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Evaluation of sugar beet (*Beta vulgaris* L.) cultivars for some biochemical and agronomic traits under drought stress

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Abstract

This experiment aimed to evaluate quantitative and qualitative characteristics, tolerance to water deficit, and stability of white sugar in sugar beet cultivars. The experimental design was a split plot based on a randomized complete block design with three replications, where the irrigation levels (normal and water shortage) were assigned to the main plots and 18 sugar beet cultivars were assigned to the subplots. The result revealed that Palma achieved the maximum root and white sugar yield under normal and water deficit; furthermore, the highest indices of YP, YS, MP, STI, HM, YI, DI, REI and MRP belonged to the Palma cultivar. The results of the AMMI analysis based on white sugar yield showed that the additive effects of genotype and environment and the multiplicative effect of G×E accounted for 75.52, 17.05 and 6.76 % of the total data variance. Based on AMMI stability value, the Delta, Pars, Paya and Novodoro cultivars were recognized as stable varieties. Also, the first 2 significant components of the interaction effect (G×E) accounted for 99.12 % of interaction effects variation. Based on the biplot results of the first 2 significant components against white sugar yield, Azare and Merak were the appropriate cultivars. Finally, Based on the multi-trait stability index, Azara, Novodoro and Merak cultivars were selected as stable genotypes. In 2 years and 2 conditions, the Palma cultivar was identified as a cultivar with high yield and drought tolerance and low stability and the Merak cultivar was identified as a cultivar with white sugar yield and acceptable stability.

Keywords

AMMI analysis; drought; MTSI index; variety; sugar beet

Introduction

Sugar beet (*Beta vulgaris* L.) is an essential crop that is solely used for sugar production (1). The global sugar production in 2020-21 was around 181 million tons, with sugar beet accounting for approximately 26 % (2). The sugar industry uses an estimated 278 million tons of sugar beet annually (3).

Drought is the most significant abiotic stress that impacts crop production. Drought seriously threatens agriculture by reducing crop production (4). The increasing drought stress will result in a water shortage for over 50 % of global agricultural land by 2050 (5). Therefore, dealing with water stress has become a major challenge recently (6).

The economic yield of sugar beet is reduced due to molecular, biochemical, physiological, morphological and ecological disturbances under water deficit conditions (1, 7). Therefore, special attention should be paid to this issue so that the quantitative and qualitative of sugar beets are not negatively affected underwater stress conditions; one of the ways to deal with water stress in beets is the production and use of drought-tolerant cultivars (8).

Genetic diversity benefits crop improvement programs, particularly with stress-tolerant cultivars (9). The genetic potential of stress-tolerant beet varieties allows them to maintain growth under stress conditions (10). Various studies have investigated the response of different sugar beet genotypes to water deficit stress (11, 12).

Evaluation of the adaptability and stability of cultivar production under different environmental conditions is particularly important in breeding programs. Due to the different responses of the cultivars to environmental changes, their performance varies from one environment to another. Typically, each genotype has the maximum production potential in a particular environment; however, Identifying genotypes with acceptable performance in all environments can be achieved by evaluating the adaptability and stability of cultivars in various environments, Other statistical methods, such as AMMI and GGE-biplot, have also been widely used (13).

The AMMI method is a multivariate statistical method that justifies the cumulative effects of genotype, environment and G×E multiplicative effects and properly interprets G×E interaction (14). The GGE-biplot method graphically illustrates the G × E interaction to help breeders check genotypes' stability and combines stability with genotypes' performance in different environments. It also evaluates the relationship among environments to identify target environments in breeding programmes (13).

AMMI and GGE-biplot method to identify and introduce stable genotypes of sugar beet in the earlier studies have been used (10-12, 15, 16).

The study tested commercial and Iranian sugar beets for drought tolerance and identified the most productive and stable cultivar. AMMI, GGE and Multi-trait stability index (MTSI) methods were used to investigate white sugar yield stability in 2 years and conditions. The methods mentioned to identify stable sugar beet cultivars in the total of 2 conditions of normal irrigation and water shortage stress have rarely been done. Therefore, this research was designed and implemented to evaluate sugar beet cultivars for some biochemical and agronomic traits under drought stress.

Materials and Methods

Plant materials

This experiment examined 18 sugar beet (*Beta vulgaris* L.) cultivars (6 Iranian and 12 foreign) (Table. 1) under normal irrigation conditions and water shortage stress.

Table 1. The list of assessed sugar beet cultivars.

No.	Cultivar	Company	No.	Cultivar	Company
1	Laetitia	KWS	10	Shrif	SBSI
2	Isabella	KWS	11	Shokoofa	SBSI
3	Dorothea	Syngenta	12	Muraille	Desprez
4	Novodoro	Syngenta	13	Rosire	Desprez
5	Merak	Strube	14	Azare	Sesvdh
6	Ekbatan	SBSI	15	Delta	Sesvdh
7	Paya	SBSI	16	Mandarin	Maribo
8	Pars	SBSI	17	Palma	Maribo
9	Arya	SBSI	18	Flores	Maribo

Drought test

This experiment was carried out in the 2020-2021 crop year at the Agricultural Research and Natural Resources Center of the Western Azerbaijan Province, Miandoab Agricultural Research Station. The station is located 5 km northwest of the city in a geographical location of 46º90′E and 36º58′N and 1314 m above sea level. The experimental design consisted of a split plot with three replications based on a randomized complete block design. The main plots were designated to irrigation levels (non-stress and water shortage stress), while the sub-plots were designated to 18 sugar beet cultivars.

Surface plowing, discing, leveling, line drawing and planting row preparation (using a chipper) were part of the spring field preparation operations, distributing the necessary fertilizers was done based on soil analysis results; accordingly, 220 kg ha^{-1} urea fertilizer during 3 stages and 130 kg ha-1 triple superphosphate and 100 kg ha^{-1} potassium sulfate at the same time as the fall plowing was given to the field. In each plot, there were 3 planting rows with a length of 8 m; The spacing between the planting rows and plants in the row was 50 and 20 cm respectively. The planting was completed in late April.

Since sugar beet is sensitive to water stress at the germination and beginning of its growth, in the germination stage, until the complete establishment of the plant (8 -leaf stage), sufficient irrigation for all treatments was performed (once a week). Irrigation was carried out using a pressure system, a hose and a meter. In subsequent irrigations, to apply stress, irrigation was carried out after 120 mm evaporation from the class A evaporation pan, which in the normal state is approximately 60 mm (17). Theta probes measured soil moisture content changes (SM300 Royal Eijkelkamp, the Netherlands).

Measured traits

Proline Estimation

The concentration of proline was analyzed in fresh leaves by taking 100 mg from fully expanded leaves. To homogenize the samples, 10 mL of 3 % sulfosalicylic acid was used and then samples were filtered through filter paper. In a test tube, a combination of 2 mL of the supernatant, 2 mL of glacial acetic acid, and 2 mL of acid ninhydrin was prepared. The mixture was then heated at a temperature of 100 °C for an h and later transferred to an ice bath. In the next step, 4 mL of toluene was used to extract the mixtures and the mixture was vortexed for 15-20 sec. The toluene-containing chromophore was measured at 520 nm using a spectrophotometer with toluene as a reference. Proline content was measured using a standard curve and reported as μmol g^{-1} fresh weight (18).

Relative Water Content (RWC %)

To determine the fresh weight, 10 discs of sugar beet leaves with a diameter of 1 cm were distilled water at 25 °C for 4 h to determine the turgid weight (TW). To determine the dry weight (DW), the sample was placed in a hot-air oven at 80 °C for 24 h. To determine the relative water content (RWC), the following procedure was carried out:

$$\text{RWC \%} = (FW - DW)/(TW - DW) \times 100 \text{ ....(1)}$$

where fresh weight (FW); dry weight (DW); turgid weight (TW) (19).

Enzyme assay

Protein extraction was carried out using frozen sugar beet leaves. For this purpose, 0.5 g of frozen leaves were ground in liquid nitrogen. To extract protein, a 3 mL buffer was used that contained 50 mM K-phosphate buffer (with a pH of 7.0), 2 mM EDTA, 20 mM ascorbate, and 0.1 % (v/v) Triton X-100 for catalase (CAT). To measure superoxide dismutase (SOD) activity, a solution containing 100 mM K-phosphate buffer (pH 7.8), 0.1 mM EDTA, 14 mM 2-mercaptoethanol and 0.1 % (v/v) Triton X-100 was utilized. The mixture underwent centrifugation at 15000× g (4 °C) for 15 min. The CAT activity was measured at 240 nm using a spectrophotometer based on the rate of H_2O_2 usage in nmol per min per mg of protein. The SOD activity was measured by determining the enzyme's ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) into blue formazan. The results were recorded at 560 nm as nmol min-1 mg protein-1(20).

The activity of Peroxidase (POX) was measured in a 3 mL solution of Na-phosphate buffer (50 mM, pH 7.8), which contained 30 % H_2O_2 (4.51 μL), guaiacol (3.35 μL) and enzyme extract (50 μL), the decline in absorbance owing to the degradation of H_2O_2 molecules was monitored using a spectrophotometer (470 nm) for Peroxidase estimation (21).

Drought tolerance indicators

In our experiment, % of yield reduction (PYR), Mean productivity (MP) (22), Geometric mean productivity (GMP) (23), Stress tolerance index (STI) (23), Harmonicmeanindex (HM) (23), Yield index (YI) (24), Drought index (DI) (25), Tolerance index (TOL) (22), Stress susceptibility index (SSI) (26), Stress susceptibility percentage index (SSPI) (27), Yield stability index (YSI) (28), Relative drought index (RDI) (29), Relative efficiency index (REI) (30) and Mean relative performance (MRP) (30) were measured as drought tolerance indicators.

Measurement of quantitative and qualitative characteristics

Sugar beets were harvested at BGS 49, 180 days post-seeding. Plants were collected, counted and weighed. The roots were washed and pulp samples were prepared randomly.

SBSI's Sugar Technology Lab in Karaj analyzed pulp samples.

The frozen samples were thawed and blended with 177 mL of lead (II) hydroxide acetate for three minutes to achieve this.

The filtered solution is used in Betalysis to analyze sugar beet quality.

This system measured the % of sugar, as well as the content of sodium, alpha-amino nitrogen and potassium.

After determining the sugar nitrogen (alpha-amino N), sodium (Na) and potassium (K) contents, the other studied traits were estimated as follows:

$$MS = 0.0343(K + Na) + 0.094\,(N) - 0.31 \text{................(2)}$$

$$WSC = SC - (MS + 0.6) \text{..(3).}$$

$$ALC = (K + Na)/\,(N) \text{...(4)}$$

$$WSY = WSC \times RY \text{..(5)}$$

where SC is the sugar content, MS is the molasses sugar percentage, ALC is alkalinity and WSC is the white sugar content.

Statistical analysis

Bartlett's test was used to check the homogeneity of the variances of experimental errors. After confirming the homogeneity of error variance for each trait, a combined variance analysis was carried out. The analysis of variance (ANOVA) and the mean comparison (the least significant deference (LSD) at 5 % probability level) of the experiment were performed by the software SAS, version 9.4 (SAS Institute Inc., USA).

White Sugar beet yield depends on root weight and sugar content.

Therefore, because of the importance of sugar yield as the main criterion to distinguish sugar beet cultivars, multivariate stability analysis was performed graphically based on GGE biplot for this trait using GGE biplot software and AMMI analysis by GEA-R (v. 4.0, CIMMYT, Mexico). The AMMI stability value (ASV) was calculated based on the formula (31) as follows:

$$\text{ASV} = \sqrt{\frac{SSIPCA1}{SSIPCA2}(IPC1)^2 + (IPC2)^2} \text{ ....(6)}$$

Where SS is the sum of squares of IPCA1 and IPCA2, IPC1 and IPC2 are the scores of the ith genotype on the first and second principal components respectively.

To estimate the mean yield and simultaneous stability of RY, SY, WSY, SC, WSC, K+, Na+, N, MS and ECS, the MSTI index was computed based on equation 7 (32).

$$MSTI_i = \left[\sum_{j=1}^{f}\left((\gamma_{ij} - \gamma_j)^2\right)\right]^{0.5} \text{ ....(7)}$$

Where ? is the multi-trait stability index of genotype i, γ_{ij} is the score of genotype i in factor j, and γ_j is the score of the ideal genotype in factor j.

Results

The results of composite variance analysis of the data showed that in both normal irrigation conditions and water deficit stress, the difference between 18 sugar beet (*Beta vulgaris* L.) cultivars in terms of the examined traits were significant at the probability of 1 % (Table 2).

Results showed that the Palma cultivar produced the highest root yield under normal irrigation conditions (115.47 t.ha^{-1}) and drought stress (77.74 t.ha^{-1}). In comparison, the lowest root yield under normal (36.95 t.ha^{-1}) and drought stress (19.8 t.ha^{-1}) were recorded for the Sharif variety (Supplementary Table 1.).

Table 2. Combined analysis of variance of quantitative and qualitative characteristics studied in sugar beet under normal irrigation conditions and water deficit stress.

		Root yield		**Sugar content**		**Sugar yield**		**White Sugar content**		**White Sugar yield**		**Sugar extraction coefficient**	
SOV	**DF**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
Year (Y)	1	0.049^{ns}	0.019^{ns}	0.01^{ns}	0.39^{ns}	0.007^{ns}	0.25^{ns}	0.006^{ns}	2.55*	2.58^{ns}	0.94^{ns}	0.002^{ns}	30.09*
Ea	2	2.71	0.48	0.030	0.15	0.23	0.09	0.58	0.03	1.01	0.01	0.79	1.81
Genotype (G)	17	2226.72**	22029.9**	28.69**	16.91**	106.11**	60.68**	36.55**	19.09**	86.69**	51.47**	292.70**	140.77**
G×Y	17	5.36^{ns}	6.05	0.10^{ns}	0.48^{ns}	0.24^{ns}	1.74^{ns}	0.09^{ns}	1.23^{ns}	0.19^{ns}	1.26^{ns}	4.63^{ns}	39.57ns
Eb	68	30.14	45.84	2.08	3.55	5.17	3.02	1.10	5.03	3.10	0.9	20.91	25.54
CV (%)	-	7.10	12.96	7.49	9.27	15.70	16.27	6.83	12.73	14.59	10.17	5.54	5.81

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

Continues **Table 2 .** Combined analysis of variance of quantitative and qualitative characteristics studied in sugar beet under normal irrigation conditions and water deficit stress.

		Molasses sugar percentage		**Relative water content**		**Proline**		**SOD**		**POX**		**CAT**	
SOV	**DF**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**	**N**	**S**
Year (Y)	1	0.17^{ns}	0.09ns	0.23^{ns}	6.20ns	0.019ns	0.009^{ns}	5.43**	0.54ns	0.004ns	0.028^{ns}	0.00009^{ns}	0.00003^{ns}
Ea	2	0.026	0.017	0.46	37.24	0.001	0.009	0.009	0.18	0.009	0.005	0.00001	0.0002
Genotype (G)	17	4.13**	0.61**	170.54**	105.32**	0.40**	1.51**	5.16**	16.88**	0.10**	0.96**	0.0015**	0.078**
G×Y	17	0.06^{ns}	0.10**	0.162^{ns}	0.34ns	0.002^{ns}	0.007^{ns}	1.75ns	0.29ns	0.01ns	0.009^{ns}	0.00004^{ns}	0.00005ns
Eb	68	0.011	0.017	25.30	31.26	0.002	0.010	0.08	0.18	0.007	0.009	0.00002	0.0001
CV (%)	-	6.27	5.82	6.00	7.29	8.03	8.15	7.01	5.57	17.40	8.65	15.38	12.42

Note : **ns,*,** and ******, non-significant, significant at 5 and 1 % probability levels respectively. **N**= Normal **S**= Stress, **SOD** – superoxide dismutase, **CAT** – catalase, **POX** - Peroxidase.

The mean comparison of cultivars regarding sugar content showed that Isabella had the highest sugar content under normal irrigation conditions (21.6 %) and drought stress (22.4 %). Cultivar Sharif under normal irrigation conditions (14.7 %) and Shokoofa (17.2 %) underwater stress conditions achieved the least sugar content.

The highest sugar yield under non-stress and irrigation shortage conditions was assigned to Palma (21.51 t.ha^{-1}) and Merak cultivars (15.16 t ha-1) respectively. Under normal irrigation conditions (5.40 t.ha^{-1}) and water deficit (3.70 t ha-1), the Sharif cultivar produced the lowest sugar yield (Supplementary Table 1.).

In the present study, the Muraille cultivar under normal irrigation conditions (19.40 %) and the Novodoro cultivar underwater stress conditions (19.80 %) achieved the first rank in terms of white sugar content. The attribute's lowest value under normal irrigation and water stress conditions was assigned to Sharif (11.00 %) and Shokoofa (12.80 %) cultivars (Supplementary Table 1.).).

The highest white sugar yield under normal irrigation (18.63 t ha-1) and drought stress (14.11 t.ha^{-1}) were obtained from the Palma cultivar. The Sharif cultivar had the lowest under normal irrigation (4.00 t.ha^{-1}) and water stress (3.10 t.ha^{-1}) (Supplementary Table 1.).

The 2 cultivars, Mandarin and Muraille, under normal irrigation (90.60 and 91.20 %, respectively) conditions and the Palma cultivar under drought stress (98.79 %) had the maximum sugar extraction coefficient; the lowest one under normal irrigation and drought stress conditions was related to Shokoofa (70.70 %) and Aria (62.90 %) cultivars (Supplementary Table 1.).

Among the examined cultivars, Sharif, under normal irrigation conditions (5.1 %) and Mandarin, under drought stress conditions (2.74 %), produced the maximum amount of molasses. The lowest molasses content under normal irrigation conditions (1.80 %) and drought stress (1.90 %) was related to Dorothea (Supplementary Table 1.).

The results showed that the maximum relative water content under normal irrigation conditions was assigned to the Palma cultivar (90.68 %) and underwater deficit stress to the Dorothea cultivar (84.00 %). Sharif cultivar under normal irrigation conditions (74.40 %) and Pars cultivar under drought stress conditions (73.40 %) showed the lowest relative water content (Supplementary Table 1.).

Among the investigated cultivars, Laetitia (1.08 and 2.84 μmol g^{-1} FW) achieved the maximum, and Sharif (0.20 and 0.50 μmol g^{-1} FW) achieved the minimum proline content in non-stress and stress conditions (Supplementary Table 1.).

The highest amount of SOD enzyme activity was related to Shokoofa and Merak (6.61 and 10. nmol H_2O_2 mg^{-1} protein min^{-1}) cultivars under normal irrigation conditions and water deficit stress respectively. The lowest amount of SOD enzyme activity under normal irrigation conditions was recorded in cultivars Ekbatan (2.77nmol H_2O_2 mg^{-1} protein min^{-1}), Laetitia (2.87 nmol H_2O_2 mg^{-1} protein min^{-1}), and Palma (2.78 nmol H_2O_2 mg^{-1} protein min^{-1}); the lowest activity value of this enzyme underwater stress conditions was assigned to cultivar Ekbatan (2.77 nmol H_2O_2 mg^{-1} protein min^{-1}) (Supplementary Table 1.).

Two cultivars, Mandarin, under normal irrigation conditions (0.77 nmol H_2O_2 mg^{-1} protein min– 1) and Muraille, under stress conditions (1.90 nmol H_2O_2 mg–1 protein min^{-1}), obtained the highest POX enzyme activity, Azare cultivar, under normal irrigation conditions (0.31 nmol H_2O_2 mg^{-1} protein min^{-1}) and the Rosire cultivar underwater deficit stress conditions (0.69 nmol H_2O_2 mg–1 protein min^{-1}), showed the lowest activity of this enzyme (Supplementary Table 1.).

Cultivar Novodoro (0.05 nmol H_2O_2 mg^{-1} protein min^{-1}) and Rosire (0.52 nmol H_2O_2 mg^{-1} protein min^{-1}) showed the maximum content of CAT enzyme under normal irrigation conditions and water deficit stress respectively, compared to other cultivars. The lowest CAT enzyme activity was assigned to cultivar Laetitia (0.003 nmol H_2O_2 mg^{-1} protein min^{-1}) under normal irrigation conditions and to cultivar Shokoofa (0.010 nmol H_2O_2 mg^{-1} protein min^{-1}) underwater stress conditions (Supplementary Table 1.).

Drought tolerance indicators

The data variance analysis showed that the difference between the examined genotypes was significant regarding all the drought tolerance indices (Supplementary Table 2.).

The comparison of investigated cultivars regarding stress tolerance indices showed that the highest index of YP, YS, MP, STI, HM, YI, DI, REI and MRP belonged to the Palma cultivar. In contrast, The lowest values of YP, YS, MP, HM, YI, DI, REI and MRP indices were recorded for the Sharif variety. The maximum indices of PEER, TO L, SSI and SSI were recorded for the Muraille cultivar (Supplementary Table 2.).

In this study, the Aria cultivar had the lowest, and the Muraille cultivar had the highest PYR and TOL, SSI indices; furthermore, the Aria cultivar had the highest and the Muraille cultivar had the lowest YSI index (Supplementary Table 2.).

Based on cluster analysis results, 18 sugar beet cultivars and 16 estimated indicators were placed in three main groups. PYR, SSI, GMP, TOL and SSPI indices were placed in the first group, YSI and RDI indices in the second group, and YS, DI, HM, YP, STI, REI, YI, MP and MRP indices. Also, the results of cultivar grouping showed that cultivars Azare, Merak, Laetitia, Shokoofa and Aria were in the first group. The second group included Flores, Mandarin, Desire, Palma, Dorothea, Isabella, Muraille, Novodoro, Delta and Paya cultivars. Sharif, Ekbatan and Pars cultivars were in the third group. Pama cultivar positively correlated with YP, YS, MP, YI, STI, REI and MRP indices. On the other hand, the relationship between Sharif's figure and the mentioned indicators was negative (Fig. 1).

AMMI and GGE

The results of the variance analysis table based on white sugar yield showed that the difference between genotypes and environments (Additive effects) and the interaction between them (Multiplicative effects) was significant at the 1 % probability level. Genotype and environment explained 75.52 % and 17.05 % of the total data variations (Table 3.).

In this investigation, the genotype × environment interaction was divided into 2 factors or components: IPCA1 (AMMI 1) and IPCA2 (AMMI 2) and both first components were significant at a probability level of 1 %.

The contribution of the first component (IPCA1) and the second (IPCA2) was 6.02 and 0.62 % of the sum of total squares and 89.05 and 17.9 % of the sum of squares of the interaction effects of genotype in the environment respectively.

Also, 2 factors or components, IPCA1 (AMMI 1) and IPCA2 (AMMI 2) explained a total of 98.22 % of the total genotype × environment interaction variation (Table 3.). The white sugar yield of genotypes in 4 experimental environments, the values of IPCA1, IPCA, 2 components and ASV parameters are listed in Supplementary Table 1.. According to the results, the highest white sugar yield, with an average of 16.73 and 13.68 t ha-1, was achieved from Palma and Merak cultivars respectively. The lowest white sugar yield was also recorded, with an average of 3.60 t ha-1 for the Sharif cultivar.

In this study, Delta, Pars, Paya and Novodoro cultivars showed the lowest ASV values with 0.003, 0.004, 0.019 and 0.046 respectively and were considered stable genotypes regarding white sugar yield, While Muraille and Ekbatan cultivars had the highest ASV values of 2.41 and 1.76 respectively and were recognized as unstable genotypes (Table 4.).

The biplot of sugar yield was used to examine genotype adaptation and yield stability in the regions studied. A genotype with higher white sugar yield and lower genotype × environment interaction is more favorable.

Based on Fig. 2, Delta and Paya cultivars had IPCA1 values close to zero compared with other genotypes and had appropriate yield stability and general compatibility.

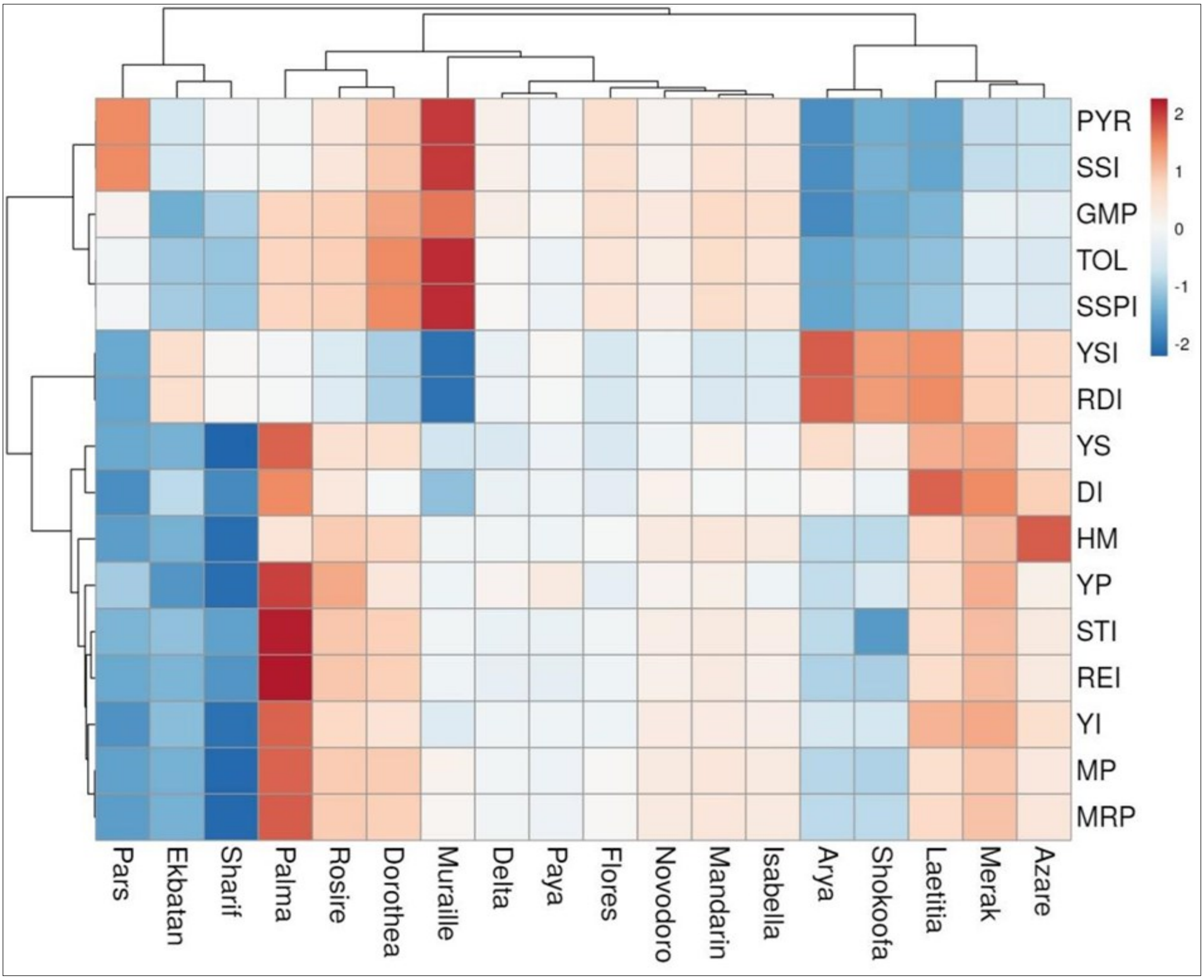

Fig. 1. Clusters analysis based on 18 genotypes and 16 indicators of drought tolerance in two years.

Table 3. Analysis of variance for sugar beet genotypes' white sugar yield using AMMI model for genotype x environment interaction.

Source	df	Sum of squares	Mean of squares	Relative variance (%)
Total	215	2920.5	13.58	
Treatments	71	2901	40.86**	
Genotypes	17	2205.7	129.75**	75.52
Environments	3	498	166**	17.05
Block	8	1.4	0.17^{ns}	0.05
Interactions	51	197.3	3.87**	6.76
IPCA	19	175.8	9.25**	6.02
IPCA	17	18.1	1.07**	0.62
Residuals	15	3.4	0.23^{ns}	
Error	136	18.1	0.13	

Note: **ns**, *****, ******: nonsignificant and significant at 5 % and 1 % probability levels respectively.

Table 4. Mean white sugar yield and AMMI stability values of sugar beet studied genotypes.

Genotypes	GM	IPC1	IPC2	ASV
Aria	7.62	-0.97	-0.23	1.189
Ekbatan	6.07	-0.77	1.03	1.764
Laetitia	12.67	-0.79	0.40	0.913
Merak	13.68	-0.27	-0.58	0.418
Flores	10.73	0.34	-0.04	0.139
Mandarin	12.07	0.44	0.15	0.257
Rosire	13.53	0.61	-0.49	0.693
Palma	16.37	0.53	0.26	0.399
Azare	11.92	-0.36	-0.09	0.164
Dorothea	13.50	0.98	0.19	1.197
Isabella	11.77	0.38	-0.46	0.382
Muraille	11.12	1.40	0.23	2.419
Novodoro	11.74	0.18	0.08	0.046
Delta	10.27	0.04	0.04	0.003
Pars	5.61	-0.04	0.05	0.004
Paya	10.06	-0.12	0.05	0.019
Sharif	3.60	-0.74	-0.16	0.696
Shokoofa	7.59	-0.83	-0.42	1.015

The Aria and Dorothea cultivars were identified as unstable genotypes due to their high positive and negative values of IPCA1. The bi-plot of the white sugar yield of genotypes versus IPCA2 values (Fig. 3) showed that the cultivars Delta and Flores had IPCA2 values close to zero, while Ekbatan cultivars had the highest IPCA2 value.

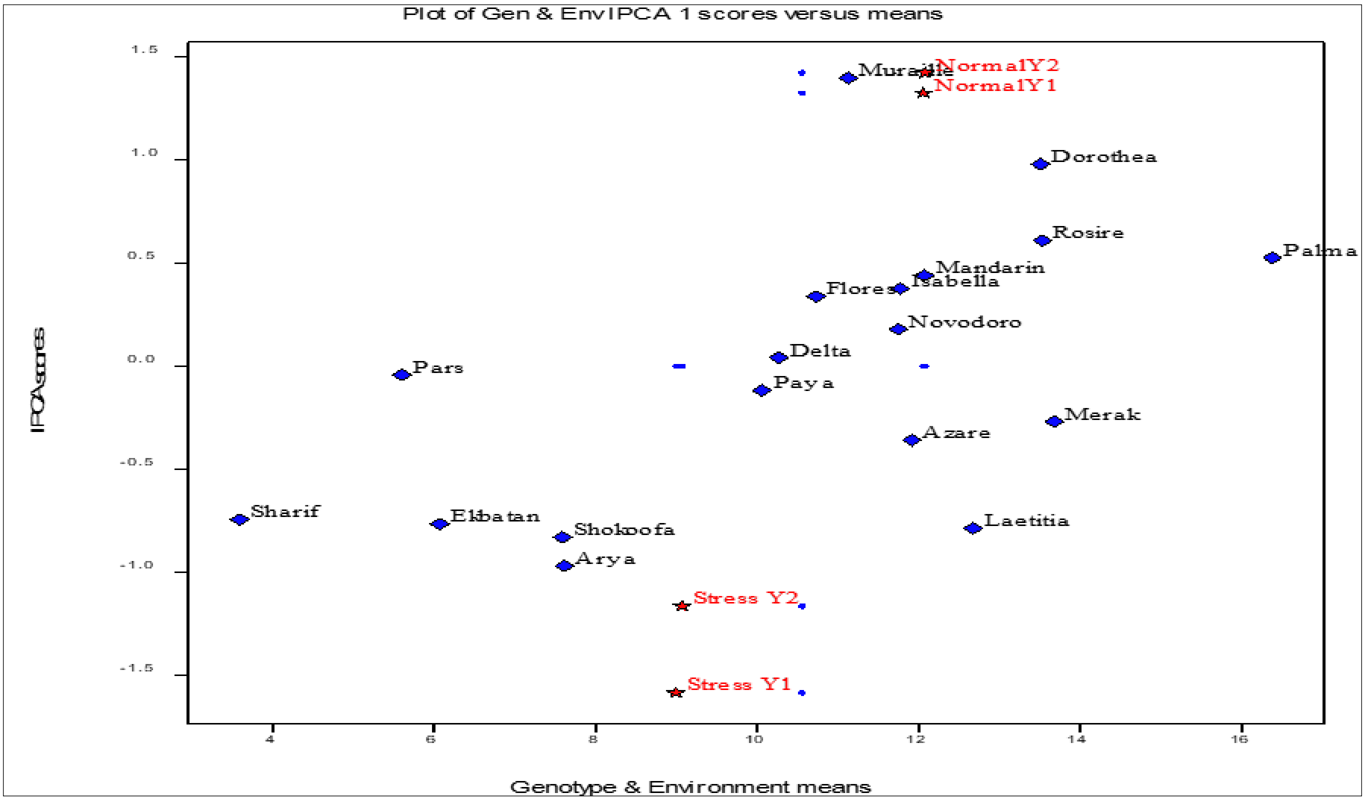

Fig. 2. Scatter plot for genotypes and environments based on white sugar yield means and a first principal component.

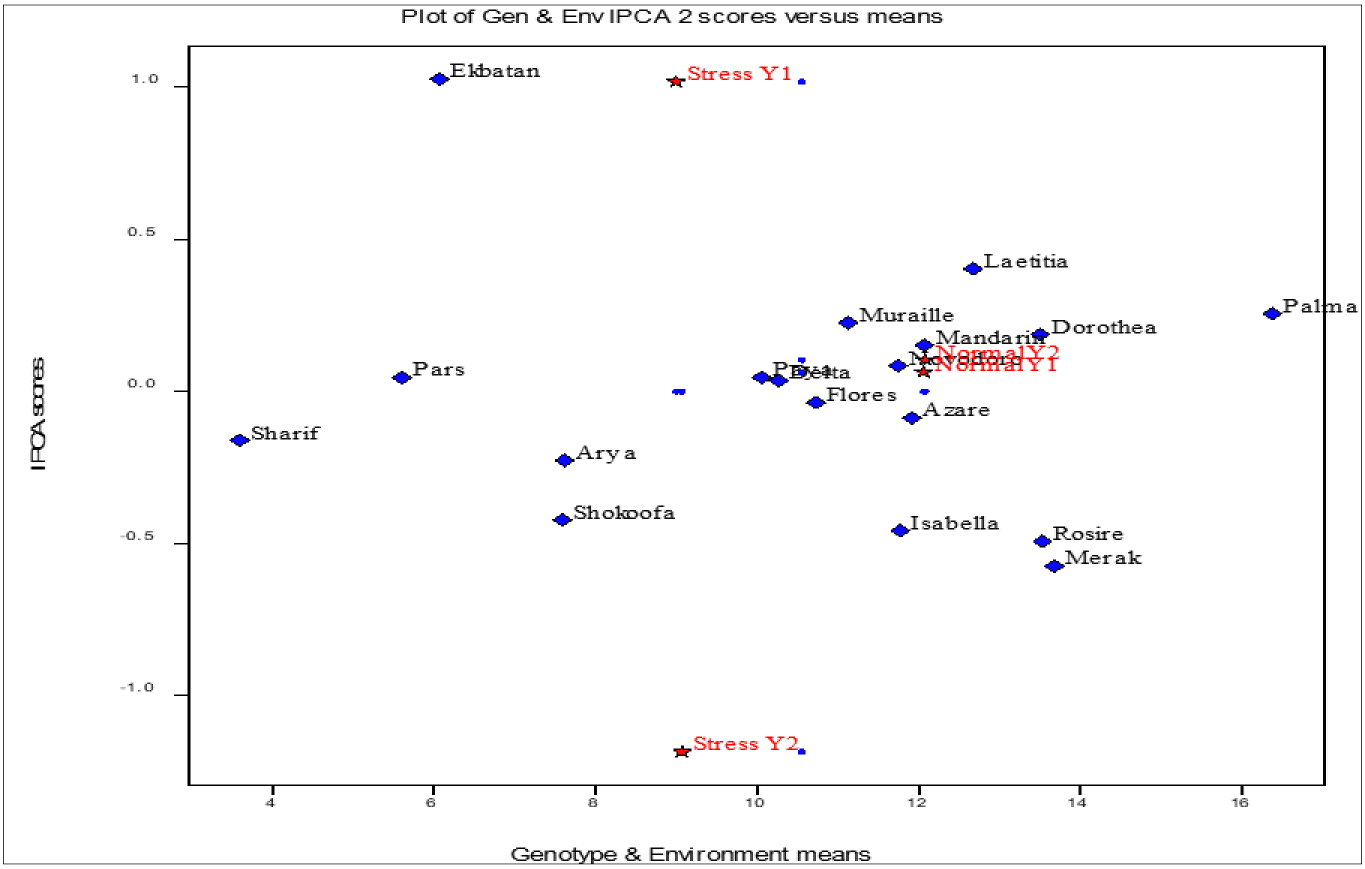

Fig. 3. Scatter plot for genotypes and environments based on white sugar yield means and a second principal component.

The polygonal display (Convex hull) resulting from GGE analysis of sugar beet cultivars in four environments is shown in Fig. 4. This diagram categorizes genotypes and environments based on the values of the first and second principal components. The cultivars with the lowest interaction effect are indicated by low values for the first and second components. This diagram (the first and second principal components of the interaction effect) accounted for 99.12 % of the variance of the interaction effect between genotype and environment. In this diagram, genotypes near a place have specific compatibility, while those near the origin have general compatibility. In the current research, the Delta Cultivar was the closest genotype to the origin of coordinates and was identified as the most

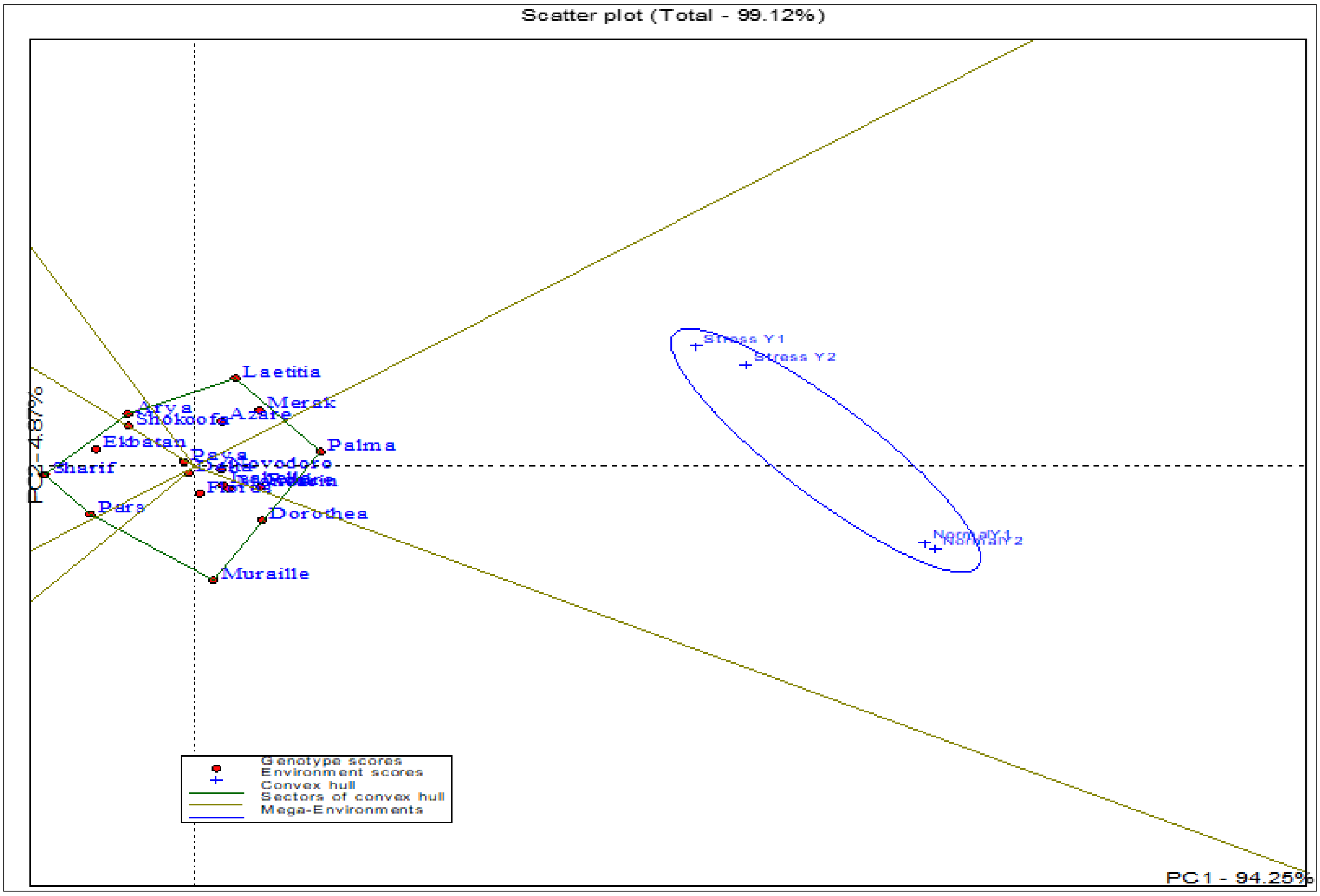

Fig. 4. Polygon of GGE biplot method to identify suitable genotypes for each environment.

stable genotype. Among the studied cultivars, the Palma cultivar showed high private adaptation for both years and conditions.

Multi-trait stability index (MTSI)

Three factors were identified based on the results of the factor analysis (based on principal components analyses), which explained 72.55 % of the total data variation. The first factor obtained 27.90 % of the total data variance with an eigenvalue of 3.34; this factor showed a positive and significant relationship with the traits of sugar yield, root yield, white sugar yield and relative water content. The second factor, which had a positive relationship with white sugar content, sugar extraction percentage, and sugar content and a negative relationship with molasses sugar %, had an eigenvalue of 2.84 and explained 23.70 % of the total variation in the data. The third factor, with an eigenvalue of 2.51, included 20.94 % of all data variations and showed a positive relationship with proline content, superoxide dismutase and glutathione peroxidase activity (Table 5.).

The MTSI stability index was calculated using factor scores. In Fig. 5, the experimental genotypes have been sorted based on their MTSI values. Genotypes with higher values of the MTSI index are favorable genotypes marked with red in the middle of the circle (20 % selection intensity).

Azara, Novodoro and Merak cultivars were selected as ideal genotypes in our research. The MTSI index is ranked from highest to lowest value. genotypes are placed in the outermost circuit to the center of the Figure respectively. F - Sharif had the lowest stability index score, showing poor stability and mean sugar yield in different environmental conditions (Fig. 5).

Discussion

The results revealed that the difference between cultivars

Table 5. Eigenvalues, relative and cumulative variance, and factor coefficients after varimax rotation in factor analysis based on principal component analysis.

	IPC1	IPC2	IPC3
Sugar yield	0.96	0.21	-0.08
Root yield	0.95	0.00	-0.22
White Sugar yield	0.90	0.38	-0.11
Relative water content	0.60	-0.12	-0.36
White Sugar content	0.09	0.95	0.25
Sugar extraction coefficient	-0.03	0.86	-0.14
Sugar content	0.16	0.71	0.47
Molasses sugar percentage	-0.36	-0.65	-0.28
Proline	0.03	0.04	0.82
Superoxide dismutase	-0.28	0.05	0.80
Peroxidase	-0.29	0.15	0.72
Catalase	-0.12	0.18	0.31
Eigenvalue	3.34	2.84	2.51
Relative Variance	27.90	23.70	20.94
Cumulative variance	27.90	51.61	72.55

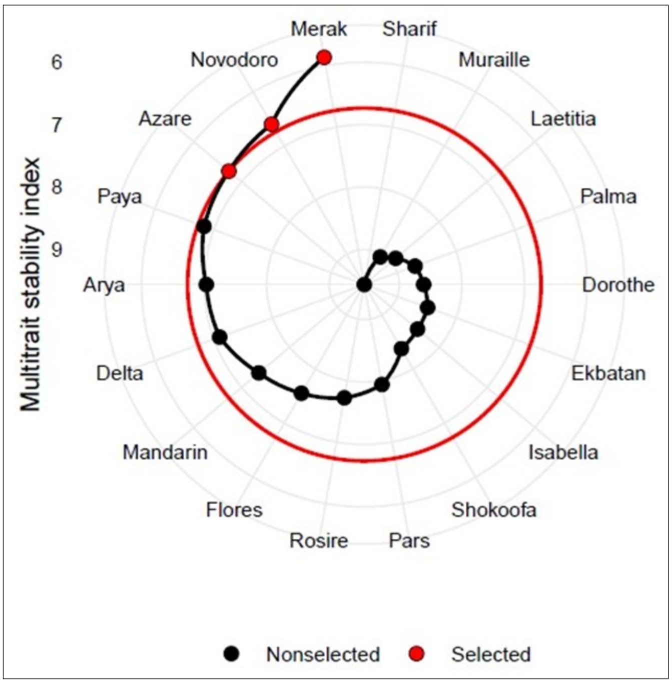

Fig. 5. Genotype ranking and selected genotypes based on MTSI index.

in normal irrigation and water deficit stress was significant regarding traits under investigation.

The existence of genetic diversity provides the possibility of selection between cultivars in terms of favorable traits; the presence of genetic diversity between sugar beet cultivars under normal irrigation and water deficit stress conditions has been documented in other studies (11, 12). Therefore, breeding programs aim to enhance sugar beet traits and reduce water usage (33).

In this study, compared to normal irrigation conditions, water stress conditions reduced root yield, sugar yield, white sugar yield and relative water content. In contrast, sugar content, white sugar content, sugar extraction coefficient, molasses sugar %, proline content, SOD, POX and SOD enzyme activity were higher underwater deficit stress conditions versus normal irrigation conditions. Abiotic stresses, including drought, cause significant morphological changes in sugar beet. Drought reduces vegetative growth, accelerates wilting of leaves, reduces stomatal conductance and the relative content of leaf water and water deficit by destroying photosynthetic pigments, reducing the speed and amount of photosynthesis. Under irrigation deficit, membrane lipid peroxidation, cell damage and compatible solute accumulation will increase, and eventually, sugar yield will decrease (34- 42).

Among the examined cultivars, Palma had the highest root yield, white sugar yield and sugar extraction coefficient in 2 environmental conditions; also, under normal irrigation conditions, the highest sugar yield and relative leaf water content were related to the Palma cultivar.

As demonstrated by the results, the Merak cultivar had the highest sugar yield, relative leaf water content and superoxide dismutase enzyme activity underwater stress conditions. The Mandarin cultivar under normal irrigation conditions and the Muraille cultivar underwater stress conditions showed the highest POX enzyme activity. It seems that the high sugar yield in the Merak variety under the condition of low water stress is related to the high leaf water content and superoxide dismutase enzyme activity. The 2 mentioned characteristics increase the tolerance to stress in the plant. In sugar beet, yield reduction underwater stress conditions can be due to changes in RWC, leaf water potential (43), leaf growth reduction and carbon dioxide assimilation (44). The first reaction of sugar beet to the water deficit is the closing of the stomata, which reduces the relative water content in the leaves, which leads to the disruption of photosynthesis and the reduction of economic yield (45).

ROS accumulation, which includes superoxide, hydroxyl radical, hydrogen peroxide and singlet oxygen, occurs in plants under stress conditions; oxidative stress is caused by ROS molecules that elicit irreversible damage to nucleic acids, proteins, pigments and lipids (46). Enzymatic and non-enzymatic antioxidant systems in plants are responsible for scavenging reactive oxygen species, Catalase, peroxidase and superoxide dismutase can be mentioned among the enzyme types of the antioxidant system (47). Previous studies on sugar beet have proven the increase in the activity of SOD and CAT enzymes due to water deficit stress (41, 48). Sugar beet cultivars with high lipid peroxidation underwater-stress conditions have low levels of antioxidant enzyme activity. In contrast, drought-resistant cultivars had elevated levels of antioxidant enzyme activity (CAT, SOD and POX) (35).

Moreover, the highest proline level was recorded for the Laetitia variety under both environmental conditions. *Proline* is a substance that accumulates in the cytosol and plays a role in regulating the osmotic pressure in the cytoplasm (49). It has been reported that drought stress increases the proline content in all organs of sugar beet, including the leaves (37). In addition, sugar beet cultivars resistant to salt were found to have higher levels of proline than sensitive cultivars (38).

Drought Stress Tolerance Indices

The results showed that the highest indices of YP, YS, MP, STI, HM, YI, DI, REI and MRP belonged to the Palma cultivar; the highness of the mentioned indices indicates the tolerance of a cultivar to drought stress; the Sharif cultivar was archived with the lowest YP, YS, MP, HM, YI, DI, REI and MRP values, which indicated its sensitivity to water stress.

The potential yield of resistant genotypes cannot be determined solely by irrigation deficit.

Tolerated genotypes are selected based on stress tolerance and susceptibility indices measured under normal and drought conditions. One of the reliable criteria for selecting drought-tolerant genotypes is yield (50).

Regarding drought tolerance indicators, Flores, Mandarin, Desire, Palma, Dorothea, Isabella, Muraille, Novodoro, Delta and Paya were placed in the same cluster. The cultivars of this group showed a positive relationship with drought stress tolerance indices such as YP, MP, YS, HM, STI, YI, DI, REI and MRP.

White sugar yield stability indicators

Based on AMMI analysis, Delta, Pars, Paya and Novodoro cultivars had the lowest ASV value and were recognized as stable genotypes in 2 years and 2 conditions. Azare, Palma, and Merak had lower ASV values among cultivars with high white sugar yield.

AMMI method analysis has been used to estimate the interaction effect of genotype with the environment and the amount of stability of genotypes in different regions and years in sugar beet in previous studies (12, 16, 33).

Based on the biplot results of the first 2 significant components against white sugar yield, cultivar Azare was one of the appropriate cultivars regarding stability and white sugar yield.

The results of the GGE graph showed that Delta, Paya and Novodoro cultivars had a high general adaptability for both environmental conditions in both years due to their proximity to the origin of coordinates. While Laetitia and Palma's cultivars showed high private adaptation for water stress conditions and Palma and Muraille cultivars for normal irrigation conditions. In this research, the Sharif cultivar was not suitable for any conditions in 2 years.

The GGE biplots were used to identify the mega-environments and the cultivars that yield the best in each. When a cultivar performs best in only one or a few specific environments, it is considered to have a narrow adaptation. Choosing a cultivar with a narrow adaptation for a specific mega-environment is recommended (15). based on the analysis of the genotype–environment interaction, it was suggested that it is impossible to identify a specific cultivar or group of cultivars that can adapt well to the environmental conditions of a temperate climate (15). They have identified cultivars suited explicitly to specific locations categorized as mega-environments.

It was identified that the RM5 genotype as superior due to its high white sugar yield and low values of the first 2 components using biplot analysis (16). Reports are on the stability of 36 modern sugar beet cultivars under Polish environmental conditions; they did not find a cultivar or cultivars with a wide adaptation to the environmental conditions (15).

In a study using biplot method, 7233-P.29 (G38) and C CMS (G49) lines and 2(6)×C (G27) and 5×C (G33) hybrids were identified as stable genotypes with acceptable yield (11).

In the calculation of Multi trait stability index, all the quantitative and qualitative characteristics measured in 2 years and 2 conditions were used to estimate the stability of cultivars Based on this, Azara, Novodoro and Merak cultivars were selected as stable genotypes.

According to plant breeders can effectively use MTSI to identify superior genotypes for multiple traits based on multi-environment data. The MTSI index is a valuable tool for breeders to identify appropriate genotypes (16). Recently, this index has been used to identify stable genotypes of sugar beet (12, 16).

Conclusion

The most important economic trait of sugar beet is the yield of white sugar; this characteristic results from all this product's quantitative and qualitative characteristics. In the present study, the Palma cultivar had the highest white sugar yield in both conditions. Merak cultivar was also in third place under normal irrigation conditions and second place underwater stress conditions regarding white sugar yield. Regarding drought tolerance indices, the Palma variables showed acceptable tolerance compared to other cultivars; however, the Merak cultivar had good performance stability among the cultivars and based on all quantitative and qualitative characteristics examined, it was considered a stable genotype. Therefore, depending on the purpose and conditions of the cultivation test, one of these 2 varieties is recommended.

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Authors' contributions

Azad Ebrahimi, Rahim Mohammadian and Heydar Azizi wrote the manuscript with support from Esmail Nabizadeh

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Supplementary Table 1. Results of mean comparison of quantitative and qualitative characteristics studied in sugar beet under normal irrigation conditions and water deficit stress

Supplementary Table 2. Comparison results of mean white sugar yield under normal and water deficit conditions and stress tolerance and sensitivity indices in sugar beet genotypes

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