



RESEARCH ARTICLE

Evaluation of enzymatic and agronomic characteristics and yield stability of sugar beet (*Beta vulgaris* L.) genotypes under normal conditions and water deficit stress

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ARTICLE HISTORY

Received: 24 November 2023

Accepted: 25 August 2024

Available online

Version 1.0 : 06 January 2025

Version 2.0 : 06 January 2025



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

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CITE THIS ARTICLE

Najari Z, Nabizadeh E, Azizi H, Mohammadian R, Hamze H. Evaluation of enzymatic and agronomic characteristics and yield stability of sugar beet (*Beta vulgaris* L.) genotypes under normal conditions and water deficit stress. *Plant Science Today*. 2025; 12(1): 1-14. <https://doi.org/10.14719/pst.3123>

Abstract

This study evaluated sugar beet (*Beta vulgaris* L.) genotypes for quantitative and qualitative characteristics under normal and water deficit conditions. The experimental design was a split plot based on randomized complete blocks with three replications. Irrigation treatments (normal, water deficit stress) were assigned to the main plots, and 18 sugar beet genotypes were assigned to the subplots. The results showed that irrigation deficit increased the content of proline, glutathione peroxidase (GPX), superoxide dismutase (SOD), and peroxidase (POX) by 57.69%, 54.78%, 50.89%, and 55.56%, and decreased root and white sugar yield by 21.35% and 11.93%, compared with normal irrigation, respectively. Under normal irrigation, genotype F-20734 produced the highest yield of white sugar, followed by genotype F-20851. However, genotype F-20851 had the maximum white sugar yield under water deficit. The results of AMMI analysis based on root yield indicate that 62.12%, 12.70%, and 2.21% of the total data variance were accounted for by the additive effects of genotype and environment and the multiplicative effect of G×E, respectively. Based on the AMMI stability value (ASV), the F-20814 genotype was recognized as a stable variety with acceptable root yield in four environments. Based on the AMMI stability value (ASV) and GGE analysis, genotype F-20814 achieved acceptable root yield and yield stability compared to other genotypes. According to the MTSI index, genotypes F-20734 and F-20851 exhibited stability across all traits and environments studied. Considering all the indicators, the F-20851 genotype can be suitable for cultivation in areas where plants face different periods and intensities of water shortage stress.

Keywords

AMMI analysis; MTSI index; stability; water deficit

Introduction

Sugar beet (*Beta vulgaris* L.) is an essential biennial herbaceous crop that can tolerate low temperatures and salinity (1). Sugar is a commonly consumed product often cultivated in semiarid regions worldwide. The impact of drought on crops is a global concern, particularly in areas with low rainfall (2). Selection for drought tolerance is essential for mitigating water stress effects on crop yield (3). Investigation of biochemical and physiological response mechanisms under drought stress conditions provides a theoretical basis for selecting and breeding drought-resistant sugar beet varie-

ties and screening drought tolerance indicators. The moisture level present in the soil has a direct impact on the physical and biological attributes of plants (4).

Much research about screening phenotypic drought tolerance indicators has been conducted worldwide (5, 6). In some countries, the yield gap between measured and actual yield in farmers' fields exceeds 30% (7). It is crucial to note that the lack of yield stability is the main consequence of GEI. It is essential to understand that as the genetic potential of crop yield increases, the plant's need for agricultural resources also increases. This heightened demand puts the plant at risk of biotic and abiotic stresses. High yield potential must be matched with increased tolerance to unfavorable environmental factors to maintain yield stability despite increased potential (8). GEI refers to the different responses of genotypes in various environments. Researchers in crop breeding find it challenging to evaluate and select superior genotypes due to genotype-by-environment interaction. Therefore, developing and releasing new high-yielding cultivars is crucial for breeders. Meanwhile, quantitative traits such as yield can be significantly impacted by GEI, having economic and agronomic importance. Breeders must evaluate and release genotypes with superior yield and adaptability in the target environment. GEI may offer opportunities to select genotypes that interact positively with specific locations (specific adaptation) or perform well in most environments (general adaptation). This can be accomplished by growing different genotypes in various environments, recording their responses, and selecting the superior and stable genotype.

Previous research utilized the AMMI and GGE-biplot methods to identify stable sugar beet genotypes (8-12).

Table 2. Physical and chemical analysis of the experimental soil

Soil texture	K	P	Ca	NH4	NO3	Mg	N(total)	O.C	T.N.V	pH	EC
	PPM					%			Ds/m		
Silt loam	255	8.05	8	13.15	19.55	3.5	0.13	0.78	8	8	2.14

The MTSI model was recently introduced to identify high-yield and stable genotypes (13). MTSI is calculated based on the distance value from the ideal genotype and by the factor analysis method. This criterion allows the selection of superior genotypes based on positive selection for desirable traits and negative selection for unfavorable characteristics. Therefore, with this index, the stability of genotypes can be measured based on several factors in different environments. According to plant breeders, they can effectively use MTSI to identify superior genotypes for multiple traits based on multi-environment data. Recently, this index has been used to identify stable genotypes of sugar beet (8, 12).

Water deficit stress is one of the most critical threats threatening the economic performance of sugar beet in the world and Iran. Identifying and introducing cultivars that are resistant to water stress and have high yield and yield stability is one of the most important strategies to deal with water stress. Therefore, this research

was designed and implemented to evaluate sugar beet cultivars for some agronomic traits and yield stability under different irrigation conditions.

Materials and Methods

Plant materials

This experiment examined 18 sugar beet (*Beta vulgaris* L.) cultivars (six Iranian cultivars: Pars, Paya, Sharif, Aria, Shkofa, and Ekbatan and 12 foreign) (Table 1) under normal irrigation and water shortage stress.

Table 1. The list of assessed sugar beet cultivars

No.	Genotype	No.	Genotype
1	Pars	10	F-20716
2	F-20722	11	Sharif
3	F-20815	12	F-20772
4	F-20817	13	Arya
5	F-20747	14	Shokoofa
6	Paya	15	F-20814
7	F-20723	16	Ekbatan
8	F-20851	17	F-20866
9	F-20734	18	F-20758

Experimental designing and applying treatments

The experiment was conducted at the Miandoab Agricultural Research Station in Western Azerbaijan, Iran during the 2020-2021 crop year. The station is 5 km northwest of the city at 46°90'E and 36°58'N, 1314 meters above sea level. Physical and chemical analysis of the experimental soil is listed in Table 2.

The experimental design was a split plot with three replications based on a randomized complete block design. The main plots consisted of two irrigation levels: non-stress and water shortage stress. In addition, the sub-plots were designated for 18 sugar beet genotypes. To prepare the seedbed in spring, operations such as plowing, disking, leveling, and drawing lines for planting rows were conducted.

The amount of fertilizer applied was determined based on the soil test results (Table 2). Based on the analysis, the experimental land was fertilized with 200 kg of urea, 130 kg triple superphosphate, and 100 kg of potassium sulfate fertilizers annually. The urea fertilizer was applied in three stages after the planting process. Triple superphosphate and potassium sulfate were applied at the same time as the fall plowing.

Each experimental unit consisted of three rows with a length of 8 m. The distance between the rows was 0.5 m, and the space between the plants in each row was 0.2 m.

The planting was completed in late April in both years of the experiment. During the growth period, customary operations such as irrigation, fertilization, cultivation, weed, pest, and disease control were implemented as needed. During the germination stage until the plant's complete establishment (8-leaf stage), sugar beet was irrigated once a week to prevent water stress since it is sensitive to it. A pressure system, a hose, and a meter were used for irrigation. In subsequent irrigations, stress treatment was induced by waiting until 120 mm of evaporation from the class A evaporation pan, which was twice the normal state of 60 mm (14). Theta probes, specifically the Royal Eijkelkamp (the Netherlands) SM300, were used to measure changes in soil moisture content.

Measured traits

Proline estimation

In this experiment, the Bates et al. (15) method was used to measure the proline content of leaves. The proline concentration in fully expanded leaves was analyzed by taking 100 mg leaf samples. They were mixed with 3% sulfosalicylic acid and filtered to homogenize the samples. A mixture of supernatant, glacial acetic acid, and acid ninhydrin was heated, transferred to an ice bath, and then mixed with toluene. The 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 $\mu\text{mol g}^{-1}$ fresh weight.

Enzyme assay

To extract protein from frozen sugar beet leaves, 0.5 g of these leaves were ground in liquid nitrogen. Then, a 3 mL buffer containing 50 mM K-phosphate buffer (pH 7.0), 2 mM EDTA, and 20 mM ascorbate was used.

To measure the activity of superoxide dismutase (SOD), a solution consisting of 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 used. The mixture was centrifuged at $15,000 \times g$ (4°C) for 15 minutes.

To measure the activity of peroxidase (POX), a 3 mL solution of Na-phosphate buffer (50 mM, pH 7.8) was used. The solution contained 30% H_2O_2 (4.51 μL), guaiacol (3.35 μL) and enzyme extract (50 μL). The degradation of H_2O_2 molecules was then monitored using a spectrophotometer (at 470 nm) to estimate the activity of peroxidase (16).

Glutathione peroxidase (GPX) was measured using the spectrophotometric method. The reaction mixture (250 μL) contained 2 mM glutathione, 1 mM NADPH, 1 mM EDTA, 2 mM t-butyl hydroperoxide, 0.5 U glutathione reductase, 10 μg of extracted proteins in 100 mM sodium phosphate buffer at pH 7.0. The NADPH oxidation rate was measured at 340 nm for 15 minutes. To investigate the impact of peroxidase, inhibitors of GPX, 1 mM azide, 10 mM mercaptosuccinic acid (MSA), or 100 μM ethacrynic acid (EA) were added to the reaction mixture (16).

Root and white sugar yield

Sugar beets were harvested at BGS 49 and sent to SBSI's Sugar Technology Lab in Karaj for analysis. Pulp samples

were prepared, thawed, blended with lead (II) hydroxide acetate, and filtered in Betalysis to analyze sugar beet quality. The system measured sugar, sodium (Na), alpha-amino nitrogen (N), and potassium (K). After determining these contents, other traits were estimated as follows:

$$MS = 0.0343(K + Na) + 0.094(N) - 0.31 \dots(1)$$

$$WSC = SC - (MS + 0.6) \dots\dots(2)$$

$$ALC = (K + Na) / (N) \dots\dots\dots(3)$$

$$WSY = WSC \times RY \dots\dots\dots(4)$$

Where, SC = sugar content, MS = molasses sugar %, ALC = alkalinity, and WSC = white sugar content.

Statistical analysis

Bartlett's test was utilized to verify if the experimental errors' variances were homogenous. A combined variance analysis was conducted after verifying the homogeneity of error variance for each trait. The SAS version 9.4 software (SAS Institute Inc., USA) was used to analyze the experiment's variance (ANOVA) and mean comparison (LSD).

Because of the importance of root yield as the main criterion to distinguish sugar beet cultivars in this study, the stability of sugar beet root yield was determined using AMMI analysis, GGE biplot, and MSTI index available in the metan package of R software.

The AMMI stability value (ASV) was calculated using the formula described by Purchase et al. (17):

$$ASV = \sqrt{\frac{SSIPCA1}{SSIPCA2}} \left((IPC1)^2 + (IPC2)^2 \right) \dots\dots(5)$$

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

In this study, the MSTI index was used to estimate the mean yield and simultaneous stability of proline, GPX, POX, SOD, ESC (extraction sugar coefficient), root yield, and white sugar yield based on Eqn. 6 (13).

$$MSTI_i = \left[\sum_{j=1}^f ((\gamma_{ij} - \gamma_j)^2) \right]^{0.5} \dots\dots\dots(6)$$

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

Results

The combined data variance analysis showed that the year significantly affected POX content and root yield ($P < 0.01$). The difference between irrigation treatments was significant ($P < 0.01$) regarding all investigated traits except ESC. The interaction of year and irrigation only affected the yield of the root ($P < 0.01$). The effect of genotype and the interaction between genotype and environment significantly ($P < 0.01$) influenced all traits examined in this re-

Table 3. Combined analysis of variance of the studied traits in two years and normal and water deficit conditions

SOV	Df	Mean of squares						
		Proline	GPX	SOD	POX	Root Yield	ESC	White Sugar Yield
Year (Y)	1	0.01 ^{ns}	0.001 ^{ns}	0.009 ^{**}	4771.52 ^{ns}	16.20 ^{**}	45.19 ^{ns}	3.17 ^{ns}
Y× Replication	4	0.005	0.001	0.001	2454.16	0.03	31.53	0.79
Irrigation (I)	1	30.21 ^{**}	21.84 ^{**}	0.14 ^{**}	132891.5 ^{**}	294.72 ^{**}	53.52 ^{ns}	80.24 ^{**}
Y×I	1	0.001 ^{ns}	0.004 ^{ns}	0.003 ^{ns}	3529.75 ^{ns}	1323 ^{**}	28.50 ^{ns}	2.25 ^{ns}
Error _a	4	0.017	0.004	0.0001	2616.10	0.07	27.52	0.66
Genotype (G)	17	1.19 ^{**}	1.82 ^{**}	0.015 ^{**}	9551.12 ^{**}	3472.13 ^{**}	185.71 ^{**}	139.28 ^{**}
Y×G	17	0.013 ^{ns}	0.01 ^{**}	0.002 ^{**}	2700.19 ^{ns}	1.05 ^{ns}	29.18 ^{ns}	0.79 ^{ns}
I×G	17	0.34 ^{**}	1.12 ^{**}	0.011 ^{**}	6422.63 ^{**}	348.09 ^{**}	122.35 ^{**}	15.06 ^{**}
Y×I×G	17	0.020 ^{**}	0.006 ^{ns}	0.001 ^{ns}	2841.98 ^{ns}	0.30 ^{ns}	30.42 ^{ns}	1.03 ^{ns}
Error _b	215	0.019	0.007	0.001	2138.65	1.09	30.19	0.75
Coefficient of variation %		9.61	10.32	18.51	23.49	5.459	6.53	8.07

ns, *, and ** were on significant, significant at level 5 and 1% respectively. **GPX**= Glutathione peroxidase, **SOD**= superoxide dismutase, **POX**= Peroxidase, **ESC**= Extraction sugar coefficient.

search (Table 3). The significance of this interaction indicates the different reactions of genotypes to different environments. The variance analysis and mean comparison of genotypes were conducted for each environment separately. In Addition, GP and POX were significantly influenced by the interaction of year and genotype ($P < 0.01$) (Table 3). As previously stated, the genotype's interaction with the environment influenced all investigated traits. The significance of this interaction indicates genotypes'

different reactions to different environments. So, the variance analysis and mean comparison of genotypes were conducted for each environment separately. The analysis of combined data from two years showed significant differences between genotypes for all traits examined apart from the ESC (results not shown).

Proline

In sugar beet genotypes, water deficit increased proline content by 113.92% compared to normal irrigation

Table 4. Mean comparison of the studied treatment for quantitative and qualitative traits of sugar beet in two years

Irrigation	Proline		GPX		SOD		POX		Root Yield		White sugar yield	
	(mg g ⁻¹ FW)		(μmol g ⁻¹ FW)		(nmol g ⁻¹ FW)		(μmol g ⁻¹ FW)		(t ha ⁻¹)		(t ha ⁻¹)	
	N	S	N	S	N	S	N	S	N	S	N	S
	0.55b	1.30a	0.52b	1.15a	4786.3b	9746.5a	0.040b	0.090a	62.11a	51.18b	11.35a	10.14b
Genotype												
Pars	0.63e	1.15d	0.60be	0.75hi	6132.4c	7496.1b-e	0.021de	0.055ij	37.82m	31.31m	7.02o	5.59h
F-20722	0.25i	1.79a	0.52ef	0.95f	8811.6b	5455.3de	0.001e	0.019l	56.67k	42.41k	10.83k	8.31g
F-20815	0.78c	1.15d	0.39hi	0.67ij	9362.3a	14800.5ab	0.012de	0.110f	74.12d	60.63e	14.27f	11.84bc
F-20817	0.31gh	1.45c	0.40fji	1.16e	5917.0c	14084.1ab	0.024de	0.048j	66.74f	57.02f	13.26g	10.97bcd
F-20747	0.42f	1.22d	0.50efg	1.20de	5852.1c	12421.4a-d	0.017de	0.143c	72.28e	70.21c	14.42e	11.99b
Paya	0.25i	1.01e	0.38hi	0.78gh	4846.6d	6479.3cde	0.030cde	0.118ef	64.04g	49.61g	11.43j	9.59defg
F-20723	0.26i	1.29d	0.40ghi	0.71hij	4790.8d	8076.8b-e	0.079abc	0.125de	62.57i	49.83i	12.40h	10.11def
F-20851	0.85b	1.69ab	0.69bc	3.19a	4736.3d	14712.4ab	0.043cde	0.034k	85.68a	72.05ab	16.7b	14.5a
F-20734	0.84b	1.69ab	0.30i	0.66j	4713.0d	6237.6cde	0.006de	0.131d	85.63a	62.12d	16.9a	11.8bc
F-20716	0.98a	1.79a	0.84ab	1.18de	4591.2d	13387.1abc	0.038cde	0.050j	80.61c	70.11c	15.4d	13.4a
Sharif	0.23i	0.55g	0.45egh	0.96f	4230.5e	9793.5b-e	0.102a	0.159b	19.31o	16.85o	3.62q	2.40i
F-20772	0.84b	1.78a	0.46fgh	2.21b	3821.6f	9782.6b-e	0.096ab	0.084g	61.61j	46.62l	11.8i	9.1efg
Arya	0.44f	1.25d	0.34i	0.74hij	3789.6f	19393.3a	0.038cde	0.016l	61.89j	41.11l	7.3n	11.5bc
Shokoofa	0.96a	1.46c	0.69cd	1.74c	3451.1g	9311.4b-e	0.049b-e	0.073h	56.42k	47.12h	7.8m	10.5cde
F-20814	0.69d	1.65b	0.90a	1.25d	3260.2g	9424.8b-e	0.047b-e	0.065h	83.03b	73.85a	16.0c	13.5a
Ekbatan	0.35g	0.75f	0.67cd	0.85g	2773.0h	3900.5e	0.019d-e	0.089g	33.11n	32.06n	4.7p	6.0h
F-20866	0.66de	1.24d	0.38hi	0.86g	2562.0h	4882.2e	0.055a-e	0.064hi	63.53h	60.08e	11.8i	10.8bcd
F-20758	0.26hi	0.42g	0.31i	0.86g	2511.3h	5805.0de	0.042cde	0.241a	49.83l	45.07j	9.5l	8.8fg

In each column, the mean with common letters has no significant difference at the 5% probability level. **N**= Normal, **S**= Stress, **GPX**= Glutathione peroxidase, **SOD**= superoxide dismutase, **POX**= Peroxidase, **ESC**= Extraction sugar coefficient.

(Table 4).

Comparing the mean proline content of genotypes under normal irrigation, F-20716 and Shokoofa had the highest values, with an average of 0.98 and 0.96 mgg⁻¹ FW, respectively. The genotype Sharif, F-20722, Paya, and F-20723 had the lowest proline content, averaging 0.23, 0.25, 0.25, and 0.26 mgg⁻¹ FW, respectively (Table 4).

A comparison of genotypes under water deficit revealed that genotypes F-20716, F-20722, and F-20772 had the highest proline content, with an average of 1.83, 1.79, and 1.78 mgg⁻¹ FW, respectively. It is worth noting that there was no significant difference between these genotypes and the two other genotypes, F-20851 and F-20734. The two genotypes with the lowest leaf proline content were Sharif and F-20758, with average values of 0.55 and 0.42 mgg⁻¹ FW, respectively (Table 4).

Antioxidant properties

The study found that water deficit led to significant increases in the activity of glycol peroxidase, polyphenol oxidase, and superoxide dismutase, with respective increments of 118.86%, 82.1%, and 103.61% compared to normal irrigation (Table 4).

Guaiacol peroxidase (GP)

Under normal irrigation, the F-20814 genotype had the highest GP enzyme activity, averaging 0.90 μmolg⁻¹ FW. However, the difference between the mentioned genotype and F-20716 was insignificant regarding the activity of the GP enzyme. Three genotypes, F-20734, F-20758, and Arya, had the lowest GP peroxidase enzyme activity, averaging 0.31, 0.31 and 0.34 μmolg⁻¹ FW, respectively (Table 4).

The comparison of the mean genotypes in terms of GP enzyme activity in water deficit conditions showed that two genotypes, F-20851 and F-20734, had the highest and lowest activity of the GP enzyme with an average of 3.19 and 0.66 μmolg⁻¹ FW, respectively (Table 4).

Polyphenol oxidase (POX)

The comparison of POX activity content between different genotypes revealed that the Sharif genotype demonstrated the highest content of POX enzyme with an average of 0.102 mgg⁻¹FW. However, concerning POX activity, there was no statistically significant difference between the Sharif genotype and the genotypes F-20772, F-20723, and F-20866. On average, the F-20722 genotype had the lowest POX enzyme activity at 0.002 mgg⁻¹ FW (Table 4).

Genotype F-20758 had the highest POX enzyme activity (0.242 mgg⁻¹ FW) under irrigation deficit, while Shokoofa and F-20722 had the lowest (0.017 and 0.020 mg g⁻¹ FW, respectively) (Table 4).

Superoxide dismutase (SOD)

Under full irrigation, the F-20815 genotype had the highest SOD enzyme activity (9362.3 μmolg⁻¹FW). The genotypes Ekbatan and F-20866 F-20758 had the lowest SOD activity, averaging 2773.0, 2562.0, and 2511.3 μmolg⁻¹ FW, respectively (Table 4).

Underwater scarcity led to the highest SOD enzyme

activity in the Arya genotype, with an average of 19393.3 μmolg⁻¹ FW. There was no significant difference between this genotype and genotypes F-20815, F-20851, F-20716, and F-20747. In this study, two genotypes, Ekbatan and F-20866, had the lowest SOD activity, with averages of 3900.5 and 4882.2 μmolg⁻¹ FW, respectively. It should be noted that the amount of superoxide SOD increased in all studied genotypes in response to water stress (Table 4).

Root yield

In this study, water stress reduced root yield by 17.38% compared to normal irrigation (Table 4).

A comparison of the average of genotypes regarding root yield under normal irrigation conditions showed that two genotypes, F-20851 and F-20734 had the highest root yield, averaging 85.68 and 85.63 t ha⁻¹, respectively. The Sharif genotype had the lowest root yield in this condition, with an average of 16.85 t ha⁻¹ (Table 4).

Under drought stress conditions, genotype F-20814 had the highest root yield, averaging 73.85 t ha⁻¹. The difference between the mentioned genotype and the F-20851 genotype was insignificant regarding root yield. During water scarcity, the Sharif genotype produced the lowest root yield of 19.84 t ha⁻¹ (Table 4).

White sugar yield

In this study, genotypes grown under water stress exhibited an 11.93% decrease in white sugar yield (Table 4).

The genotype's mean under normal conditions was compared, and the F-20734 genotype produced the highest white sugar yield, averaging 16.90 t ha⁻¹. In contrast, the Sharif genotype had the lowest white sugar yield, at 3.62 t ha⁻¹ (Table 4).

Under water deficit stress, the three genotypes F-20851, F-20814, and F-20716 showed the highest white sugar yield, with averages of 14.50, 13.50, and 13.40 t ha⁻¹, respectively. Among these genotypes, Sharif had the lowest white sugar at an average of 2.40 t ha⁻¹ under the mentioned conditions (Table 4).

Correlation and cluster analysis

The correlation coefficients for the traits are presented in Fig. 1. Based on this, the correlation of proline content with GPX was positive and significant under normal irrigation conditions and water deficit stress. Also, proline content under water deficit conditions negatively and significantly correlated with POX. The correlation between SOD content in both irrigation treatments and POX was negative and significant. Under both irrigation treatments, the correlation of root yield with proline was positive and significant; root yield under normal irrigation conditions had a negative and significant correlation with POX, and, under water stress, had a positive and significant correlation with GPX and SOD (Fig. 1). In both irrigation treatments, proline with white sugar yield and root yield had a positive correlation and a negative and significant correlation with POX. Under complete irrigation treatment, the correlation of ESC with root yield was positive, and POX was negative and significant. Under both irrigation treatments, the cor-

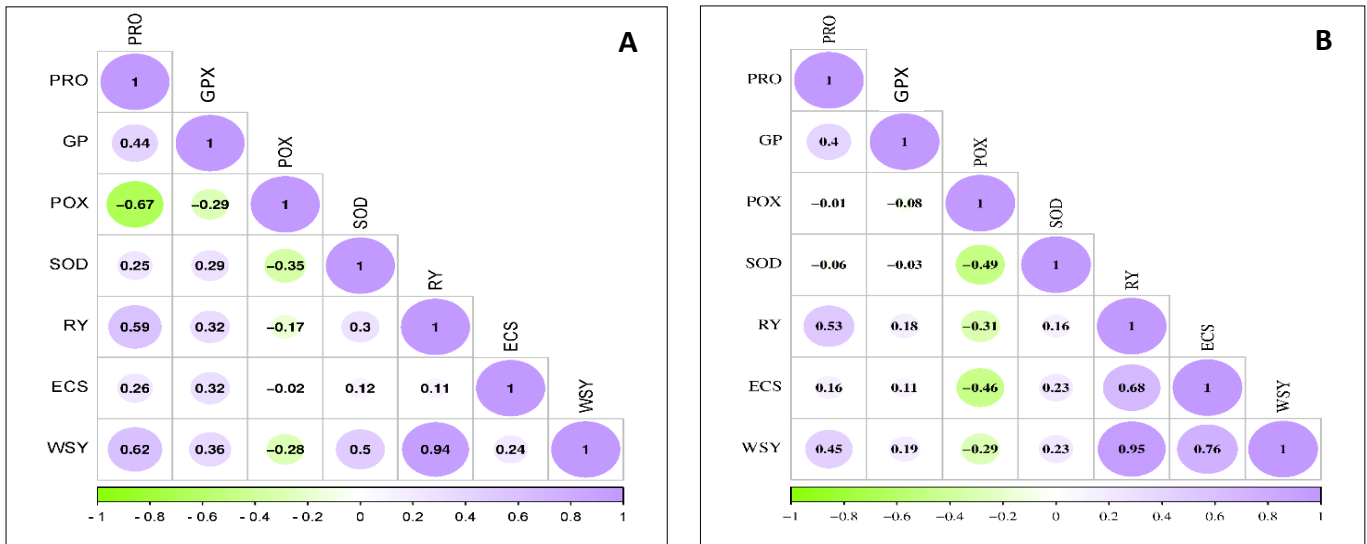


Fig. 1. Correlation of studied traits in sugar beet under normal conditions (A) and water shortage stress (B). **GPX**= Glutathione peroxidase, **SOD**= superoxide dismutase, **POX**= Peroxidase, **ESC**= Extraction sugar coefficient.

relation of white sugar yield with proline content and root yield was positive and significant. Under fully irrigated conditions, the correlation between white sugar yield and ESC was positive and significant. In contrast, in water deficit stress, white sugar yield showed a positive and significant correlation with GPX and SOD (Fig. 1).

Fig. 2 displays the results of grouping genotypes and traits using the heatmap. In this study, 18 genotypes investigated were classified into three groups under nor-

mal irrigation and water deficit stress treatments, and the examined traits also formed two separate groups (Table 5). The first cluster had higher levels of POX, proline, GPX, and SOD and lower levels of root yield and white sugar yield compared to the average of all clusters (Fig. 2).

The genotypes F-20815-N, F-20722-N, F-20747-N, F-20817-N, F-20723-N, F-20814-N, F-20816-N, F-20851-N and F-20734-N were in the second cluster. This cluster had higher values for root yield, white sugar yield, and ESC but

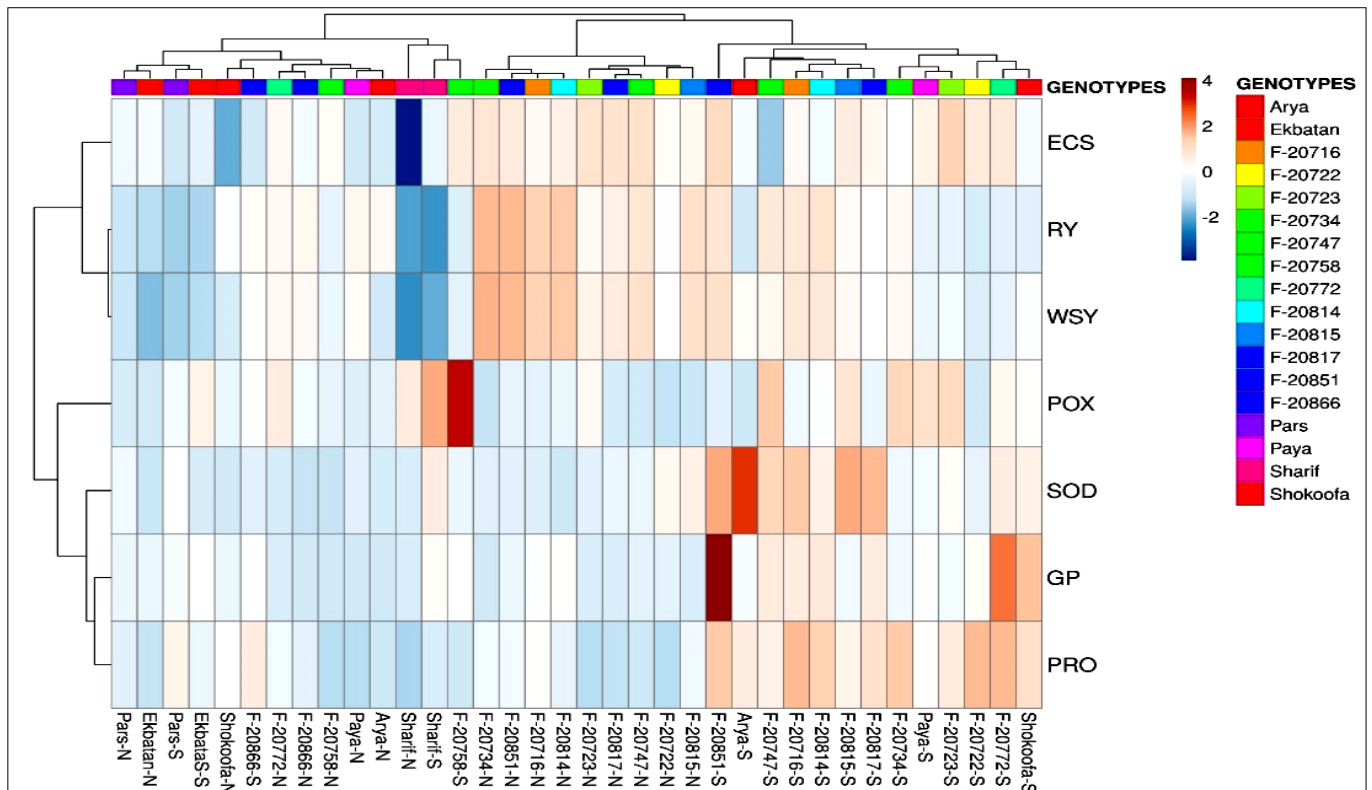


Fig. 2. Heat diagram of the grouping of the examined genotypes and traits.

mal irrigation and water deficit stress treatments, and the examined traits also formed two separate groups (Table 5).

A significant difference was observed between the three clusters that grouped genotypes regarding all the examined traits (Table 5). The first group includes genotypes Shokoofa-S, F-20722-S, F-20772-S, F-20723-S,

lower values for POX, proline, GPX, and SOD compared to the average of all groups. Cluster number three includes F-20758-S, Pars-N, Paya-N, Sharif-N, F-20772-N, Arya-N, Shokoofa-N, Ekbatan-N, F-20866-N, F-20758-N, Pars-S, Sharif-S, Ekbatan-S, F-20866-S and F-20758-S. The third cluster had lower values for proline, GPX, root yield, SOD,

Table 5. The mean square and comparisons of the mean of the groups resulting from the cluster analysis

	Proline	GPX	POX	SOD	Root Yield	ESC	White Sugar Yield
Between groups	3.21**	2.02**	0.009*	1.48**	2361.67**	125.40**	127.84**
Within groups	0.08	0.22	0.002	0.08	182.47	18.72	5.83
Cluster 1	1.47a	1.26a	0.07a	11044a	57.13b	86.07a	11.34b
Cluster 2	0.59b	0.53b	0.029b	5781.6b	74.13a	87.82a	14.49a
Cluster 3	0.61b	0.59b	0.086a	4786.7b	45.20c	81.66b	7.86c
Mean	0.98	0.793	0.061	7227.06	58.82	85.18	11.23

GPX= Glutathione peroxidase, SOD= superoxide dismutase, POX= Peroxidase, ESC= Extraction sugar coefficient

ESC, and white sugar yield, and higher POX content compared to the average of all clusters (Fig. 2).

AMMI stability

Table 6 demonstrates significant differences in root yield among genotypes and environments (additive effects) and the interaction effect of genotypes in the environment (multiplicative effects) at the 1% probability level. Genotype and environment were responsible for 62.22% and 17.01% of the total variance of data variance, respectively (Table 6). This study separated the genotype x environment interaction into two significant components: IPCA1 (AMMI 1) and IPCA2 (AMMI 2). The first component (IPCA1) contributed 5.64%, and the second component (IPCA2) contributed 0.32% to the total sum of squares. Furthermore, IPCA1 contributed 95.11%, and IPCA2 con-

Table 6. Analysis of variance based on AMMI model for sugar yield of sugar beet genotypes

Source	Df	Sum of squares	Mean of squares	Relative variance (%)	G×E Relative variance (%)
Total	215	67258	312.8		
Treatments	71	67247	947.1**	17.00	
Genotypes	17	58848	3461.6**	62.12	
Environments	3	2124	707.9**	12.70	
Block	8	0	0	0	
Interactions	51	6276	123.1**	2.21	
IPCA1	19	5969	314.2**	5.64	95.11
IPCA2	17	305	18**	0.32	4.86
Residuals	15	1	0.10ns	0.00	
Error	136	10	0.11		

ns, * and **, non-significant, significant at 5 and 1% probability levels, respectively, Df: Degrees of freedom, G×E: Genotype×Environment

tributed 4.86% to the sum of squares of genotype interaction effects in the environment.

The study reports the root yield mean for genotypes in 4 environments and IPCA1, IPCA2, and ASV parameters in Table 3. Our findings indicate that the F-20851, F-20814, F-20716, and F-20734 genotypes were related to the highest average root yield of 78.99, 78.03, 75.55, and F-20734 t ha⁻¹, respectively. On the other hand, the Sharif genotype recorded the lowest root yield of 18.39 t ha⁻¹. According to the study, genotypes Pars, F-20758, F-20866, F-20817, and F-20814 demonstrated the lowest ASV and were considered stable regarding root yield. On the other hand, geno-

Table 7. Root yield mean and ASV for sugar beet genotypes in five environments

Genotype	Root yield (t ha ⁻¹)	Yield rating	IPCA1	IPCA2	ASV
Arya	51.51	13	-4.05	0.92	79.13
Ekbatan	29.13	17	-2.17	-2.89	42.50
F-20716	75.55	3	0.64	-0.02	12.49
F-20722	49.66	14	1.21	0.07	23.60
F-20723	56.28	10	0.98	0.10	19.25
F-20734	74.23	4	2.51	-0.23	49.07
F-20747	71.34	5	-0.61	0.33	11.96
F-20758	47.74	15	-0.26	0.10	5.08
F-20772	54.14	11	1.35	-0.21	26.50
F-20814	78.03	2	0.59	0.13	11.44
F-20815	67.50	6	1.09	0.10	21.30
F-20817	61.95	8	0.54	0.12	10.51
F-20851	78.99	1	1.10	0.02	21.60
F-20866	62.27	7	-0.51	0.36	9.94
Pars	34.67	16	0.05	0.22	1.05
Paya	56.94	9	1.23	-0.05	24.01
Sharif	18.39	18	-1.35	0.53	26.41
Shokoofa	51.76	12	-2.35	0.40	45.90

types Arya, F-20734, Shokoofa, and Ekbatan had the highest values of the mentioned stability parameters and were recognized as unstable genotypes (Table 7).

Biplot

The root yield and IPCA1 and IPCA2 values were used to group the genotypes in this study. The genotype with high root yield and close to zero IPC1 and IPC2 values is considered a suitable genotype with high yield stability. Based on Fig. 3, the biplot shows the average root yield of various genotypes plotted against their IPCA1 values. The genotypes Pars, F-20758, F-20866, F-20817, and F-20814 had IPCA1 values close to zero, indicating they are stable and compatible with the general trend. On the other hand, the genotypes Arya, F-20734, and Shokoofa had high IPCA1 values, suggesting that they are unstable.

Fig. 4 shows the biplot of the mean root yield of each genotype plotted against their IPCA2 value. Genotypes Paya, F-20716, and F-20851 had IPCA2 values close to zero, indicating stability. On the other hand, the Arya and Ekbatan genotypes were identified as unstable, with the most significant distance from zero. It should be noted that the F-20716 genotype had high root function and IP-

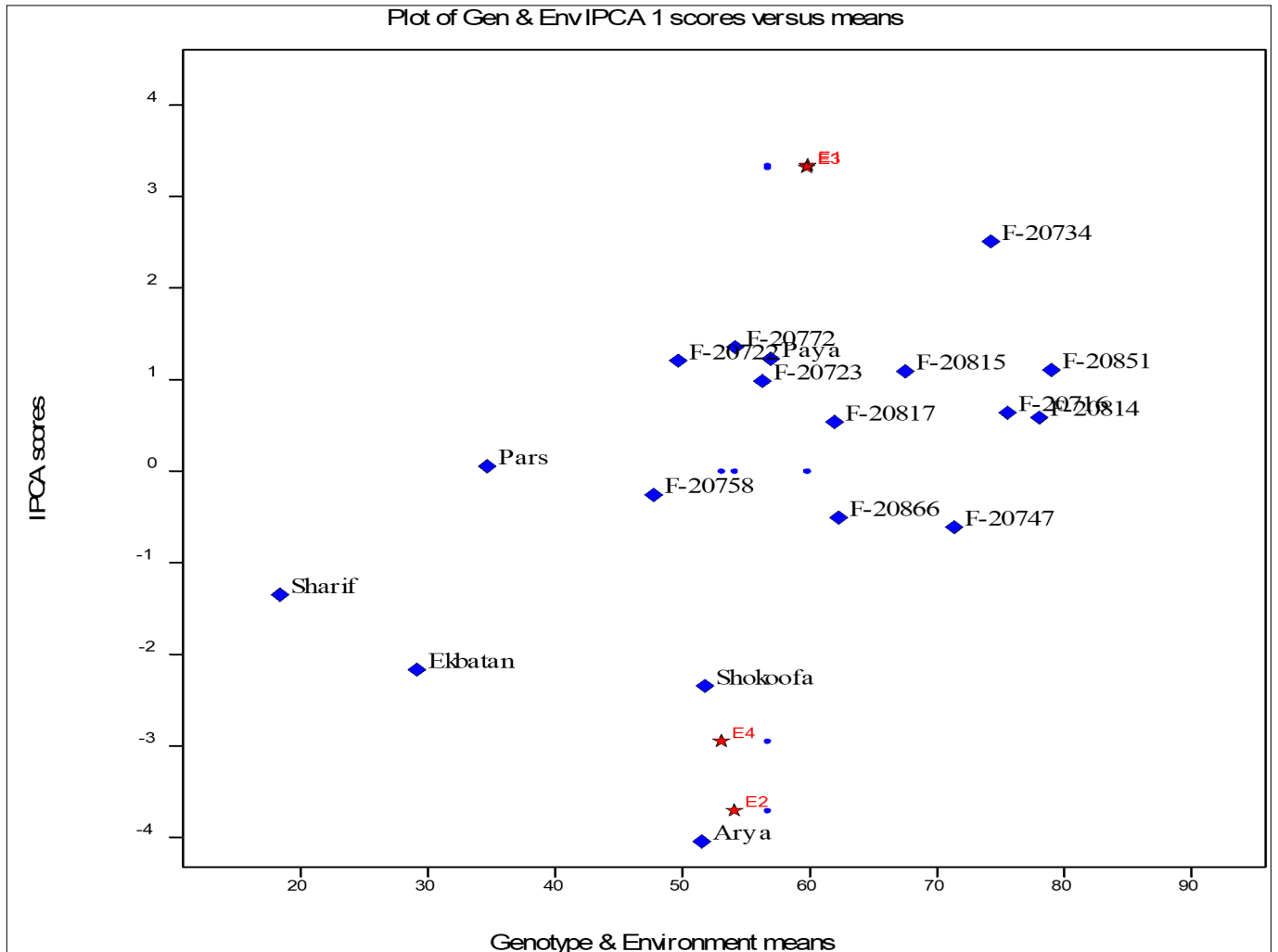


Fig. 3. Scatter plot for genotypes and environments based on root yield means and a first principal component.

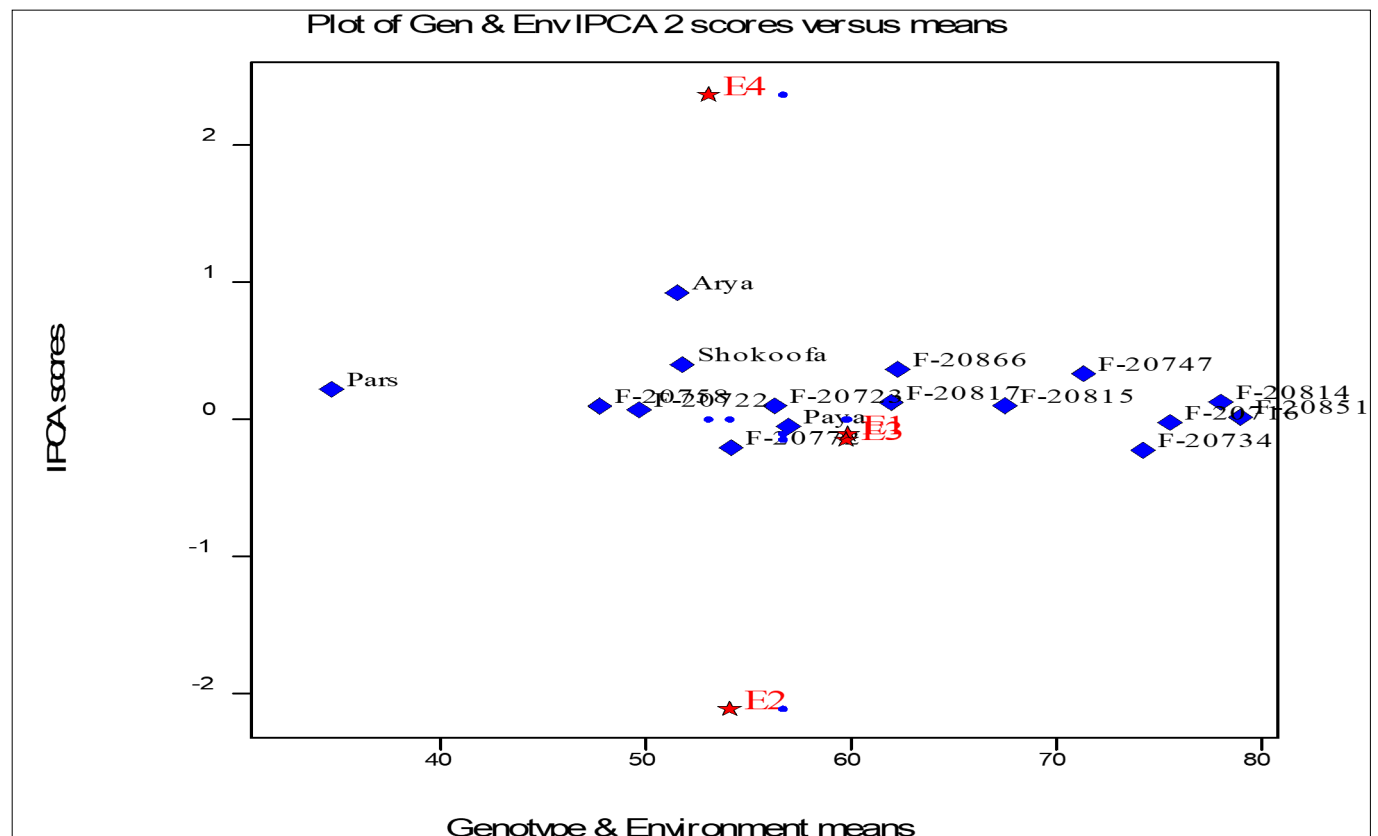


Fig. 4. Scatter plot for genotypes and environments based on root yield means and a second principal component.

CA1 and IPCA2 values.

GGE model

Fig. 5 shows the Convex hull from GGE analysis of sugar beet genotypes across four environments. Genotypes and environments are determined based on the first and second principal component values. Points close to the origin of these components have the most minor GGE interaction. This diagram represents the interaction effect's first and second principal components, explaining 99.57% of the variance resulting from genotype and environment. Genotypes near a location have private compatibility, while those near the origin have general compatibility. In this study, most of the genotypes were located around the origin of the coordinates. However, two genotypes, F-20758 and F-20817, showed a smaller distance from the coordinates' source than others.

point is called the average environment coordination line (the average representative of the coefficients of the first two components of the interaction effect in the GGE biplot model) (Fig. 5).

The genotypes closer to the circle's center of this line produce the highest yield; in contrast, genotypes perpendicular to the environmental function's average line are less stable and significantly impact G×E interaction. In our study, the F-20716, F-20814, and F-20851 genotypes had higher root yields and were recognized as stability genotypes due to their proximity to the ACE line compared to other genotypes. In contrast, the Arya genotype had the most significant distance from the ACE line and was less stable (Fig. 6).

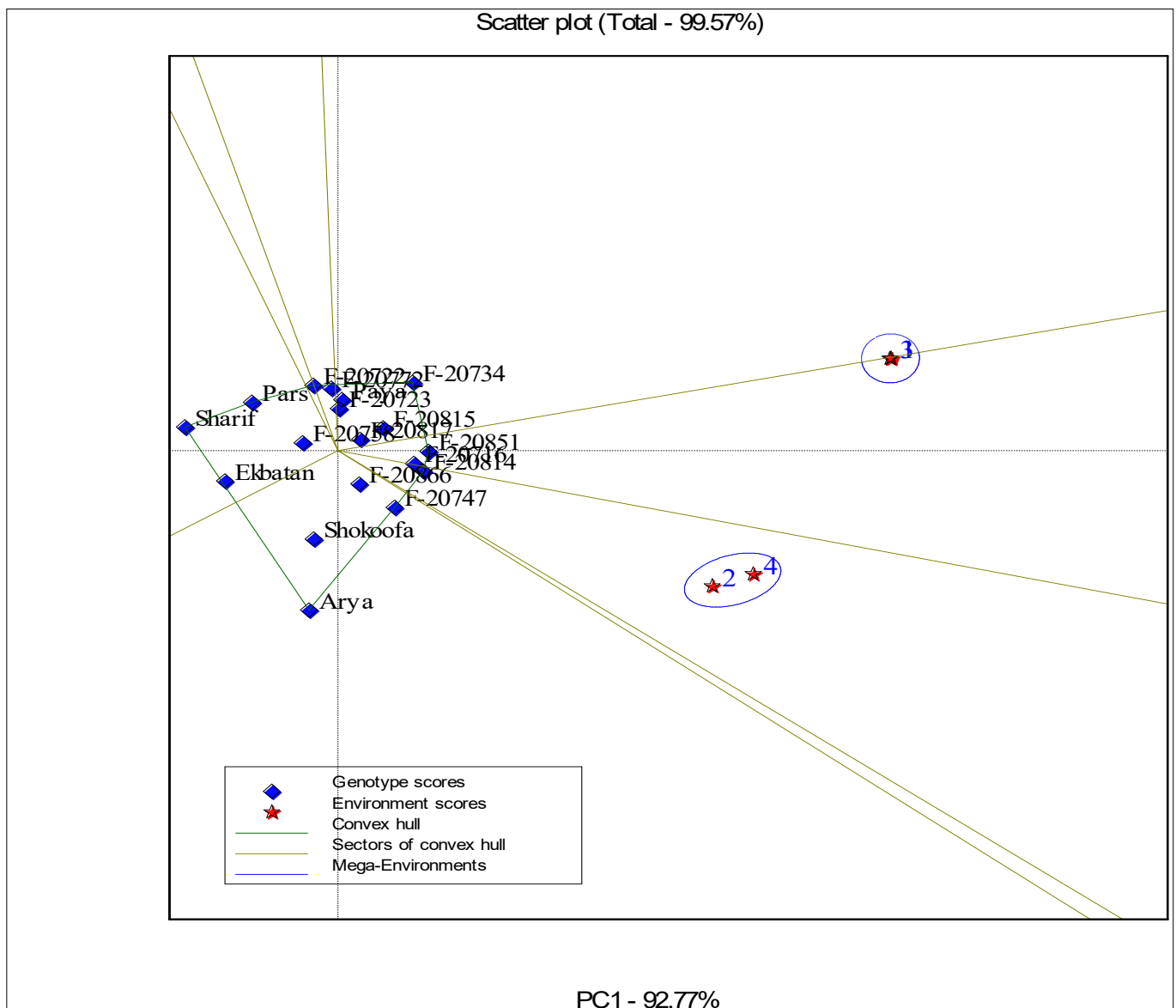


Fig. 5. Polygon of GGE biplot method to identify suitable genotypes for each environment.

According to the study, genotypes F-20851, F-20734, F-20814, and F-20716 are suitable for conventional irrigation, while genotypes F-20814, F-20851, F-20747, and F-20716 are the most suitable for water shortage environments. The Ekbatan, Arya, and Sharif genotypes had the most significant distance from the coordinate origin and were unsuitable for any environment. The diagonal line passing through the center of the biplot and the ideal

Fig. 5 shows concentric circles on the biplot to evaluate the ideal genotype. These circles visually assess the distance between the ideal and studied genotypes. The central placement of the ideal genotype helps visualize the distance between the studied genotypes and the ideal variety. Our research indicates that the most suitable genotypes for the four tested environments are those with high root yield and consistent performance. Therefore,

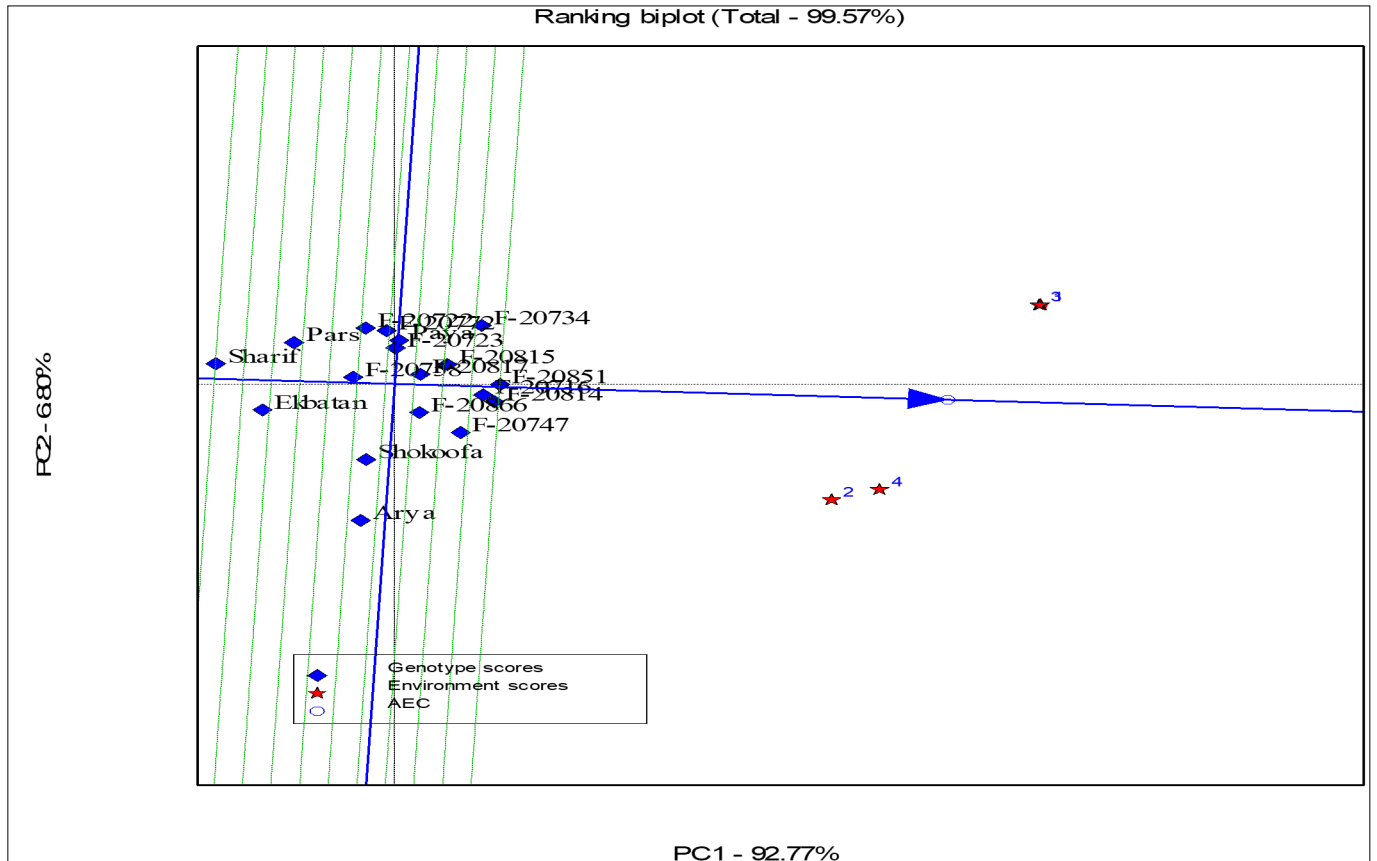


Fig. 6. Genotype ranking based on average white sugar yield and stability.

genotypes F-20716, F-20814, and F-20851 were found to have the slightest distance from the ideal genotype compared to the others and were more favorable than all the studied genotypes. On the other hand, the Sharif genotype

had the most significant distance from the ideal genotype. The Sharif genotype's root yield and yield stability were unfavorable compared to other genotypes (Fig. 7).

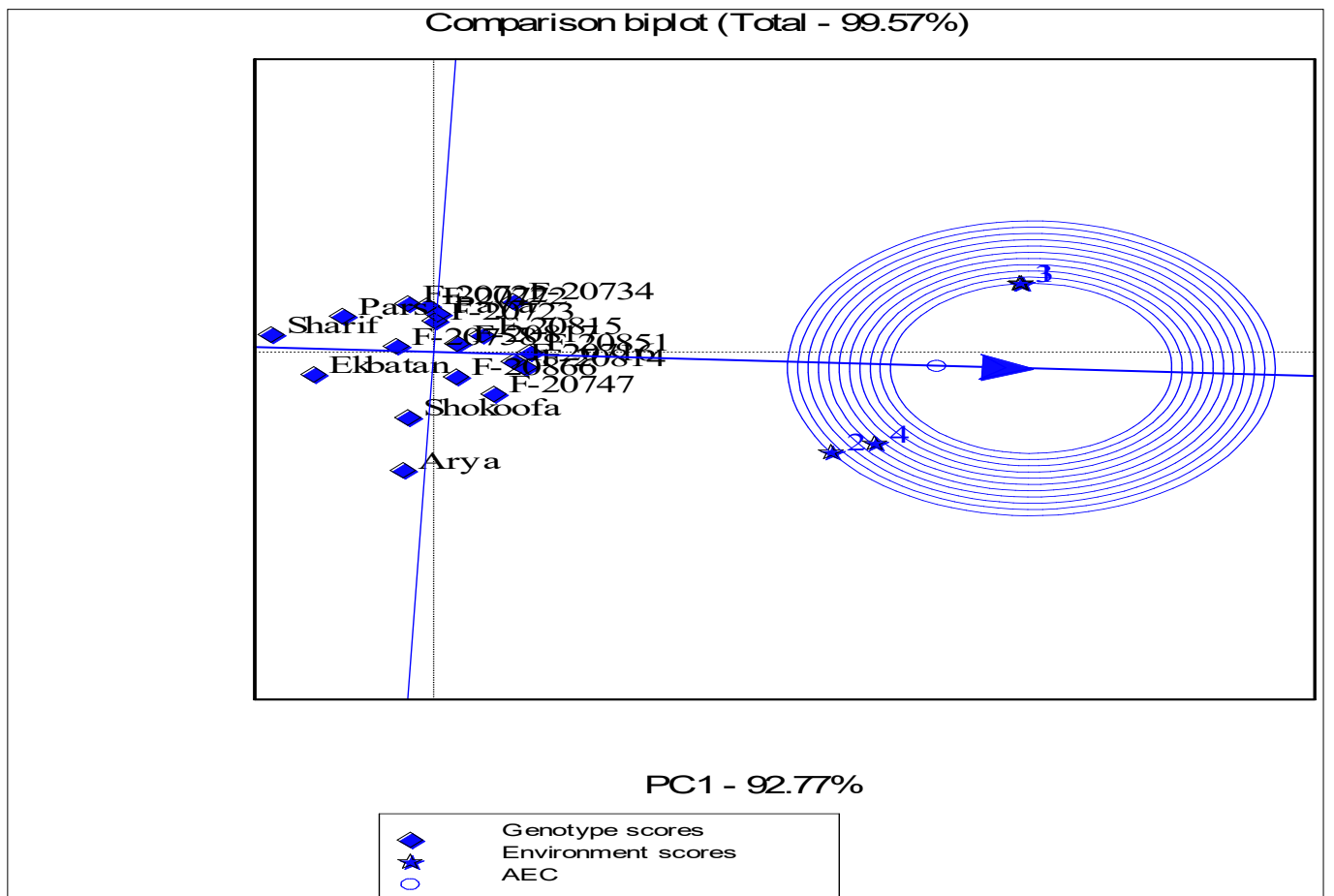


Fig. 7. Evaluation of studied genotypes compared to the ideal genotype based on root yield.

Multi-trait stability index (MTSI) Plant materials

This study identified two factors with eigenvalues greater than one, explaining 64.13% of the total data variance. The first factor had high and positive factor coefficients for white sugar yield, extraction coefficient of sugar, and root yield, with eigenvalues of 2.33, justifying 33.28% of the total variance. The second factor accounted for 35.85% of the total variance in the data and had an eigenvalue of 2.16. It had high positive coefficients for proline, guaiacol peroxidase, polyphenol oxidase, and superoxide dismutase (Table 8). The MTSI stability index of the genotypes was calculated using the factor scores. All the sugar beet genotypes' characteristics were considered while calculating the MTSI.

Table 8. Factor analysis based on principal component analysis: Eigenvalues, factor coefficients, relative and cumulative variance, after varimax rotation

Traits	Factors			
	FA1	FA2	Communality	Uniquenesses
White sugar yield	0.976	0.059	0.95	0.05
Root yield	0.906	0.080	0.82	0.18
ESC	0.683	0.016	0.46	0.54
Proline	0.191	0.817	0.43	0.57
GPX	0.076	0.815	0.66	0.34
SOD	0.103	0.647	0.42	0.58
POX	-0.194	0.633	0.70	0.3
Eigenvalue	2.33	2.16		
Relative Variance (%)	33.28	30.85		
Cumulative variance (%)	33.28	64.13		

GPX= Glutathione peroxidase, **SOD**= superoxide dismutase, **POX**= Peroxidase, **ESC**= Extraction sugar coefficient.

In Fig. 8, genotypes are sorted by MTSI, with the highest in the center and the lowest on the outermost circle. Red dots indicate the genotypes selected based on MTSI values at 20% selection intensity. Our research has identified F-20734 and F-20851 genotypes as the most ideal genotypes. The Pars genotype with the lowest MTSI values was located in the circle's center and classified as inappropriate compared to other genotypes (Fig. 8).

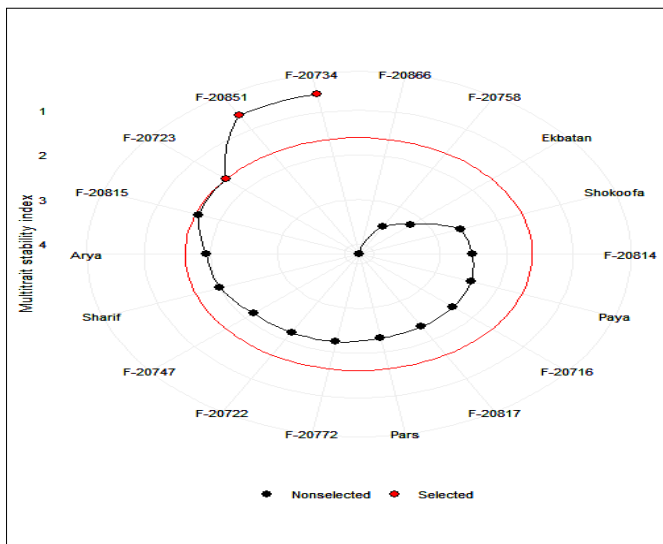


Fig. 8. Genotype ranking and selected genotypes based on MTSI index.

Discussion

Recently, breeding programs have focused on improving sugar beets' quantitative and qualitative characteristics and reducing irrigation water consumption (18). The morphology of sugar beets can be altered significantly by abiotic stressors, such as drought. The results indicate that an irrigation deficit increased the content of proline, GPX, SOD, and POX by 57.69%, 54.78%, 50.89%, and 55.56%, respectively. Additionally, it decreased root and white sugar yields by 21.35% and 11.93%, respectively.

Drought reduces vegetative growth, accelerates leaf wilting, decreases stomatal conductance and leaf water content, and impairs photosynthesis by destroying photosynthesis pigments. In addition, under water deficit, sugar beet's quantitative and qualitative characteristics are reduced due to membrane lipid peroxidation caused by oxidative stress, cell damage, and accumulation of compatible solutes (2, 19).

There was favorable genetic diversity in the genotypes for the examined traits under both irrigation treatments, except for ESC. Genetic diversity allows selection between cultivars in terms of favorable traits. The genetic diversity between sugar beet cultivars under normal and water deficit stress conditions has been documented in previous research (8, 10).

Under normal irrigation, genotype F-20716 showed the highest content of proline and GPX. Results revealed that the genotype with the highest content of PRO and GPX was F-20716, while the genotypes with the highest content of SOD and POX were F-20815 and Sharif, respectively. The highest root yield was recorded for two genotypes, F-20734 and F-20851. Furthermore, F-20734 and F-20851 genotypes were ranked first and second in white sugar yield.

Under water deficit stress, genotypes F-20851 and F-20716 produced the maximum content of proline and SOD. The genotype with the highest GPX content was F-20851, while the genotype with the highest POX content was F-20758. F-20814 and F-20851 genotypes produced maximum root yield. The three genotypes with the highest white sugar yield were F-20851, F-20814, and F-20716.

Results revealed that positive and significant correlations were observed between white sugar yield and proline content, root yield (under both irrigation treatments), ESC (under normal irrigated treatments), and GPX and SOD (under water deficit stress). According to the heatmap chart results, the genotypes with higher root yield and ESC were also high in white sugar yield under normal irrigation conditions; under water stress, genotypes with high sugar yields had elevated levels of proline, POX, SOD, and GPX, in addition to root performance and ESC.

It was observed that under normal irrigation conditions, the genotypes obtained the maximum white sugar yield (F-20734 and F-20851), which had a higher root yield. While under stress conditions, genotypes F-20851, F-20814, and F-20716, which had the maximum white sugar yield and root yield, had higher proline content and

antioxidant enzyme activity than other genotypes. It can be concluded that genotypes can maintain their yield potential under water stress, which has superior antioxidant properties.

When exposed to stressful conditions, plants accumulate reactive oxygen species (ROS), which can irreversibly harm their nucleic acids, pigments, proteins, and lipids (20). To safeguard themselves against ROS, plants have both enzymatic and non-enzymatic antioxidant systems in place. Catalase, peroxidase, and superoxide dismutase are some examples of enzymes present in this system (21). Previous studies have shown that water deficit stress in sugar beet increases the activity of antioxidant enzymes (22, 23). Sugar beet cultivars with high lipid peroxidation under water-stress conditions exhibit low levels of antioxidant enzyme activity. Drought-resistant cultivars show elevated levels of antioxidant enzyme activity, namely CAT, SOD, and POX (24). However, it has been reported that water deficit stress does not affect the activity level of antioxidant enzymes in sugar beet genotypes (25).

In our study, proline showed a positive correlation with white sugar yield in both irrigation treatments. Proline regulates cytoplasmic osmotic pressure by accumulating in the cytosol. The previous studies have proven that water stress conditions lead to an increase in proline content in sugar beet leaves and other organs (26, 27). It has been discovered that sugar beet salinity stress-resistant genotypes have high levels of proline in their leaves (28, 29).

The F-20851 genotype produced the highest white sugar yield in both irrigation treatments. This genotype is recommended for cultivation in various irrigation regimes. Evaluation of genotypes only under water shortage stress conditions cannot be a valid criterion for evaluating the yield of tolerant genotypes; evaluating genotype yield under normal and water shortage stress and assessing their stability is a more valid criterion (30). The accumulation of desirable alleles is achieved by selecting potential high-yielding final lines based on their reactions under both conditions (31).

In four environments, genotypes F-20851, F-20814, F-20716, and F-20734 produced the highest root yield; based on the ASV index, the most stable genotypes were Pars, F-20758, F-20866, F-20817, and F-20814. The genotype with F-20814 had satisfactory root yield and stability of yield.

Based on biplot results, the genotypes Pars, F-20758, F-20866, F-20817, and F-20814 had IPCA1 values close to zero. In contrast, Paya, F-20716, and F-20851 had IPCA2 values close to zero; in addition to the high root yield of the mentioned genotypes, their interaction with the environment was minimal.

Various researchers have suggested using the AMMI analysis model to estimate the interaction effect of genotype with the environment and to evaluate the stability of cultivars and different sugar beet genotypes (8, 10, 12).

Two components were identified from GGE analy-

sis, explaining 99.57% of the variation in genotype-environment interaction. The first component was more significant (92.77%). According to this, the genotypes of F-20758 and F-20817 demonstrated a high level of general compatibility for all the given conditions. In addition, genotypes F-20851, F-20734, F-20814, and F-20716 showed special compatibility with normal irrigation, while F-20814, F-20851, F-20716, and F-20747 showed special compatibility with water deficit conditions.

It was observed that genotypes F-20851, F-20814, and F-20716 were suitable for both irrigation treatments. None of this experiment's Ekbatan, Arya, and Sharif genotypes were detected under suitable conditions. Genotypes with higher root yields and proximity to the ACE line, such as F-20716, F-20814, and F-20851, were recognized as stability genotypes. In addition, genotypes F-20716, F-20814, and F-20851 were closest to the ideal genotype.

Evaluation of root yield stability in 18 sugar beet genotypes by AMMI and GGE analysis revealed that the F-20814 genotype had high root yield and acceptable stability and had the smallest distance from the ideal genotype. The GGE biplots offer a valuable tool to identify the optimal growing conditions for specific cultivars. When a cultivar performs exceptionally well in only one or a few particular environments, it is considered to have a narrow adaptation. Cultivars with a narrow adaptation are best suited for a particular mega-environment. Therefore, choosing the suitable cultivar based on the specific growing conditions can result in higher yields (9).

According to Studnicki et al. (9), it is not feasible to identify a particular cultivar or group of cultivars that can adapt to the environmental conditions prevalent in temperate climates. Instead, they have suggested that cultivars be selected based on their suitability to specific locations, categorized as mega-environments. Rajabi et al. (12) found that the RM5 genotype had a high yield of white sugar and low values on the first two components based on biplot analysis. Studnicki et al. (9) investigated the stability of 36 modern sugar beet cultivars under Polish environmental conditions. However, they found no cultivar that showed broad adaptation to the ecological conditions. In the biplot study by Abbas and Bocianowski (10), 7233-P.29 (G38) and C CMS (G49) lines, as well as 2(6)×C (G27) and 5×C (G33) hybrids, were identified as stable genotypes with acceptable yield.

Evaluation of genotype stability for all traits (MTSI) revealed F-20734 and F-20851 as the most stable genotypes across environments. These genotypes also had higher proline content, enzyme characteristics, root yield, and white sugar yield than others. They were also grouped in the highest-performing group for root yield, ESC, and white sugar yield.

The MTSI index is a valuable tool for breeders to evaluate suitable genotypes. It helps them identify the most appropriate genotypes for their breeding programs; the index can identify superior genotypes for multiple traits based on multi-environment data (12, 32). Various studies have used this index to identify stable sugar beet cultivars (8, 12).

Conclusion

The present research showed that water deficit stress increased proline content and antioxidant enzyme activity and decreased root and white sugar yields. Genotype F-20851 obtained the highest root and white sugar yield in both irrigation treatments and had higher proline content and antioxidant enzyme activity than other genotypes under water deficit stress. Therefore, one of the criteria for selecting superior genotypes under water stress conditions, in addition to root function, is the proline content and the activity of antioxidant enzymes. The presence of a significant positive correlation between proline, GPX, and SOD with white sugar yield under water deficit stress supports this argument. Based on the stability analysis results using AMMI and GGE methods, genotype F-20814 showed the highest stability in root yield across all environments; the F-20814 and F-20851 genotypes also showed the lowest distance from the ideal genotype. Finally, based on the MTSI index, the two genotypes, F-20734 and F-20851, were the most stable in the investigated environments regarding all the investigated characteristics. It can be concluded that the F-20851 genotype is suitable for cultivation in areas with varying humidity levels due to its high white sugar yield in both environmental conditions and yield stability, as well as other examined traits.

Acknowledgements

We immensely thank Islamic Azad University for its generous financial assistance.

Authors' contributions

ZN, RMHA wrote the manuscript with support from EN and HH. All authors read and approved the manuscript.

Compliance with ethical standards

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

Ethical issues: None

AI Declaration

None

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