



Selection for Drought Tolerance in Sugar Beet Genotypes (*Beta vulgaris* L.)

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ABSTRACT: In this research, the response to drought stress of five sugar beet promising, drought tolerant genotypes along with a foreign tolerant genotype called IR7 and a domestic, non-tolerant variety called Gadook as the control varieties was studied in a split-split plot experiment based on a randomized complete block design with three replications in two locations, Karaj and Kermanshah, Iran, in 2012. Main plots consisted of two irrigation levels (well-watered and water-limited), sub-plots consisted of two levels of salicylic acid application (spraying and non-spraying) and sub-sub-plots included seven genotypes. Fourteen drought tolerance indices used in this study were: stress tolerance index (STI), stress susceptibility index (SSI), tolerance index (TOL), harmonic mean (HM), geometric mean productivity (GMP), mean productivity (MP), yield index (YI), yield stability index (YSI), drought resistance index (DI), abiotic tolerance index (ATI), stress non-stress production index (SNPI), modified stress tolerance index (MSTI), relative drought index (RDI) and stress susceptibility percentage index (SSPI). These were calculated and adjusted based on white sugar yield under stress (YS) and optimal irrigation (YP) conditions. Results of this study showed that the indices STI, K1STI, K2STI, MP, GMP, HM and YI can be used as the most suitable indicators for screening drought tolerant genotypes in two locations, Iran. Using the biplot and cluster analyses, the genotypes 2 and 3 were found to be the most drought tolerant genotypes with stable performance in Kermanshah and Karaj, respectively.

Keywords: Genotypes, Sugar beet, Drought tolerance indices, Multi-locations

INTRODUCTION

Sugar beet is an important field crop in the agricultural system in Iran. Drought is the most important limitation to sugar beet (*Beta vulgaris* L.) production in Iran and other areas world. As increased irrigation is not a viable answer to the problem, an economically and environmentally desirable solution is new varieties with decreased sensitivity to water deficits. However, there is little genotypic information on drought tolerance in sugar beet, and breeders are not equipped to make these selections. The objectives of this study were to assess the degree of genotypic diversity for drought-related tolerance indices and to measure the strength of association between these indices and crop performance. Drought tolerance indices are described in a companion paper. Assessing the genetic resources to improve drought tolerance in sugar beet. In many cases, increased irrigation inputs are not available option either because the water resources are not available or

that they are too expensive. In a world limited by supplies of freshwater, the trend is towards greater restrictions on agricultural water use (Pimentel et al., 1998). Improvements in sugar beet drought tolerance are therefore sought through plant breeding: genotypes with better ability to access soil water and with improved water use efficiency could increase yields in an economic and environmentally sustainable way. To produce these new cultivars, plant breeders must be able to identify germplasm with increased drought tolerance, and must be able to select for this trait on a large scale, cheaply and efficiently. In other crops where breeding for drought tolerance has been a focus for many locations, empirical breeding methods have been the most successful. That is, breeders select for high yield in the target environments without necessarily knowing what mechanisms bring about greater yields. In some areas, the impact of water deficit on the cultivation of sugar beet production is limited.

However, in wet years it can be appropriate crop produced through irrigation, but in the years in which there's limited water plant is under drought stress, therefore tolerant cultivars are needed in the years (Orojnia, 2010). A great demand for providing food for growing population and also establishing nutrition safety require the possible increase of agricultural production in different regions of the world. Thus more precise planning for the use of available water resources, particularly in agricultural use is necessary (Mohamadian, 2010). Low irrigation provides an optimal strategy under water shortage. Although irrigation reduces yield below, but it should be borne in mind that the decrease in yield is dependent on the time of low water (Khirabi *et al.*, 1987). Drought is a common phenomenon in warm and dry environment and selection for drought tolerance is one way to reduce the effects of water on crop yield (Sarmadnia, 1993). Cultivation of sugar beet and implementation of fundamental and advanced techniques requires that one of the main strategies for sugar beet breeding at Sugar Beet Seed Institute, Karaj, Iran, to be development and recommendation of drought tolerant genotypes. On the other hand, application of proper methods of planting, irrigation, fertilization and extension of resistant cultivars and agronomic practices will help sugar beet farmers to produce acceptable yield in stress conditions (Khayamim, 2010).

Variation in plant response to drought genetically manipulated enhanced preliminary for improving the appearance of the plant and increased production of stress (Rains *et al.*, 2003). Understanding the physiological response to stress, our ability to identify genes involved in stress tolerance will improve (McNeil *et al.*, 1999). If the selection of genotypes based on indices specified in the plant cell or tissue, it would be more appropriate and more reliable. The use of reliable traits for separating hybrids can be effective in the process of breeding resistant produce cultivars tolerant to obtain more quickly gives (Ashraf, 2004). In recent years, improvements to increase drought tolerance in sugar beet breeding a need was felt but so far the yield damages caused by the drought is regarded as an important factor (Jaggard *et al.*, 1998). Mohammadian (2001) in evaluation of drought resistance indices and their correlation with other traits conclude that are differences among genotypes for many these indices several selection criteria have been proposed to select genotypes based on their yield in stress and non-stress environments. Fischer *et al.* (1998) suggested that relative drought index (RDI) is a positive index for indicating stress tolerance. Lan (1998) defined a new index of drought resistance index (DI), which was commonly accepted to identify genotypes producing high yield under both stress and non-stress conditions.

Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between stress and irrigated environments and mean productivity (MP) as the average yield of genotypes under stress and non-stress conditions. The geometric mean productivity (GMP) is often used by breeds interested in relative yield, since drought stress can vary in severity in field environments over years (Fernandez, 1992). Fischer and Maurer (1978) suggested the stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. Gavuzzi *et al.* (1997) defined yield index (YI), by genotype yield on average yield of stress condition and yield stability index (YSI) suggested by Bouslama and Schapaugh (1984) in order to evaluate the stability of genotypes in the both stress and non-stress conditions. Stress tolerance index (STI) was defined as a useful tool for determining high yield and stress tolerance potential of genotypes (Fernandez, 1992). To improve the efficiency of (STI) a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka (2002) which corrects the (STI) as a weight. Moosavi *et al.* (2008) introduced stress susceptibility percentage index (SSPI), stress non-stress production index (SNPI) and abiotic tolerance index (ATI) for screening drought tolerant genotypes in stress and non-stress conditions. The nature of (ATI) and (SSPI) are such that they rely on crop survival mechanisms in stress conditions, although these genotypes can have either high or low yields in two conditions. Genotypes can be categorized into four groups based on their yield in stress and non-stress environments: genotypes express uniform superiority in both stress and non-stress environments (Group A); genotypes perform favorably only in non-stress environments (Group B); genotypes yield relatively higher only in stress environments (Group C); and genotypes perform poorly in both stress and non-stress environments (Group D). The optimal selection criterion should distinguish Group A from the other three groups (Fernandez, 1992). Clarke *et al.*, (1992) showed that yield-based SSI index did not differentiate between potentially drought resistant genotypes and those that possessed low overall yield potential. Similar limitations were reported by White and Singh (1991). Selection through TOL chooses genotypes with low YP but with high YS (group C) hence, TOL cannot distinguish between group C and group A (Fernandez, 1992). MP is mean yield for a genotype in two stress and non-stress conditions. MP can select genotypes with high YP but with relatively low YS (group B) and it fails to distinguish group A from group B. By decreasing TOL and increasing MP, the relative tolerance increases (Rosielle and Hamblin, 1981; Fernandez, 1992).

A high STI demonstrates a high tolerance and the most important advantage of STI is its ability to separate group A from others. GMP is more powerful than MP in separating group A and has a lower susceptibility to different amounts of YS and YP, so MP, which is based on arithmetic mean, will be biased when the difference between YS and YP is high. The geometric mean is often used by breeders interested in relative performance since drought stress can vary in severity in field environments and over years (Fernandez, 1992). For HM, the higher the HM is, the higher the relative tolerance of the cultivar will be. In the case of the last index, if $RDI > 1$, the genotype is relatively drought tolerant and if $RDI < 1$, it is drought susceptible (Fischer et al., 1979). The index ATI that can select group C with more emphasis on YP than SSI and TOL. The SSPI index is suitable for better understanding of yield variations and identification of relatively tolerant genotypes (stable yield under stress and non-stress conditions), whereas the SNPI index is suitable for selection of relatively resistant genotypes with relative stability and high yield under stress and non-stress conditions (Moosavi *et al.*, 2008). The purpose of this study was to evaluate the genotypic hybrids of sugar beet, screening drought tolerance quantitative indices

and identification of drought tolerant genotypes. The findings presented here should help focus attention on key indices and their methods of measurement, which could be further developed for practical use by breeders to select lines with increased drought tolerance.

MATERIALS AND METHODS

A. Experimental Design

Five promising, drought tolerant sugar beet genotypes along with a foreign tolerant genotype called IR7 and one non-tolerant, domestic variety called Gadook as the control varieties were studied under non-stress (based on water requirement) and water stress conditions (Table 1). Evaluation of drought tolerance of genotypes was carried out in a split-split-plot experiment based on a randomized complete block design with three replications in two locations, Karaj and Kermanshah, Iran, in 2012. Main plots consisted of two irrigation levels (well-watered and water-limited conditions) and sub-plots consisted of two levels of salicylic acid application (spraying and non-spraying), and sub-sub-plots consisted of 7 genotypes (5 promising genotypes and two controls). All genotypes evaluated in this study were diploid.

Table1: Titles Promising hybrids of sugar beet evaluated in experiments.

No.	Origin	No.	origin
1	(436*231)*SBSI.DR I-HSF.14-P.7	5	(I13*A 37.1)*110-HSF.52
2	(SB17*SB36)*SBSI.DR I-HSF.14-P.7	6	GADOUK 88 (Control)
3	(I13*A 37.1)*SBSI.DR I-HSF.14-P.7	7	IR7(Control)
4	(I13*A 37.1)*7221-HSF.43		

B. Irrigation conditions

The time of irrigation was determined using the rate of evaporation from the class A evaporation pan. Irrigation was equally applied in both conditions up to the 6-8-leaf stage (usually once a week) by furrow irrigation method in Karaj and sprinkler irrigation method in Kermanshah and then the stress treatment was imposed. For measuring inlet and outlet water, volumetric flow meters and WSC flumes were used in Kermanshah and Karaj, respectively. In both Karaj and Kermanshah locations, irrigation in non- stress and stress treatments was applied after 90 mm and 200 mm evaporation from the pan, respectively. The amounts of irrigation water in non-stress condition in Karaj and Kermanshah, were 11672.458 m³ and 12200m³, respectively, and those in stress conditions were 8987.1256m³ and 9200m³,

respectively. More rainfall and relatively cool conditions in the spring resulted in reduced water consumption in Karaj.

C. Characteristics of experiment locations

Karaj experiment was conducted at Motahari Research Station (35°59' N latitude, 51°6' E longitude and 1300m altitude) with an average annual temperature of 14.9°C and annual rainfall of 387.2mm in 2011. The climate of this area is Mediterranean hot and dry with cold winters and humid, hot and dry summers. Kermanshah was conducted at Water Research Station in Mahidasht, Kermanshah (46°48' N latitude, 34°16' E longitude and 1365m altitude). Fifteen-year average rainfall was about 400mm. The average annual temperature is 13.9°C and because of dry moisture regime, five months are dry.

D. Planting and harvesting crops

Plots, consisting of three 6 m-long rows (9m² with a row spacing of 50 cm and plant spacing of 15-18 cm) were thinned. The date of sowing was Mid-April in both locations. Spraying of sugar beet leaves with salicylic acid in both normal and stress conditions in two locations was performed at 6-8-leaf and 10-12-leaf stages. On the other hand, in order to unify the stress conditions and prevent the impact of water used for spraying Salicylic acid, the same amount of water was sprayed on plants in stress condition. The final harvest was done early November. It was performed by random sampling from the roots for getting beet pulp. After freezing, the samples were sent to Sugar Technology Laboratory of Sugar Beet Seed Institute, Karaj, Iran. In laboratory, percentage of sugar was determined by Polari meter. In this method, 26 grams of pulp is mixed with 177 ml of lead soacetate. The resulting mixture is filtrated using filter paper. Then, based on the rotation of polarized light, the sugar of the extracts is determined.

E. Evaluation of drought resistance indices

Drought resistance indices were determined after measuring white sugar yield in non-stress (Y_p) and stress (Y_s) conditions. Fourteen drought tolerance indices were calculated using the following equations: (Fischer and Maurere, 1978; Fischer *et al.*, 1998; Fernandez, 1992; Rosielle and Hamblin, 1981; Bouslama and Schapaugh, 1984; Lan, 1998; Moosavie *et al.*, 2008; Farshadfar and Stuka, 2002)

1. Stress susceptibility index = SSI = $([1 - (Y_s/Y_p)]) / ([1 - Y_s/Y_p])$ where $[1 - Y_s/Y_p]$ is the stress intensity. The genotypes with SSI < 1 are more resistant to drought stress conditions.
2. Stress tolerance index = STI = $(Y_s \times Y_p) / (Y_p)^2$. The genotypes with high STI values will be tolerant to drought stress.
3. Geometric mean productivity = GMP = $\sqrt{(Y_s \times Y_p)}$. The genotypes with high value of this index will be more desirable.
4. Mean productivity = MP = $(Y_s + Y_p) / 2$. The genotypes with high value of this index will be more desirable.
5. Tolerance = TOL = $(Y_p - Y_s)$. The genotypes with low values of this index are more stable in two different conditions.
6. Harmonic index = HM = $2(Y_p) \times (Y_s) / (Y_p + Y_s)$. The genotypes with high HM value will be more desirable.
7. Yield index = YI = Y_s / \bar{Y}_s . The genotypes with high value of this index will be suitable for drought stress condition.

8. Yield stability index = YSI = Y_s / Y_p . The genotypes with high YSI values can be regarded as stable genotypes under stress and non-stress conditions.

9. Drought resistance index = DI = $[(Y_s \times Y_p) / Y_p] / \bar{Y}_s$.

10. Relative drought index = RDI = $((Y_s / Y_p) / (\bar{Y}_s / \bar{Y}_p))$

11. Abiotic tolerance index = ATI = $[(Y_p - Y_s) / (\bar{Y}_p / \bar{Y}_s)] \times [\sqrt{Y_p - Y_s}]$.

12. Stress susceptibility percentage index = SSPI = $[(Y_p - Y_s) / \bar{Y}_p] \times 100$

13. Stress non-stress production index = SNPI $[\sqrt[3]{(Y_p + Y_s) / (Y_p - Y_s)}] \times [\sqrt[3]{Y_p \times Y_s \times Y_s}]$

14. Modified stress tolerance index = MSTI = K_i STI, $K_1 = (Y_p)^2 / (\bar{Y}_p)^2$, $K_2 = (Y_s)^2 / (\bar{Y}_s)^2$. Where K_i is the correction coefficient.

In the above formulas, Y_s and Y_p represent white sugar yield in stress and non-stress conditions, respectively. Also, Y_s and Y_p are mean white sugar yield of all genotypes in stress and non-stress conditions, respectively. The genotypes can be categorized into four groups based on their white sugar yield in stress and non-stress environments: genotypes express uniform superiority in both stress and non-stress conditions (Group A), genotypes with high white sugar yield only in non-stress conditions (Group B), genotypes give relatively higher white sugar yield in stress conditions (Group C), and genotypes with low white sugar yield in both stress and non-stress conditions (Group D). The optimal selection criterion should distinguish Group A from the other three groups. Three-dimensional plots among Y_s, Y_p and STI, showed the interrelationships among these three variables to separate genotypes of group A from the other groups (Fernandez, 1992).

F. Statistical analysis

Analysis of variance for the indices was performed using statistical software MSTAT-C. Mean comparison of indices were performed based on least significant difference test LSD. Correlation among indices and white sugar yield in the conditions, Principal component analysis (PCA), Biplot analysis, Cluster analysis and Three dimensional plots drawing were performed by SPSS ver.19, Statistic ver.8.

RESULTS AND DISCUSSION

A. Analysis of variance

Table 2. shows the analysis of variance for drought resistance indices as well as white sugar yield in both stress and non-stress conditions in Kermanshah and Karaj. This Table shows the locations effects on white sugar yield of genotypes under non-stress and that ATI indices at (P = 0.05) and TOL, SSPI and SNPI indices at (P = 0.01) were significantly different.

Location is always the most important source of yield variation (more than 80%) (Yan *et al.*, 2000). Location was the most important source of yield variation (relative to genotype), but the high magnitude of location, which is irrelevant to cultivar evaluation and environment investigation (Fox and Rosielle, 1982; Gauch and Zobel, 1996). The large proportion of variability explained by the environments has been reported by other researchers such as Yan *et al.*, (2000),

Dehghani *et al.*, (2006) and Cirilo *et al.*, (2009). The effect of salicylic acid spraying on white sugar yield and Genotype \times spraying interaction was significant ($P < 0.05$). The Table of analysis of variance shows that the variance of genotypes under stress condition was less than non-stress conditions, which is in agreement with the results of Abelmula *et al.* (1998) and Ramirez-Vallejo *et al.*, 1998 and Orojnia, 2010.

Table 2 : Analysis of variance for white sugar yield of stress and non-stress conditions and drought tolerance indices in both locations Kermanshah and Karaj.

Sources of variation	Degrees of freedom	Mean-square				
		Yp	Ys	STI	K ₁ STI	K ₂ STI
Location	1	58.600*	8.203 ns	1.141 ns	5.477 ns	7.187 ns
Location*Replication	4	6.775	4.866	0.301	3.194	6.301
Spraying conditions	1	17.664*	3.167 ns	0.343 ns	1.567 ns	1.084 ns
Location*Spraying conditions	1	1.536ns	0.826 ns	0.0002 ns	0.113 ns	0.614 ns
Error type 1	4	1.900	3.591	0.045	0.250	0.918
Genotypes	6	11.838**	10.861**	0.536**	2.193**	4.719**
Location*Genotypes	6	5.167ns	11.627**	0.533**	1.836**	3.857*
Spraying conditions*Genotypes	6	1.292ns	2.895 ns	0.040 ns	0.333 ns	0.480 ns
Location*Spraying conditions*Genotypes	6	3.358ns	4.198 ns	0.154 ns	1.182*	3.063 ns
Error type 2	48	2.653	1.939	0.070	0.470	1.364
Coefficient of Variation		19.75	28.68	42.49	82.12	114.26

* $P < 0.05$; ** $P < 0.01$; ns:non significant

Sources of variation	Degrees of freedom	Mean-square				
		SSI	TOL	Mp	HM	GMP
Location	1	0.030ns	110.676**	5.738ns	0.385ns	0.077ns
Location*Replication	4	0.136	0.878	5.601	6.989	8.694
Spraying conditions	1	0.078ns	5.878ns	8.947ns	4.346ns	10.914ns
Location*Spraying conditions	1	0.331ns	0.110ns	1.154ns	0.403ns	0.121ns
Error type 1	4	0.434	6.338	1.161	2.160	1.813
Genotypes	6	0.648*	4.991ns	10.101**	11.522**	9.313**
Location*Genotypes	6	0.615*	3.121ns	7.617**	10.728**	7.654**
Spraying conditions*Genotypes	6	0.596*	3.494ns	1.219ns	2.654ns	0.949ns
Location*Spraying conditions*Genotypes	6	0.335ns	3.206ns	2.976ns	3.686ns	3.000ns
Error type 2	48	0.227	3.353	1.458	2.100	1.821
Coefficient of Variation		48.35	54.02	18.43	24.65	21.49

High levels of genotypic the better identification of differences between genotypes. Thus, the effect of genotypes was significant ($P < 0.01$) in both conditions of stress and non-stress conditions. Effects of genotypes on STI, K₁STI, K₂STI, MP, HM, GMP, YI and DI indices were significant at $P < 0.01$ and on SSI, YSI and SNPI indices at ($P < 0.05$).

Genotype \times Location interactions were significant for white sugar yield-based indices of STI, K₁STI, MP, HM, GMP and YI at $P < 0.01$ and for K₂STI, SSI and SNPI at ($P < 0.05$). Dividing the target environments into meaningful environments and deploying different genotypes for different environments is the only way to utilize Genotype \times Environments interaction (Yan and Tinker, 2005).

Sources of variation	Mean-square					
	Degrees of freedom	YSI	YI	DI	ATI	SSPI
Location	1	0.734ns	0.0002ns	0.774ns	429.952*	2490.314**
Location*Replication	4	0.18	0.204	0.129	38.352	43.919
Spraying conditions	1	0.008ns	0.141ns	0.039ns	213.551ns	166.276ns
Location*Spraying conditions	1	0.028ns	0.043ns	0.038ns	9.910ns	27.622ns
Error type 1	4	0.066	0.153	0.232	116.301	181.705
Genotypes	6	0.078*	0.426*	0.422**	62.576ns	155.085ns
Location*Genotypes	6	0.069ns	0.458**	0.378*	23.709ns	63.423ns
Spraying conditions*Genotypes	6	0.066ns	0.124ns	0.184ns	32.815ns	128.261ns
Location*Spraying conditions*Genotypes	6	0.39ns	0.178ns	0.178ns	54.869ns	118.226ns
Error type 2	48	0.031	0.084	0.106	53.507	123.440
Coefficient of Variation		29.16	29.04	49.31	62.25	55.93

* $P < 0.05$; ** $P < 0.01$; ns:non significant

Sources of variation	Mean-square		
	Degrees of freedom	SNPI	RDI
Location	1	365.375**	0.014 ns
Location*Replication	4	35.947	0.049
Spraying conditions	1	0.53 ns	0.007 ns
Location*Spraying conditions	1	4.458 ns	0.082 ns
Error type 1	4	56.986	0.232
Genotypes	6	83.072*	0.217 ns
Location*Genotypes	6	91.244*	0.181 ns
Spraying conditions*Genotypes	6	37.148 ns	0.195 ns
Location*Spraying conditions*Genotypes	6	24.690 ns	0.108 ns
Error type 2	48	30.764	0.098
Coefficient of Variation		52.44	31

For plant breeding, genotype concerns broad adaptations of benefit throughout a growing region, whereas genotype \times location interaction concerns narrow adaptations that can be exploited only by subdivision into two or more environments. Because genotype \times location interaction is often larger than genotype effect, understanding interactions and implementing environments can be strategic (Gauch 2006). Significant effects of genotypes ($P < 0.01$) and Genotype \times Location interactions on white sugar yield-based indices ($P < 0.01$) show that it would be very difficult to identify a common widely adapted sugar beet hybrid across environments. The high magnitude of Location effect (large variation), Shows that the Iran region is highly variable from location to location. The genotype-by-location analysis of data showed that the variability due to the genotype \times location interaction on for white sugar yield ($P < 0.01$) that was larger than the variability among genotypes ($P < 0.01$).

The large genotype \times location, relative to genotype, suggests the possible existence of differences among mega-environments (Yan *et al.*, 2000; Dehghani *et al.*, 2006). Therefore, this result showed that it is necessary to partition the region into sub regions to make hybrid recommendations. These results were also consistent with those obtained by other researchers (Yan *et al.*, 2000; Dehghani *et al.*, 2006, 2009; Fan *et al.*, 2007). The relative contributions of genotype and genotype \times location interaction effects to the total variation for white sugar yield found in this study indicated that it would be very difficult to achieve an indirect response to selection across all of the hybrids of environments from selection in a few environments, ignoring the observed genotype \times location interactions. However, genotype \times location interaction makes it difficult to select the best performing and most stable hybrids and is an important consideration in plant breeding programs because it reduces the progress from selection in any one environment.

B. Comparison of mean

Tables (2, 3, 4, 5, and 6) show the comparison of means of drought resistance indices, and also white sugar yield in both stress and non-stress conditions in Kermanshah and Karaj. According to the Table, mean white sugar yield under non-stress condition in Kermanshah decreased 18.4% compared to Karaj while in stress condition it was increased 12.9% which represents suitability of Kermanshah for cultivation of drought tolerant sugar beet genotypes. In other words, the extent of negative effects of the stress applied on white sugar yield was higher in Kermanshah than in Karaj. Mean comparison of the traits in conditions sprayed with Salicylic acid showed that the white sugar yields of genotypes in Karaj in non-stress and stress conditions were 10.53% and 7.68%, respectively, higher than those in Kermanshah. It represents positive effects of spraying salicylic acid in increasing white sugar content compared to root yield in Karaj. According to Table C. 4, genotypes number 1, 2 and 3 had the highest white sugar yield and differed significantly ($P < 0.05$) from the control genotypes under non-stress condition in Kermanshah and Karaj.

In stress condition, respectively genotypes number 2, 1, 4 and 3 showed the highest white sugar yield, respectively, whereas the genotype 5 had the lowest value without a significant difference with control genotypes. In terms of drought resistance indices HM, GMP, SNPI, YI and DI, genotypes number 2, 1, 3 and 4 were the most tolerant genotypes and the genotype 5 had the lowest amount and displayed no significant difference with control genotypes. In terms of MP index, the highest value was observed in genotypes number 2, 1 and 3 whereas the genotype number 5 had the lowest value with no significant difference with control genotypes. In terms of YSI index, genotypes number 4, 2 and 3 had the most highest values whereas the genotypes number 1 and 5 had the lowest values without being significantly different from the control genotypes. In terms of STI, K1STI and K2STI indices, genotypes number 2 and 1 had the highest values while the genotype 5 had the lowest value which did not significantly differ from the control cultivars. In terms of SSI index, genotypes number 1 and 5 showed the highest value whereas the genotypes number 2, 3 and 4 had the lowest value which were significantly different ($P < 0.05$) from the control genotypes.

Table 3: Mean comparison effect location in terms drought resistance indices in both Locations Kermanshah and Karaj.

Treatment locations	SNPI	SSPI	ATI	TOL	Y _S (ton/ha)	Y _P (ton/ha)
Location Kermanshah	12.66 a	14.42 b	9.489 b	2.242 b	5.169	7.411b
Location Karaj	8.491 b	25.31a	14.01 a	4.538 a	4.544	9.081 a

*Means the same letters based on least significant difference test LSD, no significant difference ($P < 0.05$)

Sources of variation	Mean		
	Degrees of freedom	SNPI	RDI
Location	1	365.375**	0.014 ns
Location*Replication	4	35.947	0.049
Spraying conditions	1	0.53 ns	0.007 ns
Location*Spraying conditions	1	4.458 ns	0.082 ns
Error type 1	4	56.986	0.232
Genotypes	6	83.072*	0.217 ns
Location*Genotypes	6	91.244*	0.181 ns
Spraying conditions*Genotypes	6	37.148 ns	0.195 ns
Location*Spraying conditions*Genotypes	6	24.690 ns	0.108 ns
Error type 2	48	30.764	0.098
Coefficient of Variation		52.44	31

Table 4: Mean comparison the locations × spraying conditions in × genotype interaction in terms of index K1STI and spraying conditions in × genotype interaction in terms of index SSI.

Treatment	K ₁ STI	Treatment	K ₁ STI	Treatment	SSI
P ₁ S ₁ G ₁	2.313 ab	P ₂ S ₁ G ₁	0.3443def	S ₁ G ₁	1.168 abc
P ₁ S ₁ G ₂	2.603 a	P ₂ S ₁ G ₂	0.3903def	S ₁ G ₂	0.9947 abcd
P ₁ S ₁ G ₃	0.7673 cdef	P ₂ S ₁ G ₃	0.6383 def	S ₁ G ₃	0.6613 cd
P ₁ S ₁ G ₄	0.4523 def	P ₂ S ₁ G ₄	0.3637 def	S ₁ G ₄	0.6237 cd
P ₁ S ₁ G ₅	0.2197 f	P ₂ S ₁ G ₅	0.2437 f	S ₁ G ₅	1.277 ab
P ₁ S ₁ G ₆	0.3577def	P ₂ S ₁ G ₆	0.5020 def	S ₁ G ₆	0.6408 cd
P ₁ S ₁ G ₇	0.2187f	P ₂ S ₁ G ₇	0.3620 def	S ₁ G ₇	1.316 a
P ₁ S ₂ G ₁	2.387 ab	P ₂ S ₂ G ₁	0.7310 cdef	S ₂ G ₁	0.9162 abcd
P ₁ S ₂ G ₂	1.166cdef	P ₂ S ₂ G ₂	1.383 bcde	S ₂ G ₂	0.5322 d
P ₁ S ₂ G ₃	1.105 cdef	P ₂ S ₂ G ₃	0.7447 cdef	S ₂ G ₃	1.137 abc
P ₁ S ₂ G ₄	1.848 abc	P ₂ S ₂ G ₄	0.4637 def	S ₂ G ₄	0.7525 bcd
P ₁ S ₂ G ₅	1.430 bcd	P ₂ S ₂ G ₅	0.3593 def	S ₂ G ₅	0.9260 abcd
P ₁ S ₂ G ₆	0.1143f	P ₂ S ₂ G ₆	1.074 cdef	S ₂ G ₆	1.385 a
P ₁ S ₂ G ₇	0.2810 ef	P ₂ S ₂ G ₇	0.5133 def	S ₂ G ₇	1.459 a

C. Correlation analysis

Correlation coefficients between white sugar yield and drought tolerance indices in both Kermanshah and Karaj (Table 7 and Table 8) can be good criteria for screening the best genotypes and indices used. White sugar yield in stress condition in both Kermanshah and Karaj were significantly and positively correlated with the indices YSI, YI, DI, STI, K1STI, K2STI, HM, MP,

GMP, RDI and SNPI and significantly and negatively correlated with SSI. White sugar yield in non-stress condition in Kermanshah was significantly and positively correlated with the indices YI, DI, STI, K1STI, HM, MP, GMP and SNPI indicating that these criteria were more effective in identifying high white sugar yield genotypes under different water conditions.

Table 5: Mean comparison genotypes in terms of indices of drought tolerance in both locations Kermanshah and Karaj.

Treatment	YP	YS	STI	K ₁ STI	K ₂ STI	SSI
G ₁	9.847 a	5.474 a	0.8722 ab	1.444 a	1.599 ab	1.042 abc
G ₂	9.179 ab	6.238 a	0.905 a	1.386 a	2.100 a	0.7634 bc
G ₃	8.577 abc	5.148 ab	0.6637bc	0.8138 b	0.9480 bc	0.8989 bc
G ₄	7.878 bcd	5.342 ab	0.6538 c	0.7819 b	0.9572 bc	0.6881 c
G ₅	7.725d	3.917 c	0.4752 cd	0.5633 b	0.4728 c	1.102 ab
G ₆	7.157 d	4.292 bc	0.4087 d	0.5121 b	0.7479 bc	1.013 abc
G ₇	7.361 cd	3.584 c	0.3868 d	0.3438 b	0.3303 c	1.387 a

Treatment	MP	HM	GMP	YSI	YI	DI	SNPI
G ₁	7.660 a	6.758 a	7.173 a	0.5613 bcd	1.107 ab	0.7071 abc	11.76 ab
G ₂	7.708a	7.237 a	7.456a	0.6846 ab	1.280 a	0.9451 a	13.89 a
G ₃	6.862 ab	6.231 a	6.528 ab	0.6343 abc	1.063 ab	0.7113 abc	12.32 ab
G ₄	6.610 bc	6.217 a	6.405 abc	0.7078 a	1.096 ab	0.8039 ab	12.40 ab
G ₅	5.821 cd	4.986 b	5.369 cd	0.5312 cd	0.802 c	0.4759 cd	8.326 bc
G ₆	5.724 cd	5.017 b	5.968 bcd	0.6087abcd	0.9003bc	0.5827 bcd	8.325 bc
G ₇	5.472 d	4.710 b	5.068 d	0.4878 d	0.7529 c	0.4063 d	7.020 c

*Means the same letters based on least significant difference test LSD, no significant difference ($P > 0.05$).

Table 6: Mean comparison locations × genotypes interaction in terms indices of drought tolerance in both locations Kermanshah and Karaj.

Treatment	YP	YS	STI	K ₁ STI	K ₂ STI	SSI	TOL	MP
P ₁ G ₁	9.865 a	7.303 a	1.309 a	2.350 a	2.827 a	0.8378 bcd	2.562 cdef	8.584 a
P ₁ G ₂	8.762 abc	6.992 ab	1.153 a	1.885 ab	2.855 a	0.6763d	1.770 ef	7.877 ab
P ₁ G ₃	7.427 bcd	5.463 bc	0.7687 bc	0.9362 cd	1.164 b	0.8533 bcd	1.973 def	6.450 cde
P ₁ G ₄	7.335 bcd	5.943 abc	0.8203 b	1.150 bc	1.289 b	0.2298 d	1.392 f	6.639 bcde
P ₁ G ₅	7.010 cd	4.493 cd	0.6097 bcde	0.8250 cd	0.6605 b	1.112 bc	2.547 cdef	5.767 ef
P ₁ G ₆	5.123 e	3.302 de	0.2018 f	0.2360 d	0.2952 b	1.173 bc	1.822 def	4.213 g
P ₁ G ₇	6.315 de	2.685 e	0.3085 ef	0.2498 d	0.1057 b	1.813 a	3.630 bcde	4.500 fg
P ₂ G ₁	9.828 a	3.645 ds	0.4350 def	0.5377 cd	0.3722 b	1.247 b	6.183 a	6.737 bcde
P ₂ G ₂	9.597 a	5.483 bc	0.6573 bcd	0.8867 cd	1.344 b	0.8505 bcd	4.115 abc	7.540 abc
P ₂ G ₃	9.717 a	4.833 cd	0.5587 bcde	0.6915 cd	0.7322 b	0.9440 bcd	4.883 ab	7.275 abcd
P ₂ G ₄	8.422 abc	4.740 cd	0.4772 cdef	0.4137 cd	0.6250 b	0.8463 bcd	3.682 bcde	6.581 bcde
P ₂ G ₅	8.410 abc	3.340 ds	0.3407 ef	0.3015 d	0.2790 b	1.091 bc	5.070 ab	5.875 def
P ₂ G ₆	9.190 ab	5.282 c	0.6155 bcde	0.7882 cd	1.201 b	0.8227 bcd	3.908 bcd	7.236 abcd
P ₂ G ₇	8.407 abc	4.483 cd	0.4650 cdef	0.4377 cd	0.5550 b	0.9313 bcd	3.923 bcd	6.445 cde

Treatment	YP	YS	STI	K ₁ STI	K ₂ STI	SSI	TOL	MP
P ₁ G ₁	9.865 a	7.303 a	1.309 a	2.350 a	2.827 a	0.8378 bcd	2.562 cdef	8.584 a
P ₁ G ₂	8.762 abc	6.992 ab	1.153 a	1.885 ab	2.855 a	0.6763d	1.770 ef	7.877 ab
P ₁ G ₃	7.427 bcd	5.463 bc	0.7687 bc	0.9362 cd	1.164 b	0.8533 bcd	1.973 def	6.450 cde
P ₁ G ₄	7.335 bcd	5.943 abc	0.8203 b	1.150 bc	1.289 b	0.2298 d	1.392 f	6.639 bcde
P ₁ G ₅	7.040 cd	4.493 cd	0.6097 bcde	0.8250 cd	0.6665 b	1.112 bc	2.547 cdef	5.767 ef
P ₁ G ₆	5.123 e	3.302 de	0.2018 f	0.2360 d	0.2952 b	1.173 bc	1.822 def	4.213 g
P ₁ G ₇	6.315 de	2.685 e	0.3085 ef	0.2498 d	0.1057 b	1.813 a	3.630 bcde	4.500 fg
P ₂ G ₁	9.828 a	3.645 ds	0.4350 def	0.5377 cd	0.3722 b	1.247 b	6.183 a	6.737 bcde
P ₂ G ₂	9.597 a	5.483 bc	0.6573 bcd	0.8867 cd	1.344 b	0.8505 bcd	4.115 abc	7.540 abc
P ₂ G ₃	9.717 a	4.833 cd	0.5587 bcde	0.6915 cd	0.7322 b	0.9440 bcd	4.883 ab	7.275 abcd
P ₂ G ₄	8.422 abc	4.740 cd	0.4772 cdef	0.4137 cd	0.6250 b	0.8463 bcd	3.682 bcde	6.581 bcde
P ₂ G ₅	8.410 abc	3.340 ds	0.3407 ef	0.3015 d	0.2790 b	1.091 bc	5.070 ab	5.875 def
P ₂ G ₆	9.190 ab	5.282 c	0.6155 bcde	0.7882 cd	1.201 b	0.8227 bcd	3.908 bcd	7.236 abcd
P ₂ G ₇	8.407 abc	4.483 cd	0.4650 cdef	0.4377 cd	0.5550 b	0.9313 bcd	3.923 bcd	6.445 cde

Table 7: Correlation coefficients between drought tolerance indices in Kermanshah.

	YP	YS	YSI	YI	DI	STI	K ₁ STI	K ₂ STI	HM	SSI	MP	TOL	GMP	ATI	RDI	SSPI	SNPI
YP	1	.907**	.538	.907**	.838*	.976**	.969**	.924**	.944**	-.537	.973**	-.068	.906**	.749	.537	.067	.841*
YS		1	.837*	1.000**	.989**	.976**	.956**	.937**	.995**	-.836*	.980**	-.481	.985**	.434	.836*	-.344	.973**
YSI			1	.837*	.899**	.703	.659	.649	.782*	-1.000**	.713	-.865*	.794*	-.054	1.000**	-.754	.863*
YI				1	.989**	.976**	.956**	.937**	.995**	-.836*	.980**	-.481	.985**	.435	.836*	-.344	.973**
DI					1	.936**	.912**	.908**	.970**	-.898**	.941**	-.600	.970**	.301	.898**	-.476	.980**
STI						1	.984**	.954**	.992**	-.702	.999**	-.281	.965**	.600	.702	-.147	.931**
K ₁ STI							1	.972**	.974**	-.658	.985**	-.247	.973**	.634	.658	-.099	.882**
K ₂ STI								1	.947**	-.648	.933**	-.296	.961**	.511	.648	-.171	.899**
HM									1	-.782*	.994**	-.392	.983**	.520	.782*	-.250	.958**
SSI										1	-.713	.865*	-.794*	.054	-1.000**	.754	-.863*
MP											1	-.295	.971**	.595	.713	-.156	.933**
TOL												1	-.448	.531	-.865*	.956**	-.557
GMP													1	.468	.793*	-.293	.937**
ATI														1	-.055	.673	.287
RDI															1	-.754	.862*
SSPI																1	-.445
SNPI																	1

*. Correlation is significant ($P < 0.05$), **. Correlation is significant at ($P < 0.01$)

Table 8: Correlation coefficients between drought tolerance indices in Karaj.

	YP	YS	YSI	YI	DI	STI	K ₁ STI	K ₂ STI	HM	SSI	MP	TOL	GMP	ATI	RDI	SSPI	SNPI
YP	1	.254	-.102	.259	.098	.527	.725	.374	.403	.240	.744	.507	.532	.918**	-.069	.580	-.027
YS		1	.926**	1.000**	.978**	.947**	.801*	.925**	.969**	-.863*	.835*	-.705	.953**	-.106	.941**	-.627	.839*
YSI			1	.925**	.963**	.756*	.517	.758*	.854*	-.974**	.581	-.900**	.778*	-.434	.997**	-.859*	.927**
YI				1	.978**	.948**	.802*	.923**	.971**	-.862*	.838*	-.701	.954**	-.101	.940**	-.624	.841*
DI					1	.877**	.694	.886**	.932**	-.931**	.731	-.800*	.878**	-.280	.978**	-.720	.844*
STI						1	.950**	.959**	.953**	-.671	.954**	-.457	.991**	.187	.783*	-.359	.669
K ₁ STI							1	.905**	.836*	-.428	.965**	-.182	.924**	.436	.555	-.074	.431
K ₂ STI								1	.880**	-.720	.851*	-.550	.919**	.021	.791*	-.441	.590
HM									1	-.761*	.899**	-.568	.969**	.044	.877**	-.476	.788*
SSI										1	-.460	.945**	-.675	.562	-.971**	.905**	-.862*
MP											1	-.198	.960**	.449	.610	-.103	.564
TOL												1	-.459	.768*	-.889**	.985**	-.768*
GMP													1	.201	.798*	-.374	.735
ATI														1	-.417	.789*	-.283
RDI															1	-.835*	.902**
SSPI																1	-.776*
SNPI																	1

*. Correlation is significant ($P < 0.01$), **. Correlation is significant at ($P < 0.05$)

It was also significantly and positively correlated with the index ATI in Karaj. SSPI had positive, significant correlations with TOL in both Karaj and Kermanshah and negative, significant correlations with RDI and YSI in Karaj. SNPI also exhibited negative correlation with index SSI in the two locations, but it was positively correlated with indices YS, YSI, YI, DI, HM and RDI. Farshadfar *et al.* (2012) reported that YI, YSI, STI, GMP, MP and HM were significantly and positively correlated with stressed yield and these indices showed that genotypes may be ranked only on the basis of their yield under stress and so does not discriminate genotypes of group A. Toorchi *et al.* (2012) showed that correlations between MP, GMP, YS and YP were positive. Dehghani *et al.* (2009) reported that GMP, MP and STI were significantly and positively correlated with stressed yield. Farshadfar *et al.* (2001) believed that the most appropriate index for selecting stress tolerant cultivars is an index which has partly high correlation with yield under stress and non-stress conditions. In a study conducted by Farshadfar and Elyasi (2012), grain yield in the stress and non-stress conditions were positively correlated with YSI, YI, DI, MSTI, RDI. Ehdaie and Shakiba (1996) found in wheat that there was no correlation between SSI and yield under optimum condition.

D. Three dimensional plots and cluster analysis

In order to identify drought tolerant genotypes, three-dimensional plots were drawn based on YP, YS and STI (Fig. 1. and Fig. 2).

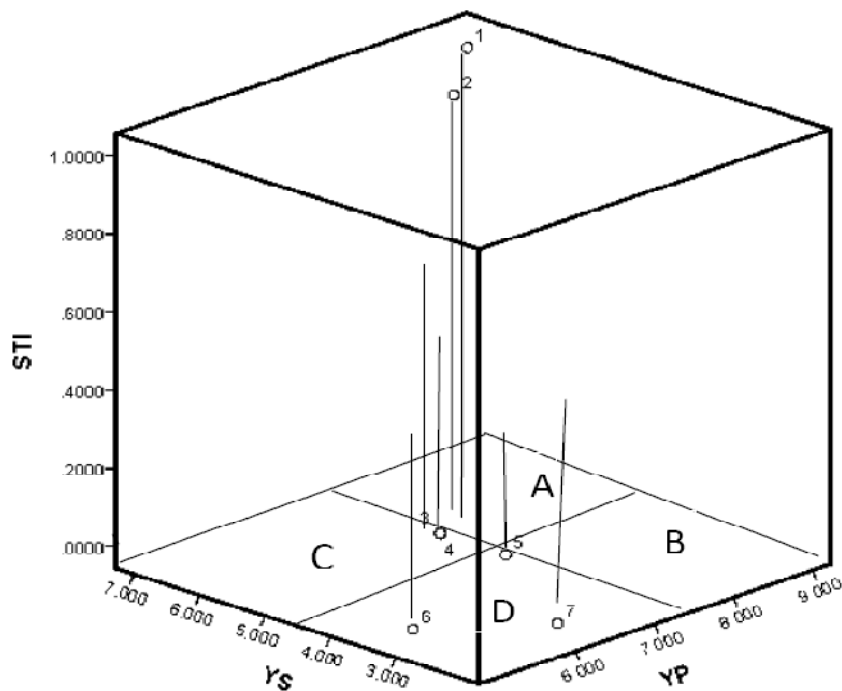


Fig. 1. The three dimensional plots among STI, Yp, Ys in Kermanshah.

Three-dimensional plots are presented to show the interrelationships among these three variables to separate the genotypes of group (A) (high white sugar yield genotypes in both stress and non-stress conditions) from the other groups (B, C and D), and to illustrate the advantage of STI index as a selection criterion for identifying high white sugar yielding and stress tolerant genotypes.

In three-dimensional plot in Kermanshah, the genotypes number 1 and 2 and in Karaj the genotypes number 2, 6 and 3 were included in group A, these accessions revealed stable white sugar yield in stress and non-stress conditions. In Kermanshah, the genotypes number 6, 7 and 5 and in Karaj the genotype number 5 were in group D that performed poorly in both conditions.

Cluster analysis based on indices in both locations tended to group the genotypes, into three groups: group 1, tolerant; group 2, semi-tolerant and group 3, sensitive genotypes (Fig. 3 and Fig. 4). In this analysis, the first group had the highest YP, YS, STI, MP, HM, GMP, YI, DI, K1STI, K2STI, RDI and SNPI and was thus considered to be the most desirable cluster for both growth conditions in Kermanshah and Karaj. The second group had average indices values. In the third group, all genotypes had high SSI, thus they were susceptible to drought and only suitable for non-stress conditions.

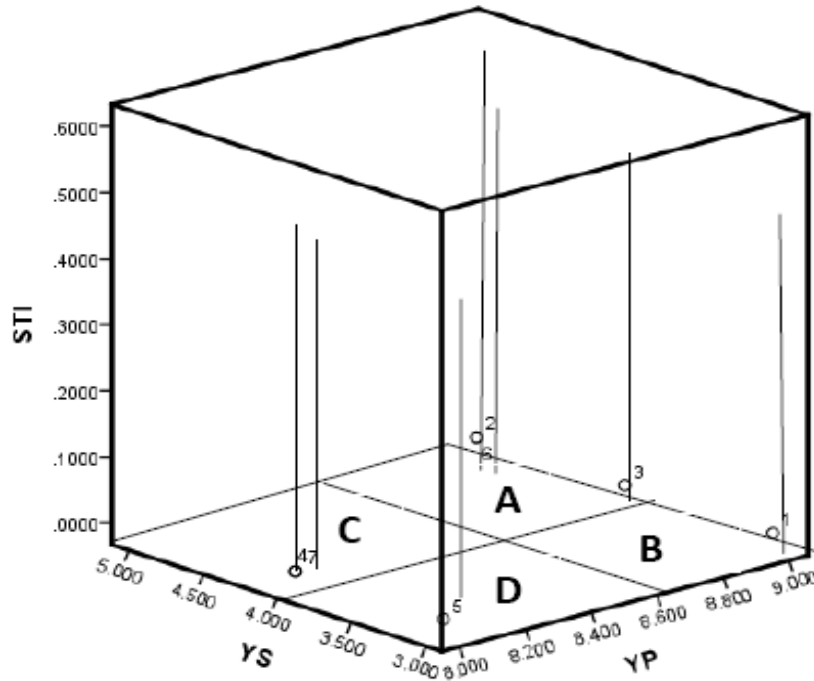


Fig. 2. The three dimensional plots among STI, Yp, Ys in Karaj.

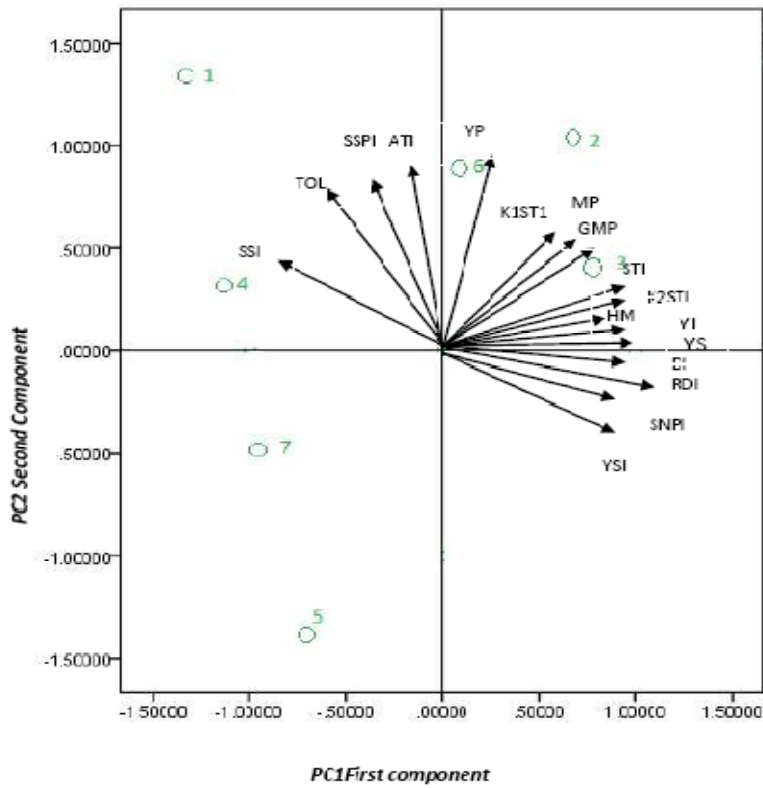


Fig. 3. Biplot based on first and second components of drought tolerance indices in Kermanshah.

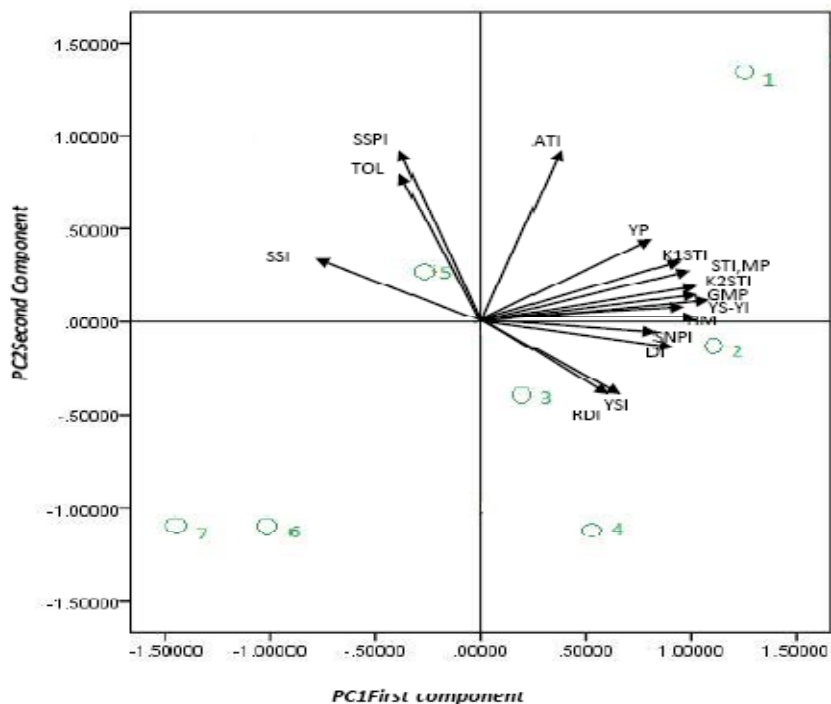


Fig. 4. Biplot based on first and second components of drought tolerance indices in Karaj.

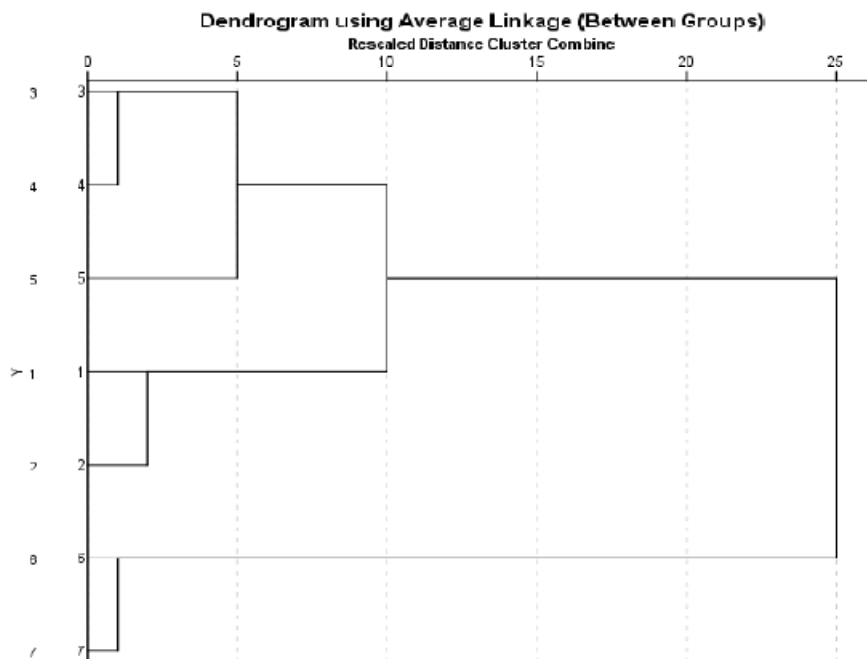


Fig. 5. Dendrogram using Average Linkage between groups showing classification of genotypes based on tolerance indices in Kermanshah.

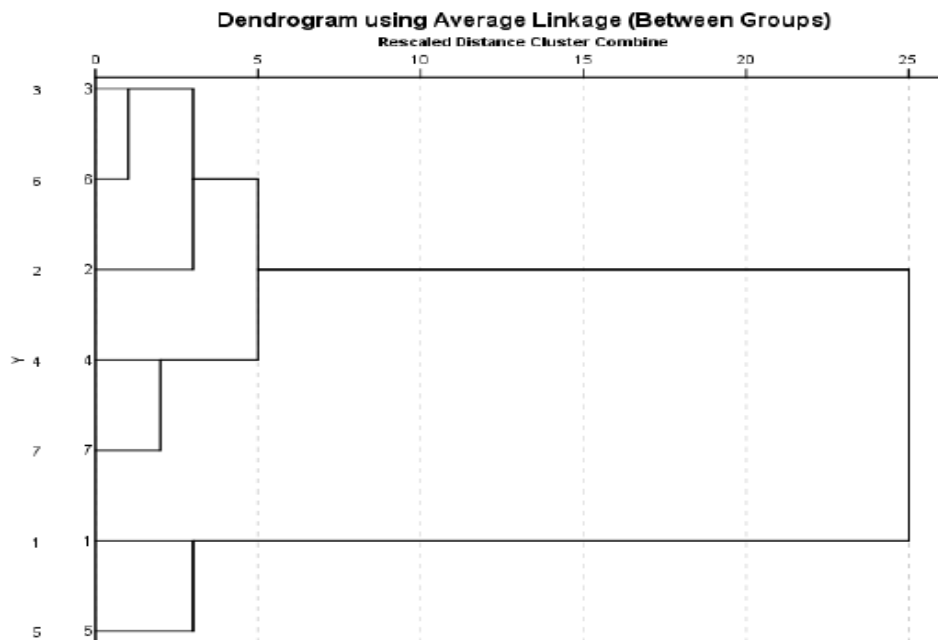


Fig. 6. Dendrogram using Average Linkage between groups showing classification of genotypes based on tolerance indices in Karaj.

In general, cluster analysis classified the genotypes number 2 and 3, in both Kermanshah and Karaj, as drought tolerant genotypes and their stability was higher than the other genotypes.

E. Biplot analysis

To better understand the relationships, similarities and dissimilarities among drought tolerance indices and assessment of drought tolerant genotypes, Principal Component Analysis (PCA), based on the indices correlation matrix was used. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only. Principal Component Analysis for drought tolerance indices and white sugar yield in two conditions showed that the first component explained 77.017 % and 71.119% of the variation in the data matrix in Kermanshah and Karaj, respectively. It was shown in both Kermanshah and Karaj that YS had a high positive correlation with YI, DI, HM, GMP, STI, K2STI, RDI, YSI, SNPI, K1STI and MP. YS showed a relatively high negative correlation with SSI, TOL and SSPI. Thus, the first component can be named as stress-resistant component and it separates the stress-resistant genotypes from stress-susceptible ones. The second component explained 20.898% and 25.609% of total variability in Kermanshah and Karaj, respectively, and revealed in both locations a high positive correlation of YP with ATI, SSPI, TOL and SSI.

Therefore, the second component can be named as stress-susceptible component which separates the high yielder from the low yielder genotypes. Biplot for the first two components properly explained and confirmed the results of genotypes grouping based on cluster analysis and relationships of drought tolerance attributes with YS and YP. The best cultivars should have a large principal component score in the first component (PC1, high grain yield) and a small principal component score in the first component (PC2, high stability) (Yan, 2001). Biplot diagram (Fig. 3 and Fig. 4) showed that in Kermanshah and Karaj, the first component score was higher and the second component score was lower for genotypes number 2, 3 and 4 in Kermanshah and genotypes number 2, 3 and 6 in Karaj. Thus, selection of these genotypes with high PC1 and low PC2 are suitable for both stress and non-stress conditions (Fig. 3 and Fig. 4). Farshadfar *et al.* (2012) and Dehghani *et al.* (2009) obtained similar results in multivariate analysis of drought tolerance in different crops. Yan *et al.*, 2000, suggested using biplot, three-dimensional plots and cluster analysis as the most appropriate techniques for analysis the multi-location trials data, for identifying drought tolerant genotypes and for elucidating the relationships of drought tolerance attributes with yield in non-stress and stress conditions.

On the other hand, in both locations, relationships of GMP, STI, K1STI, K2STI, MP, HM and YI with Yp and Ys were properly illustrated, and considering the direction of and the angles between vectors of these attributes, these indices were found to be more suitable. The relationships of DI, SNPI, RDI and YSI with YS and relationships among ATI, SSPI, TOL and SSI were also revealed by biplot.

CONCLUSION

In summary, significant genotype \times location interaction for white sugar yield in stress conditions, showed the influence of changes in the location environment on the white sugar yield performance of the genotypes evaluated. Based on biplot analysis, the indices GMP, STI, K1STI, K2STI, MP, HM and YI exhibited strong correlation with YS and YP. Therefore, they can discriminate drought tolerant genotypes with high white sugar yield at the same manner under stress and non-stress conditions (group A of Fernandez) in both locations (Kermanshah and Karaj). With regard to these indices and cluster analysis, in both locations the genotypes 2 and 3 were the most drought tolerant genotypes with higher stability than the other genotypes.

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