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Delineation of tolerance some wheat genotypes under severe water deficit via multi-trait selection index MTSI

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ABSTRACT

The present study was conducted at Sakha Agricultural Research Station to investigate the effects of severe water deficit on grain yield of 16 wheat genotypes during the 2022/2023 and 2023/2024 growing seasons. Changes in agronomic and physiological characteristics have resulted in a decline in grain yield from 23.1 to 9.7 (ardb/fad), representing an average decrease of 57.3% under severe water shortage. The Multi-Trait Stability Index (MTSI) is used as a modern, powerful tool selection index for superior genotypes in severe water deficit. Based on the low values of stability Index (MTSI) at a selection intensity of 25 %, four wheat genotypes were selected, and at a selection intensity of 50%, eight wheat genotypes were selected. According to the findings of this study, we recommend lines 2, 3, 5, 9, 10, and 12 to be cultivated in regions experiencing water scarcity, as these lines achieve high grain yields with minimal irrigation water. The identified genotypes demonstrated high reliability, significant yield potential, and early maturity, making them strong candidates for variety and hybrid development, as well as ideotype breeding programs aimed at ensuring food and nutritional security. These genotypes should be included in breeding programs focused on improving wheat tolerance to water deficits by enhancing the performance and stability of agronomic, physiological, and grain quality traits across various environments.

Keywords: Bread wheat; scarce water, Multi-Trait Stability Index (MTSI)

INTRODUCTION

The scarcity of freshwater resources poses a significant challenge globally, particularly for irrigated agriculture, which consumes the most freshwater (Ingrao *et al.,* 2023). Climate change is altering the global hydrological cycle, impacting agriculture through floods, droughts, and erratic rainfall. These changes are affecting the yields of major crops like maize, soybeans, rice, and wheat, and are expected to continue, reducing yields of rain-fed crops and irrigation water in water-stressed regions. Globally, it is estimated that droughts and other extreme weather events have led to a 9–10% decrease in total cereal production (IPCC, 2023). As water shortage increases and the demand for grain production rises to meet global food needs, effectively managing limited water resources to maximize benefits per unit is a critical issue (International Commission for Irrigation and Drainage, 2022). Egypt is a dry Mediterranean country that faces many significant challenges regarding water scarcity and food security. The competition for water in Egypt is intensifying due to various factors, including a rapidly growing population and water development projects on the Nile initiated by upstream countries (Gamal *et al.,* 2024).

Egypt's rapid population growth has led to insufficient food production, causing the country to rely on external imports for 35.6% of its wheat, 1.7% of its legumes, and 53.1% of its meat. Egypt is expected to continue being the world's largest wheat importer, with annual imports projected to reach 14.2 million tons by 2032/33 (Interagency Agricultural Projections Committee, 2023). The demand for water, energy, and food (WEF) is projected to increase by 30 to 50% by 2050. Egypt faces the challenge of producing more food for every cubic meter of water used, every kilowatt of energy consumed, and every unit of land available (Aly *et al.,* 2024). Wheat is a highly nutritious food that, along with rice and corn, is essential for global food security (Hachisuca *et al.,* 2023). Furthermore, it is a staple crop in the worldwide supply and the Egyptian food basket. Revealing a genetic variation in crops enhances global plant improvement by enabling breeders to identify top lineages for crossbreeding, resulting in superior crops with desirable traits. Furthermore, morphological characterization is essential for genetic improvement programs, revealing genetic variability crucial for successful crop breeding (Basnet, 2024). Traditional breeding programs have often concentrated on a single trait, primarily yield. However, this focus can create unforeseen issues. Multi-trait selection is a more comprehensive approach to a range of desirable traits. This method aims to yield other important characteristics, including disease resistance, drought tolerance, and grain quality (Sellami *et al.,* 2024).

The Multi-Trait Stability Index (MTSI) is an important tool for making selection decisions. It overcomes the limitations of traditional linear selection indices by considering the economic weight of each trait, genotypic and phenotypic variances, covariance, and multicollinearity. By taking multiple traits into account, the MTSI helps in developing improved treatments and genotypes, providing significant benefits to both breeders and agronomists (Taleghani *et al.,* 2023; Sellami *et al.,* 2024; Soni *et al.,* 2024). Varieties with the lowest MTSI values are closer to the ideotype with the ideal type, indicating superior average performance and greater stability across all analyzed variables (Olivoto and Lúcio, 2020). Optimizing multi-trait selection indices helps breeders balance trade-offs between traits like grain yield and protein content while combining desirable traits such as yield and weed competitiveness. This method promotes genetic improvement and maintains diversity in wheat populations (Silva *et al.,* 2023). MTSI is an effective technique for selecting genotypes based on multiple traits, as it offers a straightforward and robust selection process (Mohammadi and Geravandi, 2024).

The primary objectives of this study were to: 1-Assess the interactions between genotype and environment for sixteen bread wheat genotypes across multiple traits. 2-Identify superior wheat varieties with high performance and stability under limited water, using the MTSI index. 3-Focus on traits influencing yields to help breeders develop drought-resistant varieties for future generations. This research aims to facilitate the identification of suitable genotypes and the planning of new hybridizations to improve resistance to water deficit stress.

MATERIALS AND METHODS

1. Experiment Description:

This study evaluated the effects of severe water deficit, with only once irrigation vs. full irrigation, on agronomic traits, including earliness, grain yield, physiological traits, and grain quality, for sixteen bread wheat genotypes. Table 1 lists the names and pedigrees of the studied genotypes. A field trial was held at the Sakha Agricultural Research Station in Kafr El-Sheikh, Egypt, from mid-November to early May, covering the 2022/2023 and 2023/2024 growing seasons. Wheat was sown at a density of 400 seeds per square meter using the drill method in a 4.2 m² plot with six rows, each 3.5 meters long and spaced 20 centimeters apart. Table 2 presents the region experiences an arid Mediterranean climate, with annual temperatures ranging from 8.4 °C to 31.8 °C and a mean annual precipitation of 0.4 mm. These data were obtained from the Central Laboratory of Agricultural Climate (CLAC, ARC), as Maximum Temperature (C°), Minimum Temperature (C°), and Relative Humidity% (RH), Rain: Precipitation (mm day^{-1}), Wind Speed (m/s) (WS). The rate of change was calculated using the formula: (Season 1 - Season 2) / Season 1 * 100.

The soil type is clay, with an EC of 3.15 and a PH of 8.15. A randomized complete block design (RCBD) with a split-plot arrangement was implemented using three replicates. Water treatment was assigned to the main plots, while the sixteen bread-wheat genotypes (12 lines and four check cultivars) were randomly allocated to the subplots. Irrigation open-field experiments utilized a surface irrigation system with two treatments: 1 - The full irrigation applied five times at different growth stages, and 2 - A single irrigation occurring 20 days after sowing. All the agricultural practices were applied as recommended. The data were collected at the appropriate time, encompassing the following studied traits: agronomic traits, yield components, grain quality traits, and physiological traits.

1- Planting material and Recording traits:

2-1 Agronomic traits:

1. Earliness traits: Across the whole plot, the plants were noticed for the duration from sowing until approximately 50% of the spikes in the plot emerged (Number of days to heading (DH). The number of days to physiological Maturity (DM) was measured as the number of days from sowing until about 50% of the peduncles in the plot turned yellow.

2. Plant height (PH) is measured in centimetres (cm) from the soil surface to the top of the spike, excluding awns.

3.Number of spikes/m^2 (NS/m^2), by counting the total number of spikes per square meter.

4.The number of kernels per spike (NK/S) was determined by averaging the grains in ten randomly selected spikes.

5. 1000-kernel weight (TKW) in grams, a random sample of 1000 grains was taken from each plot. Each sample was hand-counted and weighed.

6.Spike kernel weight (KW/S) in grams

7.Flag Leaf area (LA cm^2).
8.Grain Yield (GY) data were collected from the plot, recorded after threshing, and converted to ardb/fed (1 ardb = 150 kg at 14.5% moisture, and 1 feddan = 4200 m^2).

Table 1. Pedigree of the sixteen tested bread wheat genotypes

No	Genotype	Pedigree	Selection history* FCRI
1	Line 1	GIZA164 / SAKHA 61 // Giza 171	S.2012-170-020S-010S-07S-0S
2	Line 2	Vorobey / Giza 171	S.2012-171-030S-018S-05S-0S
3	Line 3	Sids 13/ Sids 12	S.2012-172-020S-020S-06S-0S
4	Line 4	Sids 13/ Sids 12	S.2012-173-020S-020S-06S-0S
5	Line 5	Sids 13/6/ GIZA 158 /5/ CFN /CNO "S" // RON /3/ BB / NOR 67 /4/ TL /3/ FN / TH //2*nar 59*2	S.2012-175-020S-020S-06S-0S
6	Line 6	Vorobey /6/GIZA 158 /5/ CFN /CNO "S" // RON /3/ BB / NOR 67 /4/ TL /3/ FN / TH //2*NAR 59*2	S.2012-176-020S-020S-03S-0S
7	Line 7	Giza158/5/CFN/CNO"S"//RON/3/BB/NOR67/4/TL/3/ FN/TH//NAR59*2/6/ Vorobey	S. 2012 -099S -099S-19S -0S
8	Line 8	Sids 13 /2/ GIZA164 / SAKHA 61	S.2012-177-030S-010S-02S-0S
9	Line 9	Sids 13 /2/ GIZA164 / SAKHA 61	S.2012-178-030S-010S-02S-0S
10	Line 10	Sids 13 /2/ GIZA164 / SAKHA 61	S.2012-179-030S-010S-02S-0S
11	Line 11	GIZA 158 /5/ CFN /CNO "S" // RON /3/ BB / NOR 67 /4/ TL /3/ FN / TH //2*NAR 59*2	S10232-3S-2S-4S-0S
12	Line 12	GIZA164 / SAKHA 61	S.9242-IBR-2BR-5BR-2BR-0BR
13	Giza 171	SAKHA 93 / GEMMEIZA 9	Gz 2003-101-1Gz-4Gz-1Gz-2Gz-0Gz
14	Misr 3	ATTILA*2/PBW65*2/KACHU	CMSS06Y00582T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY
15	Sakha 95	PASTOR/SITE/MO/3/CHEN/AEGILOPS SQUARROSA(TAUS)//BCN /4/WBLL1	CMA01Y00158S-040POY-040M-030ZTM-040SY26M-0Y-0SY-0S.
16	Vorobey	CROC-1/AE.SQ(224)//OPATA-M-85/3/PASTOR	CMSS96Y02555S-040Y-020M-050SY-020SY-6M-0Y

Source: According to the data of the Wheat Research Section, * FCRI = Field Crops Research Institute, ARC (Agriculture Research Centre), Giza, Egypt.

Table 2. Meteorological data for the first and second growing seasons and the change rate %, ▲ increased, ▼ decreased.

Month	Maximum air Temperature (ºC)				Minimum air Temperature(ºC)				Relative Humidity%				Rainfall (mm/day^{-1})			
	1st	**2nd**	**Change Rate %**		**1st**	**2nd**	**Change Rate %**		**1st**	**2nd**	**Change Rate %**		**1st**	**2nd**	**Change Rate %**	
Nov	26.8	29.1	-8.5%	▲	15.4	17.0	10.8%	▲	60.5	60.8	-0.5%	▲	0.1	0.2	66.7%	▲
Dec	24.2	23.8	1.9%	▼	12.7	13.0	-2.1%	▲	68.1	73.2	-7.4%	▲	0.9	1.2	25.0%	▲
Jan	21.5	20.9	2.8%	▼	10.0	9.6	4.3%	▼	72.9	67.1	8.0%	▼	0.6	0.4	45.3%	▼
Feb	19.6	22.0	-12.3%	▲	8.4	9.5	12.8%	▲	68.4	69.3	-1.4%	▲	0.8	0.2	72.6%	▼
Mar	26.4	26.4	0.3%	▼	11.9	11.5	3.2%	▼	56.8	59.0	-3.7%	▲	0.3	0.2	34.4%	▼
Apr	30.4	31.8	-4.8%	▲	13.8	15.1	-9.6%	▲	51.2	56.9	11.1%	▲	0.2	0.1	54.2%	▼

(The Change Rate % was calculated using the formula: $((1^{st} - 2^{nd}) / 1^{st}) * 100$)

2.2 Physiological traits:

Ten flag leaves from the main stems of ten randomly chosen plants per plot were used during the heading stage for physiological studies.

1. Normalised difference vegetation index (NDVI), measured by a field portable NDVI sensor (Green Seeker® Handheld Crop Sensor, Trimble Navigation Limited, Westminster, CO, USA) between 11:30 a.m. and 2:00 p.m., at the beginning of the grain filling stage.
2. Canopy temperature (CT) was obtained using a near-infrared temperature sensor (CEM DT 8835 infrared and K-type thermometer) at the completed flowering stage of each plot from 1:00 p.m. to 2:00 p.m. on a cloudless day.

3- The relative water content (RWC%) was measured according to the method outlined by (González and González, 2001).
4- Chlorophyll A and B content (CL µg ml^{-1}) was measured using N-N-Dimethyl-formamide and a UV-VIS Spectrophotometer according to (Moran, 1982)
5- Malondialdehyde (MDA, µmols g^{-1} FW.) was measured according to the methods of (Heath and Packer, 1968).
6-Enzymatic antioxidants: Catalase activity (CAT) (µmol min^{-1} g $protein^{-1}$) and Peroxidase activity (POD) (µmol min^{-1} g $protein^{-1}$) were determined according to the method of (Lum *et al.,* 2014).

2.3 Grain quality traits.
1- Grain protein content (protein, %) was measured according to (A.O.A.C., 2000).
2- Wet and dry gluten percentage (WG%, DG% %) and grain ash% (GA) were measured by hand washing 25 g flour, according to the standard method (Anonymous, 1983).
3 - Seed oil content was extracted by Soxhlet's extractor using Petroleum ether (60 - 80 °C) was preferred for extractions that continued for not less than eight hours (rate of siphoning was 6-7/hr.), according to the methods of (A.O.A.C., 2000).

2- Statistical analysis:

3-1 Analysis of variance and pair-wise comparison:

Before running analysis of variance, the Shapiro-Wilk test was done according to (Shapiro and Wilk, 1965) to make sure that the data were normally distributed, and also the (Levene test, 1960) was run to assess the equality of individual error variances.

A combined analysis of variance (ANOVA) of a split-plot design was carried out for the data collected over two seasons for each observed trait. To identify significant differences among treatment means, it is applied the least significant difference test (LSD) is applied at a probability level of 0.05.

3-2 Multi-trait stability index (MTSI) analysis:

The Multi-trait Stability Index (MTSI) ranked genotypes for agronomic, physiological, and grain quality traits, along with tolerance to severe water deficit. The stability of each genotype across environments was estimated by WAASB (Weighted Average of Absolute score obtained by Singular value decomposition from the Best linear unbiased predictor using linear mixed effect model). The MTSI index for 16 genotypes was calculated according to (Olivoto and Lúcio, 2020) with the "metan" package in R version 4.1.1.:

$$\boldsymbol{MTSI_i} = \sum_{j=1}^{f} [(\boldsymbol{F_{ij}} - \boldsymbol{F_j})^2]^{0.5}$$

The Multi-Trait Stability Index (MTSI) for genotype i is calculated using the scores of genotype i (Fij) and the scores of the ideotype genotype (Fj). The genotype with the lowest MTSI value is closest to the ideotype, demonstrating high mean performance and stability based on the examined variables and marked with red colour in the MTSI plot. This study considered five selection strategies: First Scenario: The selection process considers all traits under study.

Second Scenario: Selection is based on grain yield and earliness characteristics, including days to heading (DH) and days to maturity (DM).

The Third Scenario is selection concentrated on grain yield and specific agronomic traits, including (PH), (NS/m²), (NK/S), (TKW), and (LA), and the Fourth Scenario is selection that emphasizes grain yield, along with physiological traits such as NDVI, CT, RWC %, chlorophyll A and B, MDA, and CAT. Fifth Scenario: Selection based on both grain yield and grain quality traits, including Wet Gluten, Dry Gluten, protein content, Ash, and oil content. These scenarios aim to evaluate the impact of various characteristics on election outcomes. A selection intensity of 25% was applied to identify four elite wheat genotypes based on their average performance and stability, using the MTSI. Additionally, a selection intensity of 50% was employed to select eight wheat genotypes for the same criteria of average performance and stability.

RESULTS

1- Weather conditions during field trials:

Table (2) presents seasonal weather data, including maximum and minimum temperatures (°C), relative humidity (RH) (%), and daily rainfall (mm/day). During the first season of 2022-2023, January and February were the coldest months with the lowest average maximum and minimum temperatures (Table 2). The average maximum and minimum temperatures during the second season (2023 - 2024) show that maximum temperatures increased by 8.3%, while minimum temperatures rose by 10.8% compared to the first season. The second season was observed to be warmer than the first (Table 2). During both growing seasons, the relative humidity varied from 51.2% to 73.2%. In the second season, it increased, reaching a minimum of 0.5% and a maximum of 11.1% (Table 2). Furthermore, the precipitation measured in millimeters per day was ineffective for two consecutive growing seasons.

2- Mean performance of studied traits and reduction percentage:

2-1 Agronomic traits:

The Levene test confirmed the homogeneity of variances for nineteen studied traits, which allowed applying the combined analysis. Tables 3a and b show the combined mean performance and least significant difference (LSD) for the earliness and agronomic traits of 16 genotypes evaluated under normal (full irrigation) and water deficit (one irrigation) conditions. Significant variations ($p < 0.05$) were observed between the irrigation treatments(I) and genotype (G), and the interaction between the Irrigation treatment and genotype (I× G) for most measured traits.

The mean performance of earliness characters was summarized in Table 3a. Data showed that Lines 2, 3, 8, 9, and 11 had the earliest genotypes for DH and DM, under both normal and stress conditions. The results indicated that lines 12 and Giza 171 exhibited the latest heading dates, taking 107.2 and 106.83 days to reach this stage, respectively (Table 3a). Their corresponding days to maturity were 153.67 and 153.77 days, respectively. Data in Table 3a show the lowest reduction % in DH and DM days (0.32, 1.50, and 2.36, 1.22, respectively), observed in wheat genotype lines 6 and 7, indicating their stable heading and maturity dates under stress conditions. Moreover, the other wheat cultivars showed moderate mean values of days-to-heading and days-to-maturity during the two seasons.

Lines 1, 5, 6, and 7, along with Voroby, exhibited the highest plant heights (PH) under normal irrigation, measuring 128.33, 131.67, 125.83, 127.50, and 129.17 cm, respectively. The same trend was also held under stress conditions (Table 3a). Additionally, Lines 2, 3, 9, and 12 showed the least reduction in PH compared to the normal conditions of plant height. Plant height of the cultivar Giza 171 was greatly affected by water shortage, decreasing plant height from 125.83 cm to 98.43 cm, with a percentage of change rate being 21.77 %. The genotypes affected by water shortage, which led to a severe decrease in flag leaf area (LA), were: Lines 6, 8, Giza 171, and Misr 3. Meanwhile, the smallest decrease is experienced by Line 10 (Table 3a).

Table 3a. Mean values of agronomic traits for 16 genotypes across the two seasons of 2022/2023 and 2023/2024.

Genotypes	DH			DM			PH			LA		
	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%
Line 1	103.67	97.83	5.63	151.50	144.50	4.62	128.33	113.33	11.69	40.20	21.72	45.97
Line 2	97.17	90.83	6.52	148.17	137.17	7.42	114.17	104.17	8.76	51.29	17.37	66.15
Line 3	98.50	93.50	5.08	149.17	140.83	5.59	112.50	103.17	8.30	40.03	14.67	63.35
Line 4	103.17	99.67	3.39	150.33	145.00	3.55	115.83	100.83	12.95	60.30	19.65	67.41
Line 5	105.17	99.00	5.86	153.57	147.00	4.34	131.67	114.17	13.29	57.16	18.47	67.68
Line 6	103.67	103.33	0.32	150.50	148.67	1.22	125.83	114.17	9.27	44.36	9.26	79.13
Line 7	100.00	98.50	1.50	148.50	145.00	2.36	127.50	112.50	11.76	50.35	17.97	64.32
Line 8	91.33	85.67	6.20	149.00	144.33	3.13	113.33	95.00	16.18	59.06	16.32	72.36
Line 9	91.50	86.67	5.28	146.67	138.83	5.34	110.83	105.00	5.26	58.04	20.25	65.11
Line 10	100.83	93.50	7.27	150.00	140.67	6.22	123.33	103.33	16.22	49.44	27.28	44.82
Line 11	95.17	87.50	8.06	147.33	137.50	6.67	115.00	100.00	13.04	66.38	29.32	55.83
Line 12	107.17	92.50	13.69	153.67	141.50	7.92	117.50	110.00	6.38	65.36	24.93	61.85
Giza 171	106.83	97.67	6.84	153.77	140.33	8.68	125.83	98.43	21.77	74.03	20.86	71.82
Misr3	104.67	98.33	6.05	151.33	143.50	5.18	115.00	95.83	16.67	52.34	15.28	70.81
Sakha 95	104.67	101.00	3.50	149.67	145.00	3.12	123.33	103.33	16.22	74.37	26.76	64.02
Vorobey	105.17	101.67	3.33	152.67	143.50	6.00	129.17	108.33	16.13	49.73	24.67	50.40
Average	101.4	95.4	5.5	150.4	142.7	5.1	120.6	105.1	12.7	55.8	20.3	63.2
P values	G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001		
LSD $_{0.05}$	G=1.51 I=0.55 G*I=2.14			G=1.53 I=0.50 G*I=2.07			G =4.49 I=1.63 G*I=6.37			G =2.45 I=0.84 G*I=3.38		

N: Normal condition (Full irrigation), S: Stress condition (one irrigation), CR%: Change Rate percentage was calculated using the formula: ((N -S) / N) * 100), DH: days to heading, DM: days to maturity, PH: plant height, LA: flag Leaf area (cm^2), G: genotypes, I: Irrigation, G*I: Genotypes * Irrigation, and LSD: least significant difference, at 0.05 probability levels.

As anticipated, higher averages for yield and its components were observed in favorable environments with continuous supplementary irrigation. In contrast, water-deficient conditions led to a reduction in yield components compared to the normal circumstances. This includes agronomic traits such as number of spikes /m^2 (NS/m^2), Kernel weight per spike (KW/S in grams), thousand kernel weight (TKW in grams), number of kernels per spike (NK/S), and grain yield (ardb per fad).

Results obtained that genotypes line 4, line 8, line 3, and line 1 exhibited a high number of spikes/m^2 under normal irrigation conditions, with the least percentage change under stress conditions being 16.21, 18.48, 24.52, and 24.79%, respectively (Table 3b). Despite their high number of spikes/m^2 under normal conditions, the highest percentage of reduction rate was recorded by Sakha 95, Voroby, and Misr 3, and line 2, with values of 49.9, 40.2, 40.1, and 42.6%, respectively (Table 3b). The results presented in Table 3b also show that kernel weight per spike (KW/S) and number of kernels per spike (NK/S) varied significantly among the different studied genotypes under the two conditions. The two genotypes of Sakha 95 and Giza 171 exhibited the highest spike kernel weights under normal irrigation~~s~~ and the highest number of kernels /spike, as well as change rate percentages of 53.0 and 43.0%, for KW/S, respectively. In contrast, Lines 4 and 8 exhibited the heaviest spike KW/S and the lowest under normal condition NK/S, resulting in the lowest change rate percentages of 25.0% and 22.8%, respectively (Table 3b).

Table 3b. Mean values of agronomic traits for 16 genotypes under two irrigation treatments combined over the two seasons 2022/2023 and 2023/2024.

Genotypes	NSm²			KW/S			NK/S			TKW			GY		
	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%
Line 1	511.3	384.5	24.8	1.9	1.4	26.6	49.3	32.2	34.6	49.9	35.2	29.4	22.5	10.4	53.7
Line 2	518.2	297.5	42.6	1.9	1.2	37.9	50.7	26.8	47.2	50.1	30.3	39.5	23.0	7.3	68.2
Line 3	531.6	401.2	24.5	2.0	1.5	28.5	53.4	33.9	36.5	51.5	37.2	27.7	24.1	11.5	52.5
Line 4	505.0	423.1	16.2	1.9	1.5	17.3	48.6	36.4	25.0	48.7	40.0	18.0	21.7	13.0	40.3
Line 5	516.8	354.1	31.5	2.0	1.3	33.2	51.1	31.1	39.2	49.3	34.2	30.7	22.3	9.7	56.6
Line 6	505.8	353.5	30.1	1.9	1.3	29.8	48.9	31.8	35.0	49.3	34.9	29.2	22.3	10.3	54.1
Line 7	471.3	287.8	38.9	1.7	1.1	34.5	43.2	24.3	43.8	45.8	30.4	33.5	18.5	7.9	57.4
Line 8	460.4	375.3	18.5	1.7	1.4	17.8	41.5	32.0	22.8	44.8	35.5	20.8	17.5	10.2	42.1
Line 9	484.9	319.0	34.2	1.8	1.2	31.5	45.4	29.6	34.7	47.8	32.3	32.4	20.7	8.8	57.3
Line 10	541.8	381.8	29.5	2.1	1.4	33.4	55.6	32.7	41.1	51.5	36.0	30.1	23.6	10.5	55.6
Line 11	473.6	341.0	28.0	1.7	1.3	26.3	43.2	30.3	29.9	46.0	33.7	26.7	19.1	9.2	51.8
Line 12	509.2	349.4	31.4	1.9	1.3	31.7	49.8	29.4	40.9	49.2	32.8	33.4	22.1	8.6	61.3
Giza 171	602.6	386.8	35.8	2.5	1.4	43.0	66.9	32.9	50.9	59.1	35.8	39.4	29.7	10.5	64.6
Misr3	582.9	349.4	40.1	2.3	1.3	42.9	62.6	31.4	49.8	55.3	34.5	37.6	28.0	10.2	63.4
Sakha 95	629.0	315.0	49.9	2.6	1.2	53.0	70.5	29.0	58.9	58.9	31.9	45.9	30.5	8.5	72.3
Vorobey	527.1	315.1	40.2	2.0	1.2	38.1	52.1	28.1	46.1	50.7	31.2	38.3	23.7	8.0	66.1
Average	523.2	352.2	32.3	1.9	1.3	32.8	52.1	30.7	39.8	50.5	34.1	32.1	23.1	9.7	57.3
p values	G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001		
LSD 0.05	G =29.36 I=11.19			G =0.14 I=0.05			G =3.85 I=1.33			G =2.59 I=0.86			G=1.76 I=0.62		
	G*I=42.75			G*I=0.19			G*I=5.34			G*I=3.52			G*I=2.46		

N: Normal condition (Full irrigation), S: Stress condition (one irrigation), CR%: Change Rate percentage (The rate of change was calculated using the formula: ((N -S) / N) * 100), NS/m²: number of spikes/m², KW/S: kernel weight per spike (gm), NK/S: Number of Kernels per Spike, TKW: Thousand Kernel Weight (gm), GY: Grain Yield (ardb/fad)G: genotypes, I: Irrigation, G*I: Genotypes * Irrigation interaction, and LSD: least significant difference, at 0.05 probability levels.

Significant differences for thousand kernel weight (TKW) were observed among the studied genotypes across the two irrigation levels in both growing seasons. The cultivar Sakha 95 recorded low TKW, under stress conditions, exhibiting a reduction of 45.9% (from 58.88 g under normal conditions to 31.86 g under water deficit conditions). This was followed by Line 2 and Giza 171, which recoded a reduction of 39.5% and 39.4%, respectively. Lines 1, 2, 3, 4, 6, 8, and 11 showed the least reduction from normal to shortage water conditions.

The studied 16 genotypes demonstrated different responses under severe water shortage compared to normal conditions, revealing a significant difference for grain yield (GY ardb/fad) under control and stress treatments (Table 3b). Lines 4 and 8 recorded the lowest reduction values (40.32 and 42.14 %) for grain yield fed^{-1} under the two studied irrigation levels. Additionally, Lines 3, 5, 6, 9, 10, and 11 exhibited moderate redaction values for GY (Table 3b). The shortage of water resulted in the highest reduction of 72.25% for the cultivar Sakha 95, dropping from 30.53 to 8.47 Ardab/fed. This was followed by line 2, which showed a 68.15% reduction, decreasing from a normal value of 22.95 Ardab/fed under normal irrigation to 7.31 Ardab/fed under stress conditions. The two genotypes Voroby and Giza 171 showed reductions of 66.11 and 64.61%, respectively (Table 3b).

2-2 Physiological Traits:

Results in (Tables 4a and 4b) show that water stress significantly impacts various studied genotypes under severe water deficit (single irrigation), affecting all physiological traits except for NDVI, Cla, CAT, and POD. It is important to realize that the insignificant effect observed from the interaction between wheat genotypes (G) and irrigation treatments (I) in Tables 4a and b may be due to the similar responses of these genotypes to reduced irrigation. The rates of change, whether an increase or decrease, under water stress, did not significantly differ among the wheat genotypes.

The reduction in Normalized Difference Vegetation Index (NDVI) values due to water deficit ranged from 14.9% (Line 1 and 5) to 24.88% (Line 9), with an average reduction of 18.62 %. Among the wheat genotypes, Lines 1 and 5 showed the lowest reduction in NDVI values, with readings of 0.74 and 0.72, respectively, under normal conditions and 0.64,0.62 with one irrigation. Conversely, Lines 8 and 9 experienced the highest reductions in NDVI, at 24.37 and 24.88%, respectively (Table 4a).

The results in (Table 4a) indicate that the Canopy Temperature (CT) increased under water deficit conditions (one irrigation) compared to normal irrigation, unlike the rest traits. The genotypes Vorobey and Giza 171 exhibited the highest increases in canopy temperature (CT), with values of -39.10 and -36.97, respectively. In contrast, Lines 2, 7, 8, 11, and 12 showed the lowest increases in CT under one irrigation condition compared to full irrigation, with values of -20.61, -18.24, -15.46, -17.61, and -22.48, respectively (Table 4a). The average reduction (CR %) for relative water content (RWC) due to water deficit was 12.87%. Specifically, the RWC decreased from 83.6% under normal conditions to 72.4% under stress conditions. The highest RWC percentages were observed in lines 2, 6, 7, 9, 10, and 11, which had the lowest reduction ratios of 13.02, 8.23, 9.55, 12.47, and 10.75 %, respectively (Table 4a). In contrast, the other genotypes exhibit a slightly higher rate of decline in relative water content.

Table 4a. Effect of the interaction between irrigation treatments and 16 genotypes for physiological traits over the two seasons 2022/2023 and 2023/2024

Genotypes	**NDVI**			**CT**			**RWC**			**Cla**		
	N	**S**	**CR%**	**N**	**S**	**CR%**	**N**	**S**	**CR%**	**N**	**S**	**CR%**
Line 1	0.74	0.64	14.19	20.07	26.30	-31.06	82.03	67.67	17.51	10.73	7.54	29.69
Line 2	0.73	0.62	15.14	21.27	25.65	-20.61	83.32	72.48	13.02	11.86	7.91	33.31
Line 3	0.74	0.61	17.53	21.23	25.98	-22.37	89.42	73.56	17.73	10.56	7.12	32.61
Line 4	0.76	0.63	16.34	21.17	25.52	-20.55	85.21	73.38	13.88	11.00	8.02	27.10
Line 5	0.72	0.62	14.19	20.43	27.48	-34.50	86.25	73.25	15.07	10.53	7.38	29.96
Line 6	0.73	0.61	16.17	20.80	26.65	-28.13	85.09	78.09	8.23	10.98	7.30	33.56
Line 7	0.73	0.60	18.86	21.83	25.82	-18.24	86.27	78.03	9.55	10.76	8.04	25.26
Line 8	0.73	0.55	24.37	22.97	26.52	-15.46	83.43	71.17	14.70	10.93	8.13	25.65
Line 9	0.70	0.53	24.88	21.97	26.70	-21.55	83.13	73.75	11.29	10.53	8.18	22.34
Line 10	0.74	0.59	20.67	20.57	26.92	-30.88	80.27	70.26	12.47	11.18	7.43	33.57
Line 11	0.67	0.54	19.11	23.00	27.05	-17.61	83.91	74.89	10.75	11.34	7.92	30.16
Line 12	0.75	0.60	19.02	22.32	27.33	-22.48	79.58	69.30	12.92	11.01	7.50	31.89
Giza 171	0.76	0.63	17.51	19.70	26.98	-36.97	77.69	66.60	14.28	10.59	7.13	32.68
Misr3	0.77	0.62	20.13	21.27	26.25	-23.43	82.45	72.81	11.69	10.85	8.39	22.68
Sakha 95	0.72	0.58	19.11	20.70	26.57	-28.34	82.06	72.43	11.74	10.69	8.09	24.28
Vorobey	0.70	0.56	20.62	19.57	27.22	-39.10	82.04	72.98	11.05	10.64	7.11	33.13
Average	0.73	0.60	18.62	21.18	26.56	-25.71	83.26	72.54	12.87	10.89	7.70	29.24
p values	G = <.001 I= <.001 G*I = 0.981			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = 0.368		
LSD 0.05	G =0.048 I=0.018 G*I=NS			G =0.91 I=0.35 G*I=1.34			G =1.17 I=0.41 G*I=1.63			G =0.645 I=0.231 G*I=NS		

N: Normal condition (Full irrigation), S: Stress condition (one irrigation), CR%: Change Rate percentage (The rate of change was calculated using the formula: ((N -S) / N) * 100), NDVI: Normalized Difference Vegetation Index, CT: Canopy temperature, RWC%: Relative water content, Cla: Chlorophyll a content, G: genotypes, I: Irrigation, G*I: interaction between Genotypes by Irrigation and LSD: least significant difference, at 0.05 probability levels and NS: Non Significant.

The analysis of chlorophyll content in flag leaves (chlorophyll a and b) revealed that all studied genotypes showed a decline with varying degrees under severe water scarcity (Tables 4a and 4b). This trend accounts for the lack of significant interaction between genotypes and irrigation treatments, as all genotypes showed a decrease in chlorophyll content in flag leaves due to water deficit, albeit at different rates. The reductions in chlorophyll a ranged from 22.34% to 33.57%, while chlorophyll b decreased between 27.83% and 55.62%.

Lines 1, 3, 5, and 10 showed the lowest percentage change rates and reductions, as well as the most favorable MDA contents from full irrigation to water shortage (-297.86, -373.65, -282.43, and -368.54%). The highest increase in MDA values under stress conditions was observed in the Vorobey genotype (762.22), which also exhibited the most significant percentage change rate (-565.84) among the sixteen studied genotypes.

Under stressful conditions, the activity of catalase (CAT) and peroxidase (POD) increased significantly. The increased average rate percentage was - 128.12 and -28.45%, respectively (Table 4b). High catalase and peroxidase activities in the leaves were recorded for Line 3, Line 4, and Line 11, indicating that these lines may be tolerant. In contrast, genotypes with lower levels of catalase and peroxidase activities in their leaves may be more sensitive.

2-3 Grain-quality traits:

Table (5) presents the combined mean performance of grain-quality traits, showing significant effects from irrigation treatments, genotypes, and their interaction. The genotypes demonstrating the least reduction in wet and dry gluten contents under stress conditions in comparison to normal irrigation were line 2, line 4, and Giza 171. The reduction percentages for wet gluten were 10.36, 16.28, and 15.70%, respectively. In terms of dry gluten, line 4 and Giza 171 showed reductions of 17.55 and 6.99%, respectively, which are better compared to other genotypes listed in Table 5. In contrast, the genotype that suffered from irrigation stress was Voroby, showing a reduction of 42.26% for wet gluten and 41.19% for dry gluten. Table (5) indicates that genotype line 1 exhibited the smallest reduction in oil content from normal irrigation to stress conditions (16.11). Conversely, lines 5 and 7 showed the highest reduction rate percentage under the irrigation treatments.The protein content increased with water stress compared to normal irrigation. The interaction effects on quality traits revealed that line 10 and Vorobey experienced the greatest increase in protein content compared to normal irrigation treatments. The rate of ash decreased by an average of 12.95 % with deficit irrigation. Lines 1 and 3 showed the lowest percentage change under water shortage conditions.

Table 4b. Effect of the interaction between irrigation treatments and 16 genotypes for physiological traits over the two seasons 2022 /2023 and 2023/2024

Genotypes	Clb			MDA			CAT			POD		
	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%
Line 1	3.52	2.03	42.19	154.19	613.45	-297.86	0.22	0.55	-154.49	2.08	2.76	-32.67
Line 2	3.92	2.26	42.48	115.65	664.61	-474.70	0.23	0.56	-139.82	2.14	2.85	-33.39
Line 3	3.18	2.13	33.08	132.46	627.41	-373.65	0.22	0.55	-156.72	2.06	2.64	-28.11
Line 4	3.58	2.42	32.37	137.44	605.11	-340.27	0.22	0.56	-152.44	2.11	2.87	-36.06
Line 5	3.02	2.18	27.83	161.05	615.91	-282.43	0.22	0.54	-139.50	2.02	2.67	-32.29
Line 6	3.39	2.34	31.06	129.35	660.60	-410.71	0.22	0.51	-131.71	2.11	2.56	-21.11
Line 7	3.64	2.51	31.15	124.02	713.22	-475.10	0.22	0.54	-149.78	2.10	2.84	-35.41
Line 8	4.13	2.34	43.24	119.94	697.38	-481.46	0.22	0.56	-149.93	2.12	2.89	-36.46
Line 9	3.59	2.32	35.35	137.28	690.32	-402.86	0.33	0.55	-64.81	2.17	2.88	-32.99
Line 10	3.52	1.84	47.72	133.88	627.29	-368.54	0.22	0.54	-147.55	2.13	2.59	-21.63
Line 11	3.57	1.96	45.06	126.58	642.47	-407.56	0.21	0.55	-158.25	2.11	2.65	-25.79
Line 12	3.77	1.98	47.39	114.02	692.50	-507.35	0.22	0.54	-144.96	2.13	2.60	-22.08
Giza 171	3.87	1.72	55.62	112.14	664.82	-492.82	0.22	0.56	-147.56	2.07	2.61	-25.68
Misr 3	3.46	2.40	30.58	128.50	698.38	-443.47	0.44	0.56	-25.50	2.22	2.93	-31.71
Sakha 95	3.67	2.47	32.73	128.36	703.83	-448.33	0.33	0.56	-69.60	2.23	2.90	-30.16
Vorobey	3.38	2.27	33.02	114.47	762.22	-565.84	0.24	0.51	-117.32	2.37	2.63	-11.04
Average	3.58	2.20	38.18	129.33	667.47	-423.31	0.25	0.55	-128.12	2.14	2.74	-28.54
p values	G = 0.117 I= <.001 G*I = 0.050			G = 0.011 I= <.001 G*I = 0.010			G = 0.052 I = <.001 G*I = 0.151			G = 0.005 I = <.001 G*I = 0.221		
$LSD_{0.05}$	G = NS I = 0.135 G*I = 0.548			G =33.85 I = 17.53 G*I = 59.51			G = 0.072 I = 0.026 G *I = NS			G = 0.15 I=0.063 G*I=NS		

N: Normal condition (Full irrigation), S: Stress condition (one irrigation), CR%: Change Rate percentage was calculated using the formula: ((N -S) / N) * 100), Clb: Chlorophyll b content, MDA: Malondialdehyde, CAT: Catalase Enzyme, POD: Peroxidase Enzyme, G: genotypes, I: Irrigation, G*I: interaction between Genotypes by Irrigation and LSD: least significant difference, at 0.05 probability levels, NS: Non Significant.

3- Genotype selection and ranking by the multi-trait stability index (MTSI):

The Multi-trait Stability Index (MTSI) assesses multi-trait performance and stability together. A lower MTSI value indicates closer alignment with the ideal genotype, signifying more remarkable performance, stability, and resilience to severe water scarcity. Figures (1, 2, 3, 4, and 5) illustrate the ranking of genotypes using the MTSI plot method. This method aids in selecting the best genotypes for tolerance to severe water deficit by incorporating data on agronomic, physiological, and grain quality traits across various environments, with selection intensities of 25% and 50%.

According to the heat map shown in (Fig. 6), the colour scale indicates that lighter shades, such as white and light red, correspond to genotypes that are resistant or moderately resistant, as reflected by their low MTSI values (Fig. 6). These resistant genotypes are ranked across five different scenarios. In contrast, darker red shades represent susceptible genotypes, which are characterized by higher MTSI values, also listed in (Fig. 6).

3-1 Ranking genotypes by MTSI in the first Scenario:

When evaluating all traits with equal weights in the first scenario, the lines identified as the most stable genotypes among the 16 bread wheat genotypes were lines 2, 9, 4, and 7. These lines had the lowest MTSI values of 2.2, 2.3, 2.6, and 2.7, respectively (Fig. 1A and 6). The MTSI value of 2.7 serves as a cutoff point (represented by the red circle in Fig. 1A), considering a selection intensity of 25%. At a selection intensity of 50%, additional lines were noted: lines 3, 10, 12, and 5, which had the next lowest MTSI scores of 2.9, 3.0, 3.3, and 3.9, as shown in (Fig.1B and 6).

Table 5. Effect of the interaction between Irrigation treatments and wheat genotypes for grain quality traits over the two seasons of 2022/2023 and 2023/2024.

Genotypes	Wet gluten %			Dry gluten %			Oil %			Protein %			Ash %		
	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%	N	S	CR%
Line 1	30.0	22.5	24.9	11.4	9.2	19.0	2.6	2.2	16.1	12.6	14.2	-12.5	1.8	1.7	7.1
Line 2	28.2	25.3	10.4	11.1	8.1	27.5	2.9	1.6	42.9	13.5	14.1	-4.1	1.8	1.5	15.7
Line 3	27.7	21.0	24.1	10.3	8.2	21.1	3.1	1.7	46.5	11.0	12.4	-12.9	1.6	1.5	6.8
Line 4	29.7	24.9	16.3	12.2	10.0	17.6	2.7	1.7	36.0	11.2	13.4	-18.9	1.8	1.6	12.7
Line 5	31.2	23.0	26.3	11.1	7.9	29.0	4.8	2.1	56.1	11.2	14.5	-29.2	2.3	2.1	7.5
Line 6	32.1	20.3	36.9	11.5	8.0	30.6	3.3	1.8	44.6	12.7	16.1	-26.7	2.0	1.8	11.0
Line 7	33.4	27.4	17.9	12.4	10.0	19.1	3.4	1.5	55.4	12.8	15.8	-23.6	1.7	1.5	8.5
Line 8	27.6	20.1	27.1	10.3	8.3	19.5	3.4	1.8	47.6	11.2	14.3	-27.6	2.0	1.4	31.7
Line 9	28.9	20.4	29.5	10.4	8.2	20.9	3.2	1.6	50.5	11.2	15.5	-38.3	1.7	1.5	14.2
Line 10	38.8	30.7	20.9	14.1	10.9	22.9	3.4	2.1	37.6	9.7	16.6	-70.6	1.8	1.5	12.8
Line 11	36.4	23.4	35.7	13.9	9.4	32.5	2.8	2.0	28.6	12.7	15.6	-22.7	2.3	2.1	9.0
Line 12	31.5	23.5	25.3	12.2	9.4	22.6	2.7	1.9	31.5	12.6	14.1	-11.9	1.9	1.7	12.6
Giza 171	26.2	22.1	15.7	9.4	8.8	7.0	3.3	1.7	49.1	12.6	12.6	0.0	1.7	1.4	18.0
Misr3	30.3	21.2	29.9	10.6	7.7	27.2	3.6	1.7	53.3	11.1	15.8	-41.9	1.6	1.5	9.8
Sakha 95	28.3	20.1	29.0	9.7	7.3	24.5	3.6	2.1	42.8	11.1	15.8	-42.1	1.9	1.6	14.8
Vorobey	27.6	16.0	42.3	10.1	6.0	41.2	3.7	2.4	33.9	9.6	14.3	-48.9	2.1	1.8	15.3
Average	30.5	22.6	25.8	11.3	8.6	23.9	3.3	1.9	42.0	11.7	14.7	-27.0	1.9	1.6	13.0
p values	G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I= <.001 G*I = <.001			G = <.001 I =<.001 G*I = <.001		
LSD 0.05	G = 0.35 I= 0.15			G = 0.187 I = 0.085			G = 0.16 I = 0.39			G = 0.111 I = 0.035			G =0.02 I=0.006		
	G*I = 0.55			G*I = 0.303			G*I = 0.159			G*I = 0.147			G*I=0.025		

N: Normal condition (Full irrigation), S: Stress condition (one irrigation), CR%: Change Rate percentage was calculated using the formula: ((N -S) / N) * 100), G: genotypes, I: Irrigation, G*I: interaction between Genotypes by Irrigation, and LSD: least significant difference, at 0.05 probability levels.

3-2 Ranking genotypes by MTSI in the second scenario:

In the second scenario, when assessing the characteristics of grain yield (GY) and earliness, specifically DH and DM, the superior genotypes identified are line 1, line 3, line 9, and line 5. These genotypes achieved the lowest values of MTSI at a selection intensity of 25 % (Fig. 2A). Accordingly, the selected genotypes are expected to exhibit early and good productivity.

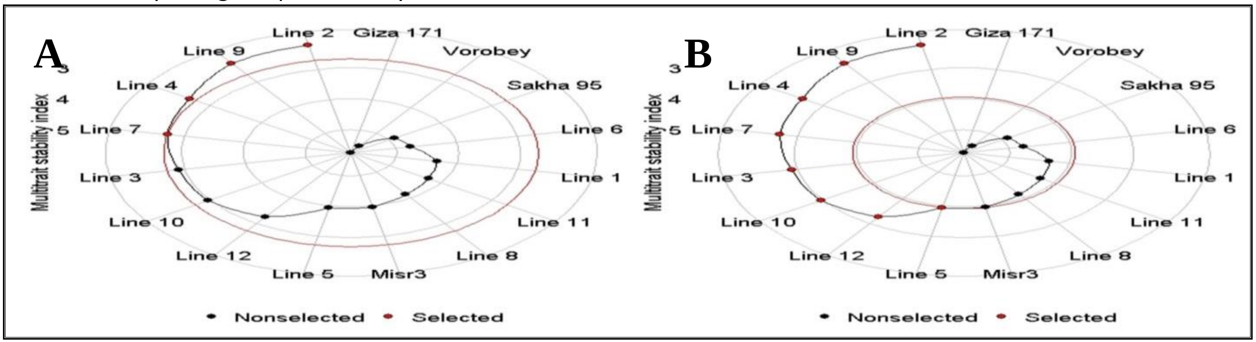

Fig. 1. Genotypes are ranked in ascending order based on the MTSI across all traits (first scenario) and environments. Red dots represent selected best genotypes with the central red circle indicating cutoff points for selection pressures of 25% (A) and 50% (B).

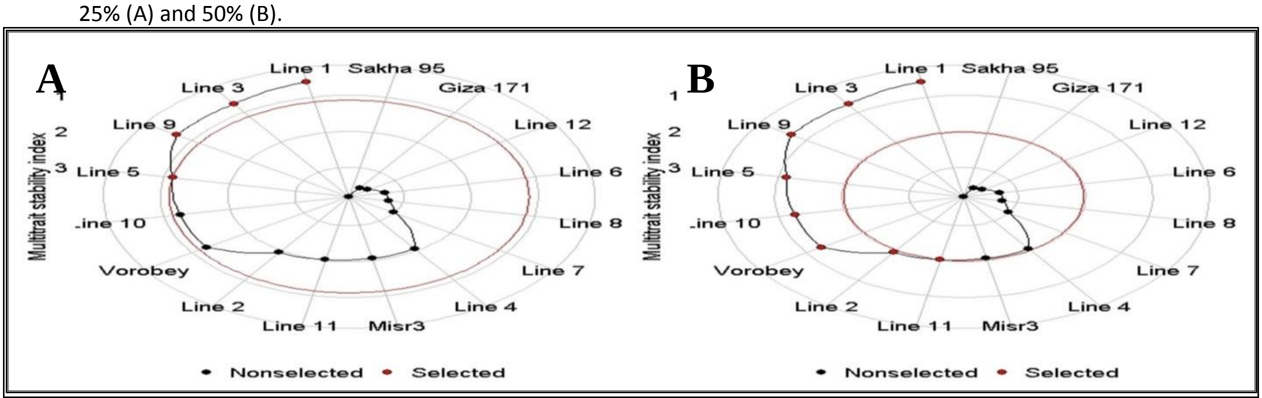

Fig. 2. Genotypes are ranked in ascending order based on (MTSI) across GY, DH, and DM (second Scenario) and environments. Red dots represent selected best genotypes with the central red circle indicating cutoff points for selection pressures of 25% (A) and 50% (B).

At a selection intensity of 50 %, additional genotypes were observed: line 10, Vorobey, line 2, and line 11, which exhibited the lowest MTSI scores among the 16 bread genotypes, as shown in (Fig. 2B and 6).

3-3 Ranking genotypes by MTSI in the third scenario

The selection process was based on lower MTSI values and 25% intensity in the third scenario, focusing on grain yield and specific agronomic traits, including PH, NS/m², NK/S, TKW, and LA (Fig. 3A). From the 16 evaluated genotypes, line 5 (MTSI = 0.38), line 3 (MTSI = 0.48), Line 10 (MTSI = 0.49), and line 6 (MTSI = 0.51) were selected based on the conditions of the present study among the 16 bread genotypes (Fig. 3A and 6).

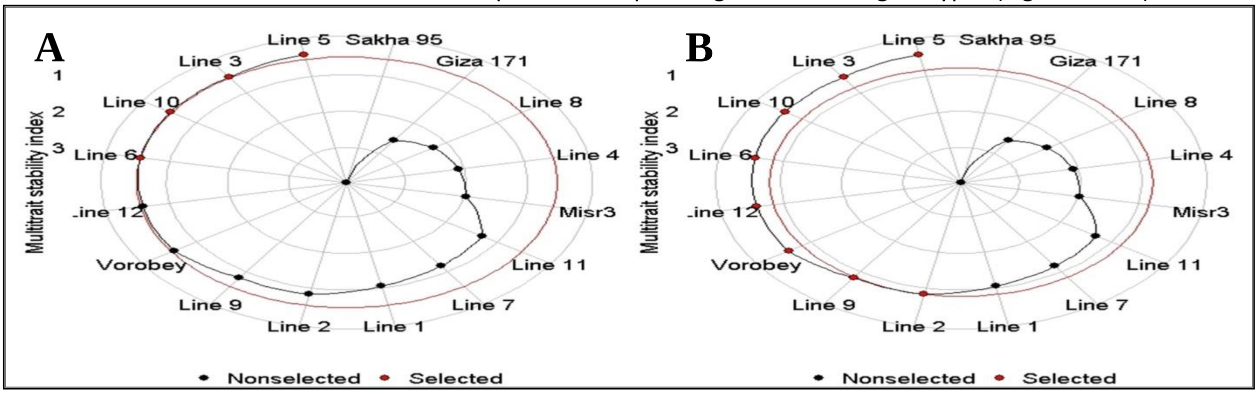

Fig. 3. Genotypes are ranked based on (MTSI) across GY and specific agronomic traits (Third Scenario). Red dots represent the selected best genotypes, with the central red circle indicating cutoff points for selection pressures of 25% (A) and 50% (B).

These genotypes demonstrated high stability and consistently strong mean performance for the traits analyzed in the third scenario. As shown in Fig. 3Bat a selection intensity of 50%, additional lines were recorded: Line 12 (MTSI = 0.51), Vorobey, Line 9 (MTSI = 0.56), and Line 2(MTSI = 0.80), among the studied 16 bread genotypes (Fig. 6).

3-4 Ranking genotypes by MTSI in the fourth scenario:

The fourth scenario is the selection that emphasizes grain yield, along with physiological traits such as NDVI, CT, RWC% %, chlorophyll a and b, MDA, and CAT at a selection intensity of 25 % and 50 % (Fig. 4A and B). In the multi-trait stability analysis, focusing on the fourth scenario, Lines 6, 2, 12, and 10 were found to be highly stable. These lines were selected at a selection intensity of 25% among the 16 bread wheat genotypes studied, and they exhibited low MTSI values (Fig. 4A and 6). When considering a selection intensity of 50%, Lines 3, 5, 7, and 9 were selected; they were identified as stable (Fig. 4B and 6).

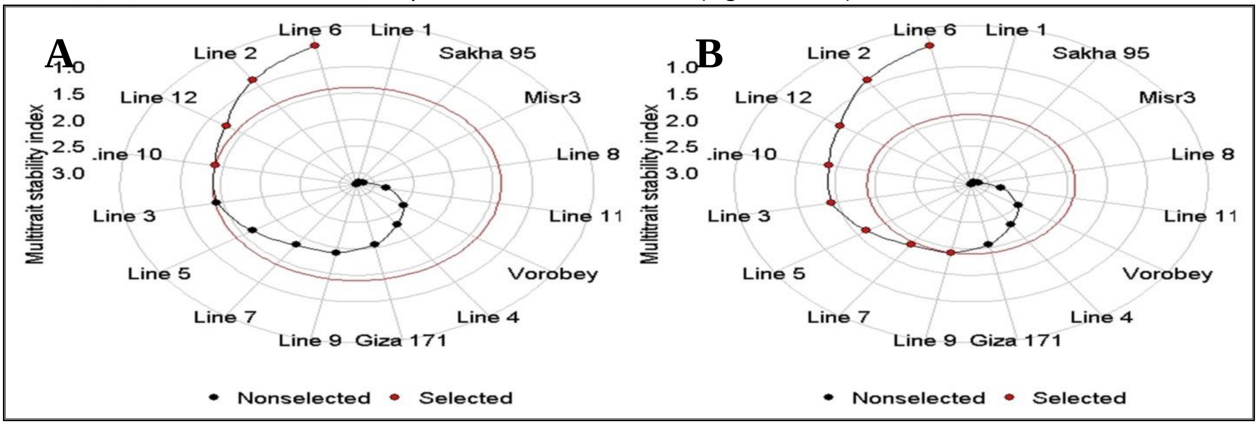

Fig. 4. Genotypes are ranked based on (MTSI) across GY and Physiological characters (fourth scenario). Red dots represent selected best genotypes with the central red circle indicating cutoff points for selection pressures of 25% (A) and 50% (B).

3-5 Ranking genotypes by MTSI in the fifth scenario

Likewise, as shown in (Fig. 5A and B), the fifth scenario selection is based on both GY and grain quality traits, which include wet gluten, dry gluten, protein content, ash, and oil content. When considering the index under water shortage conditions, lines 9,3,6 and 12 with the lowest MTSI values of 1.1 and 1.6 (Fig. 6) were identified as the most stable genotypes for grain quality traits among the 16 bread wheat genotypes at a selection intensity of 25% (Fig. 5A). Under selection intensity of 50 %, the selected genotypes were line 4, line1, line 10 and line 7 with the lowest MTSI values (Fig. 5B and 6).

It is worth noting that genotypes were very close to the cutoff point for the index in the scenarios (Figures 1B, 2B, 3B, 4B, and 5B), as indicated by the red line that spots the number of genotypes selected based on the selection pressure. These genotypes exhibit interesting characteristics, so it is worth focusing further on investigating those that are remarkably close to the cutoff point. Those genotypes considered in (Figures 1B, 2B, 3B, 4B, 5B, and 6) were Misr 3, line 8, and line 4 in the first and second scenarios, respectively. Lines 1 and 7 were nearly at the cut point in the third scenario, while Giza 171 was in the fourth scenario. Furthermore, Line 2 was close to the cut point in the fifth scenario.

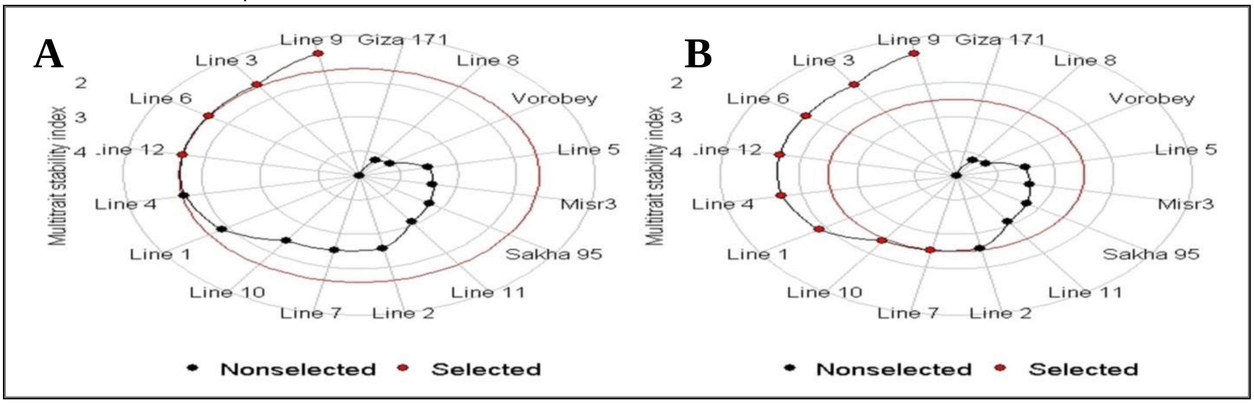

Fig. 5. Genotypes are ranked in ascending order based on MTSI across GY and grain quality traits (fifth scenario). Red dots represent selected best genotypes with the central red circle indicating cutoff points for selection pressures of 25% (A) and 50% (B).

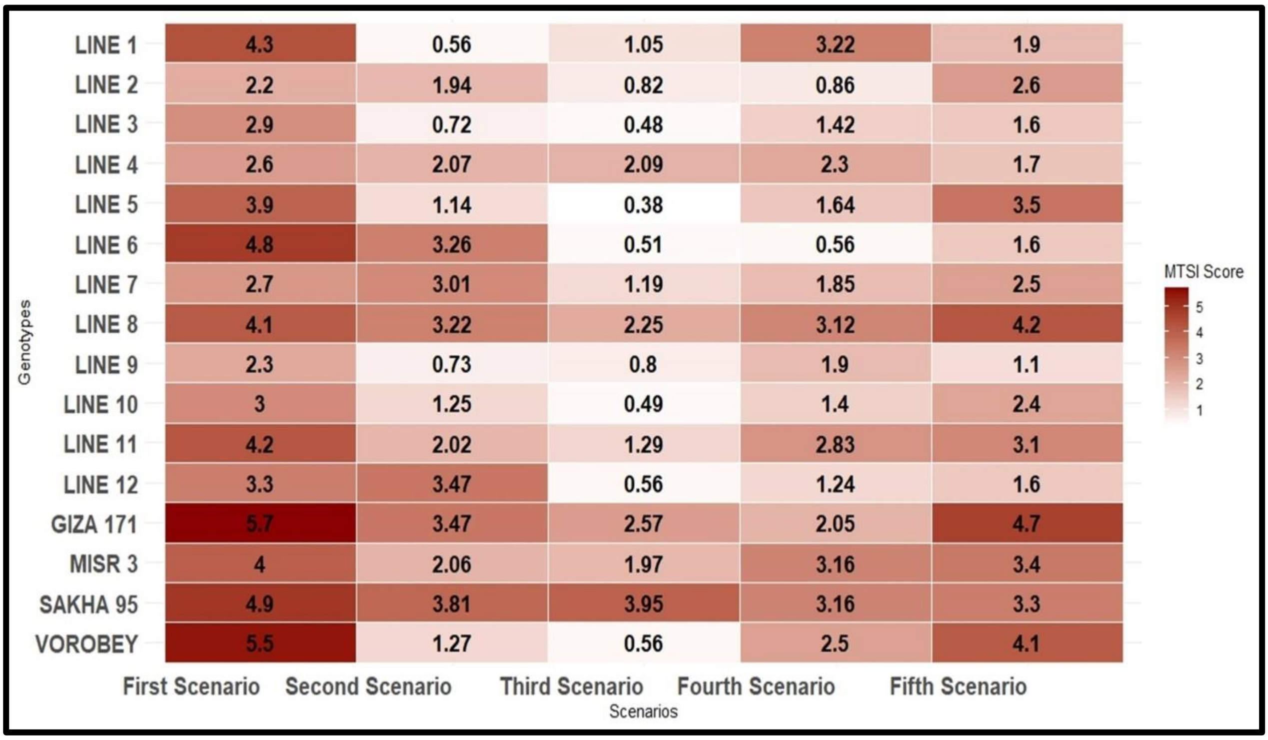

Fig. 6. Heat map to rank 16 bread wheat genotypes based on MTSI values across agronomic, physiological, and grain quality traits, along with tolerance to severe water deficit under five Scenarios.

DISCUSSION

Egypt is developing new varieties of wheat that can withstand drought and high temperatures in response to annual drought conditions. This initiative is essential due to the impacts of global warming, increasing population, and upstream water projects on the Nile. Currently, the world is facing a scarcity of freshwater, which particularly affects irrigated agriculture, the largest consumer of freshwater resources (Ingrao*et al.,* 2023; El-Aty*et al.,* 2024; Gabr*et al.,* 2024; Gamal *et al.,* 2024).

Impact of water shortage on yield, physiological, and grain quality traits:

Wheat cultivation under drought significantly affects morpho-physiological and agronomic traits, leading to reduced grain yield, particularly during critical growth periods. These factors underscore the need to identify new genotypes that yield high outputs with less water. Consequently, developing stable, high-yielding cultivars for varying drought conditions is a primary focus for plant breeding scientists (El-Hawary *et al.,* 2022; Bayisa *et al.,* 2024; Darwish *et al.,* 2024; Reddy *et al.,* 2024). The current study evaluated the grain yield, agronomic, physiological, and grain quality traits of 16 bread wheat genotypes over two consecutive growing seasons. The pooled ANOVA revealed significant interactions among genotype (G), irrigation (I), and their interaction (G x I), suggesting that these genotypes are suitable for estimating stability parameters. Significant effects for all recorded traits showed that genotype performances varied under different growing conditions, increasing diversity and facilitating the selection of the most appropriate and distinguished genotypes. Previous studies have reported similar findings (Al-Ashkar *et al.,* 2023; Darwish *et al.,* 2024; Mohi-Ud-Din *et al.,* 2024; Gab Alla and Hussein, 2025; Muhammad *et al.,* 2025; Mutanda *et al.,* 2025). These studies noted that wheat genotypes vary significantly in their yield performance under stress conditions.

Conversely, the lack of a significant interaction effect between wheat genotypes and irrigation treatments for certain traits may be due to the similar responses of these genotypes to reduced irrigation. In this case, the extent of change or decline under water stress did not differ markedly among the various wheat genotypes. This study reveals that a severe water deficit has a clear impact on crop development, shortening the generative phase and leading to earlier grain maturity. In dryland areas, early-maturing genotypes are preferred because they have the potential to withstand the extreme drought conditions that often occur toward the end of their growth period. Gab Alla and Hussein, (2025) and (Mutanda *et al.,* 2025) found that early-maturity genotypes were associated with low yields. Early maturity is a proxy trait for genotype selection with the drought avoidance mechanism. Water shortages negatively impacted key yield-affecting parameters such as plant height (PH), leaf area (LA), the number of kernels per square meter (NK/m²), kernel weight (KW), the total number of kernels per spike (NK/S), and the weight of 1000 kernels (TKW). As a result, there was an average decrease in grain yield (GY) of 57.3%, these findings are consistent with previously reported results by (Nyaupane *et al.,* 2024) who declare that drought stress during key growth stages, tillering, jointing, anthesis, and grain filling, can reduce yields by up to 46%, with severe drought during anthesis causing losses of up to 69%. It affects germination rates, leaf area, and dry weight, leading to earlier leaf senescence, increased root-to-shoot ratios, and earlier maturity. Sellami *et al.,* (2024) reported that the Water stress during the tillering stage led to a 21% reduction in tillers, 43% in flag leaf area, 12 % in plant height, and 19 % in dry matter compared to controls, highlighting the stage's sensitivity to water deficits. Drought has a significant impact on yield and its parameters, leading to greater overall damage (Bhandari *et al.,* 2024). Variation in agronomic traits is crucial for improving cultivars. In wheat, there is significant phenotypic and genetic variation for agronomic traits, demonstrating a wide range of diversity (Singh *et al.,* 2025). Drought stress during anthesis reduces the number of grains produced. This reduction leads to decreased water content in the shoot and an increase in abscisic acid levels, resulting in fewer grains being formed (Mahrookashani, 2023).

Vegetation indices like the normalized difference vegetation index (NDVI) and canopy temperature (CT) have been widely researched for predicting productivity across different crops and analytical methods. NDVI showed a stronger correlation with grain yield, while CT exhibited a significant negative correlation during drought, making both valuable high-throughput screening tools for assessing drought tolerance (Darwish *et al.,* 2024; Reddy *et al.,* 2024; Atanasov *et al.,* 2025; Yang *et al.,* 2025). Drought-tolerant genotypes are known to have cooler canopies, which reduce CT and are valued for their efficiency in transpiration and gas exchange, especially during water shortages, as they enhance leaf-cooling responses. In the current study, lines 4, 8, 11, and 3 display cooler canopies and demonstrate the least reduction in grain yield under stress conditions (once irrigation). These varieties exhibit tolerance to water stress by maintaining cooler canopies. These findings are consistent with the results of (Thakur *et al.*, 2022; Darwish *et al.*, 2024; Reddy *et al.*, 2024).

To improve their tolerance to drought, plants use osmotic adjustment mechanisms, which involve accumulating various organic and inorganic solutes. This process helps maintain cell turgor, allowing for cell expansion, growth, and development even under drought-stressed conditions (Nyaupane *et al.,* 2024). Wheat plants adapt to drought stress through various morphological, physiological, developmental, and molecular changes. When faced with environmental stress, they can quickly activate their defense mechanisms in response to future stressors (El-Hawary *et al.,* 2022; Darwish *et al*., 2024). Some physiological traits decrease under low irrigation conditions, such as chlorophyll (a, b) and leaf relative water content (RWC). Additionally, there was an increase in Malondialdehyde (MDA) levels under low irrigation treatment. MDA concentrations serve as a marker for stress. Research has shown that drought-sensitive wheat varieties tend to have higher levels of MDA content (Sallam *et al.,* 2019; Kirova *et al.,* 2021; El-Hawary *et al.,* 2022; Lamlom *et al.,* 2025). Grain yield was negatively correlated with oxidative stress markers (MDA and proline), indicating that increased oxidative stress correlates with reduced productivity. This suggests that higher levels of oxidative stress are associated with decreased productivity.

Our findings are consistent with the results of (El-Hag *et al.*, 2025; Lamlom *et al.*, 2025), which indicated that oxidative damage is inversely related to yield potential under abiotic stress. The mean performance showed that Lines had the lowest MDA under water deficit conditions, indicating their capacity for stress resistance. In contrast, the lines and genotypes that exhibited the greatest increase in MDA content during water stress suggest their potential sensitivity to water deficit. Both enzymatic antioxidants, catalase (CAT) and peroxidase (POD), increased in all lines and genotypes under low irrigation. Higher levels of CAT and POD were found in the leaves of wheat varieties tolerant to abiotic stresses like water deficit, heat, and salinity, aligning with previous findings (Kirova *et al.,* 2021; Amini *et al.,* 2023; El-Hag *et al.,* 2025). It has been previously shown that water stress leads to significant changes in grain composition, notably an increase in the protein content of the grains. Water stress affects wheat's protein composition, raising total protein levels but lowering wet and dry gluten, consistent with earlier research (El-Hawary *et al.,* 2022; Darwish *et al.,* 2024; Devi *et al.,* 2024). Drought severely impacts wheat growth and yield, so drought-tolerant genotypes that perform consistently across various environments are essential for sustainable solutions. Early phenotyping predictions of key wheat traits, like grain yield and stress tolerance, help accelerate the selection of promising breeding lines (Nyaupane *et al.,* 2024). Therefore, this study examines five scenarios with various characteristics to identify tolerant varieties.

Selecting genotypes with Multi-Trait Stability Index (MTSI)

Plant breeders strive to combine desirable traits into high-performing genotypes, but selecting ideal genotypes is challenging due to multiple traits (Pour-Aboughadareh *et al.,* 2021). Therefore, selecting genotypes based on a multi-trait stability index (MTSI) can enhance adaptability to current climatic conditions, which is crucial for hybridization programs (Olivoto *et al.,* 2019). The use of selection indices is an efficient strategy, as it allows the combination of multiple pieces of information for selection based on a complex of variables that integrate several attributes of economic interest (Silva *et al.,* 2023; Mohammadi and Geravandi, 2024). MTSI is calculated based on the genotype – idetype distance (Euclidean) derived from scores obtained through factor analysis. In terms of MTSI criteria, genotypes with lower MTSI values indicate greater stability across multiple measured traits (Bandeira *et al.,* 2025).

This study assessed the performance, drought tolerance, and ranking of 16 bread wheat genotypes using the MTSI. The evaluation was conducted across five scenarios, focusing on agronomic, physiological, and grain quality traits, with selection intensities of 25% and 50%. The MTSI allows for the selection of highly stable genotypes that exhibit superior average performance across important interacting traits (Elbasyoni *et al.,* 2023; Rajesh-Kumar *et al.,* 2025). This index is a reliable method for identifying elite genotypes based on their stability in multiple traits and their overall mean performance. Environmental conditions affect genotypic performance. Therefore, identifying genotypes that consistently perform well in different ecological conditions is essential, which can be achieved through stability analysis. The MTSI allows breeders to adjust trait levels, aligning genotypes with breeding objectives (Reddy *et al.,* 2024).

It is important to note that the lines with the lowest MTSI values across all scenarios are the breeds that should be incorporated into future breeding programs. Consequently, the focus was on enhancing wheat tolerance under water deficit via the performance and stability of agronomic, physiological, and grain quality traits across various environments (Memon *et al.,* 2023; Rajesh-Kumar *et al.,* 2025).On the other hand, genotypes with the lower MTSI values in the second and fifth scenarios emphasize the superiority regarding yield potential and water shortage tolerance via earliness traits and grain quality traits. Likewise, these genotypes present a high genetic potential regarding earliness and specific agronomic traits, including PH, NS/m², NK/S, TKW, and LA. Overall, the results should be considered, and the varieties that excel in certain traits should be included in breeding programs. These promising and distinguished varieties can help improve bread wheat's tolerance to severe water shortages (Mohammadi and Geravandi, 2024).

It's important to note that all the varieties examined had low values of the MTSI index. This indicates that the differences among the 16 genetic compositions are minimal compared to other scenarios, resulting in an insignificant outcome.

The use of selection indexes enables more efficient genotype selection across multiple traits, leading to time savings, enhanced effectiveness in breeding programs, better cultivar positioning strategies, and reduced waste of financial resources (Bandeira *et al.,* 2025; Rajesh-Kumar *et al.,* 2025).

CONCLUSION

The lines 2, 3, 5, 9, 10, and 12 are recommended for cultivation in areas facing water scarcity, as they achieve high grain yields under reduced irrigation water. These genotypes also exhibit superior physiological and agronomic traits, as well as high grain quality, under low irrigation conditions. Therefore, they can be considered for release as cultivars, pending extensive evaluation across multiple locations in Egypt.

Additionally, it is essential to incorporate these cultivars into breeding programs to improve wheat's resistance to severe water scarcity stress.

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تحديد تحمل بعض أصناف قمح تحت ظروف الاجهاد المائى الحاد باستخدام الانتخاب متعدد الصفات MTSI

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4 المعمل المركزي للبحوث في التصميم والتحليل الإحصائي، ، مركز البحوث الزراعية ، الجيزة، مصر

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أجريت هذه الدراسة في محطة بحوث سخا الزراعية لدراسة آثار نقص المياه الحاد على محصول حبوب قمح الخبز خلال موسمي الزراعة 2023/2022 و2024/2023، باستخدام تصميم القطاعات الكاملة العشوائية في نظام القطع المنشقة مره واحدة. وقد أدت التغيرات في الخصائص الزراعية والفسيولوجية إلى انخفاض محصول الحبوب من 23.1 إلى 9.7 (أردب/فدان)، بمتوسط انخفاض قدره 57.3% في ظل نقص المياه الحاد. تم استخدام مؤشر استقرار السمات المتعددة (MTSI) كمؤشر اختيار حديث للتراكيب الوراثية المتفوقة تحت ظروف العجز المائي الشديد. بناءً على القيم الصغيرة لمؤشر ثبات الصفات المتعددة (MTSI) عند شدة انتخاب 25%، تم اختيار أربعة تراكيب وراثية من القمح، وعند كثافة اختيار 50%، تم اختيار ثمانية تراكيب وراثية من القمح لمتوسط أدائها وثباتها. توصي هذه الدراسة، بزراعة السلالات 2 و3 و5 و9 و10 و12 في المناطق التي تعاني من ندرة المياه، حيث تحقق هذه السلالات غلة حبوب عالية مع الحد الأدنى من مياه الري. حيث أظهرت هذه السلالات المحددة موثوقية عالية، من حيث القدرة الانتاجية العالية، ونضجًا مبكرًا، مما يجعلها التراكيب الوراثية الاوفر حظا للاشتراك فى تحسين الأصناف والهجن، من خلال برامج التربية التي تركز على ضمان الأمن الغذائي في ظل ندرة المياه.

الكلمات المفتاحية: قمح الخبز؛ ندرة المياه، مؤشر ثبات الصفات المتعددة (MTSI)