران، *(نگارنده مسئول)، پست الکترونیک: riasat.mehrnaz@yahoo.com

[ب]استاد موسسه تحقیقات جنگلها و مراتع؛ سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران

[ج]دانش آموخته دکتری اصلاح نباتات، بخش زراعت و اصلاح نباتات دانشکده کشاورزی دانشگاه شیراز، شیراز، ایران

چکیده. به منظور بررسی اثرات سطوح مختلف تنش خشکی بر صفات مورفوفیزیولوژیکی یونجه، در فصل رویش سال ۱۳۹۲ بذر ده جمعیت یونجه زراعی از نقاط مختلف استان فارس جمع‌آوری شدند و در گلخانه مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان فارس مورد آزمایش قرار گرفتند. چهار تیمار تنش خشکی ۱۰۰، ۷۵، ۵۰ و ۲۵ درصد ظرفیت مزرعه (FC) بر ۱۰ جمعیت یونجه جمع‌آوری شده اعمال گردید. از آزمایش فاکتوریل بر پایه طرح کاملا تصادفی با سه تکرار استفاده شد. صفات وزن تر و خشک سرشاخه و ریشه، طول ریشه، طول ساقه، نسبت طول ریشه به ساقه و محتوای پرولین آزاد اندازه‌گیری شد. مقایسه میانگین بین جمعیت‌ها انجام شد، تجزیه خوشه‌ای و تجزیه به مولفه‌های اصلی بر روی میانگین جمعیت‌ها انجام و پارامترهای ژنتیکی برآورد شدند. نتایج نشان داد که تغییرات زیادی در این جمعیت‌ها در اثر تنش‌های مختلف خشکی رخ می‌دهد. اکثر صفات، وراثت‌پذیری متوسطی را نشان دادند که این موضوع دلالت بر پتانسیل مناسب این جمعیت‌ها برای انتخاب در برنامه‌های اصلاح نژادی دارد. بیشترین میزان وراثت پذیری برای طول سرشاخه (۵۲٪)، طول ریشه (۴۸/۸۸٪) و محتوی پرولین (۴۷/۷۲٪) بود. در تجزیه کلاستر، چهار جمعیت ده کهنه، دزکرد، کمال آباد و کومهر با توجه به شباهت ژنتیکی و عملکرد بیشتر در کلاستر۲ قرار گرفتند. مقایسه میانگین بین جمعیت‌ها در همه تیمارها نشان داد که جمعیت کمال آباد نیریز بالاترین ضریب تولید و رشد گیاهچه و بیشترین تطابق پذیری و مقاومت به شرایط استرس را داشت. از تنوع بالا در جمعیت‌های مذکور می‌توان برای برنامه‌های اصلاح نژادی و بهبود بهره‌وری یونجه در تولید ارقام ترکیبی استفاده نمود.

کلمات کلیدی: یونجه، تنش خشکی، وراثت پذیری، تنوع ژنتیکی، پرولین