

Phenotypic and SSR marker-based selection of M4 bread wheat (*Triticum aestivum* L.) putative mutant lines adapted to limited water availability in Egypt

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Abstract

Background: Developing high-yielding bread wheat (*Triticum aestivum* L.) cultivars adapted to limited water availability is a key strategy for sustaining wheat production in Egypt.

Hypothesis and objectives: In this study, we hypothesized that mutant lines in M3 and M4 generations could combine high productivity under well-watered and water-stress conditions. The objectives were to identify promising mutant lines under both conditions and assess genetic diversity. **Methods:** Field experiments were implemented at Experimental and Research Station, Cairo University (clayey soil), Egypt during the 2022-2023 growing seasons. The experiments were arranged in a split-plot design with four replications. Agronomic traits included spike weight, spike yield, grains per spike, 1000-grain weight, spikes m⁻² and grain yield. Heritability, GGE biplot analysis, phenotypic and genotypic correlation coefficients, path analysis SSR markers were applied.

Results and novelty: Significant differences ($p \leq 0.05$) were observed for all traits. 12 lines exhibited high grain yield relative to the respective mother. High heritability and high genetic advance determined spikes m⁻² and grain yield as effective selection criteria to detect promising mutant lines. GGE biplot analysis determined the mutant line Maryut-5100 as an ideal genotype, combining high productivity and stability performance. Grain yield was positive and significantly correlated with the studied traits. Path coefficient analysis confirmed that spike yield had the highest direct effect on grain yield, followed by spikes m⁻². SSR markers detected novel genetic (Genetic similarity 0.520-0.880). Cluster analysis differentiated the mutant lines from their mother cultivars. Analysis of molecular variance (AMOVA) exhibited 86% of the total genetic variation reflected unique genomic alterations. The putative mutant lines, particularly Maryut-5100, were recommended for confirmation trials aimed at developing high-yielding wheat cultivars adapted to limited water availability.

1. Introduction

Bread wheat is a cornerstone of global food, providing a significant proportion of the calories and proteins for the global population [1, 2]. In Egypt, domestic wheat production remains inadequate to meet the increasing demand, the widening gap between production and consumption [3]. Notable, bread wheat productivity and the harvested area in Egypt have declined from 2020 to 2024 (Fig. 1) [4].

This issue was complicated by climate change, which can reduce wheat yield by up to 60.0% [5, 6]. Water-stress conditions reduce photosynthetic efficiency by disrupting chlorophyll and increasing the accumulation of reactive oxygen species (ROS) [7, 8]. Given that rainfall in Egypt is inherently limited, erratic, and unpredictable [1, 9], forcing millions of farmers to depend on surface irrigation [10]. However, this reliance is strained by water scarcity and climatic volatility [8]. Therefore, rationalizing irrigation water use through new cultivars with reduced water requirements has become one of the most important strategies [11, 6]. In this study, genotypes were evaluated under well-watered and water-stress conditions to develop new bread wheat lines combining reduced irrigation frequency with high grain yield. Such mutant lines could contribute to water-saving while sustaining wheat productivity under limited irrigation water [12, 13]. However, breeding for drought tolerance is constrained by the low heritability of complex drought-tolerance traits and significant genotype-by-environment ($G \times E$) interactions [14].

Mutation breeding via ionizing radiation has emerged as a successful approach to overcoming some traditional breeding constraints for drought tolerance [15, 16]. Mutation breeding has contributed to inducing novel genetic variability for the improvement of grain yield and drought tolerance [17–19]. The fundamental idea of mutagenesis is to induce novel genetic variation through heritable modifications in key grain-yield components of bread wheat. This variability facilitates phenotypic selection to detect superior alleles controlling grains per spike, spike weight, spike yield and number of spikes per area, ultimately improving final grain yield potential. This approach is promising because these traits are readily measurable and observable under field conditions. However, direct selection is not enough to capture the full spectrum of induced genetic diversity. Therefore, integrating mutation breeding with Simple Sequence Repeats (SSRs) enables accurate assessment of genetic diversity. SSRs are commonly used in the wheat genome, highly polymorphic, and co-dominantly inherited, making them exceptionally effective tools for gene mapping and stress tolerance assessment [20–22]. However, few studies have used phenotypic selection with SSR markers to detect high-yielding wheat mutant lines under water stress in Egypt. Effective crop improvement requires knowledge of phenotypic and genotypic coefficients of variation, heritability, and genetic advance to improve the effectiveness of selection and predict genetic gain across segregating generations [23]. Numerous statistical techniques have been used to estimate stability and genotypes $\times$ environmental interactions, among which the GGE biplot graphic method is widely used [24, 25]. Additionally, correlation and path analyses of grain yield components are fundamental for forming selection indices to detect promising genotypes under different water regimes [26, 27].

In this study, we hypothesized that promising mutant lines in the M4 generation, developed via mutagenesis, would combine high productivity and adaptation under limited water availability, and that the combined use of phenotypic selection with SSR markers would effectively detect novel genetic variability useful for developing new bread wheat cultivars adapted under irrigation regimes. The present study aimed to evaluate the performance of new bread wheat mutant lines relative to their mother cultivars under well-watered and water-stress conditions, and assess genetic diversity among wheat genotypes using SSR markers.

2. Material and Methods

2.1.Plant material

The genetic material consisted of five commercial cultivars of bread wheat (*Triticum aestivum* L.,2n = 6x = 42, AABBDD) were officially released and can purchased through the Wheat Research Department, Field Crops Institute, Agricultural Research Center (ARC), Egypt. Therefore, no specific permission or collection permit was required for their use in the current study. The pedigree and characteristics of the mother cultivars were presented in the Table 1.

Table 1
Pedigree and Characteristics of mother cultivars

Cultivar	Pedigree	Characteristics
Sakha- 93 (P ₁)	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S	Resistant to rusts, tolerates salinity and heat, short plant height, more tillers
Sids-13 (P ₂)	KAUZ"S"//TSI//TSI/SNB"S"ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD	Resistant to rusts and water deficit, early maturing (152 d). Tolerates water salinity
Maryout- 5 (P ₃)	Giza 162 // Bch's /4/ PI-ICW 79Su511Mr-38Mr-1Mr-0Mr	High yielding and salt tolerant
Giza-168 (P ₄)	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B	Tolerates water deficit, heat tolerant, resistant to rusts, late maturing (165–170 days), medium plant height, white grain color, thin spikes
Gemmeiza-9 (P ₅)	Ald"S"/Huac"S"//CMH74A.630/5x CGM4583-5GM-1GM-0GM	Sensitive to salinity and water deficit, tall plant height, long spikes, late maturing (160 days),resistant to rusts

2.2. Characterization of the experimental site

Field experiments were conducted at Experimental and Research Station, Cairo university, Giza, Egypt (30.0295° N, 31.1885° E; WGS84), during the 2022–2023 growing seasons. The soil characteristics were presented in Table 2.

Table 2
The soil characteristics of the experimental site

Property	Soil type	Bulk density (g cm ⁻³)	Porosity (%)	Organic matter (%)	pH	EC (ds m ⁻¹)	CaCO ₃ (%)	CEC (cmol (+) kg ⁻¹)	Permanent wilting point (%)	Field capacity (%)
Value	Clay	1.3	50%	2.0%	7.8	1.0	4.0	35.0	18%	35%
CEC: Cation exchange capacity										

Soil samples were collected to estimate physical and chemical properties. This location was clayey, with moderate organic matter (2.0%), a bulk density of 1.31 g cm⁻³, a porosity of 50% and slightly alkaline reaction with a pH of 7.9. The electrical conductivity (EC) was 1.1 dS m⁻¹, calcium carbonate (CaCO₃) content was 5.0%, and the cation exchange capacity (CEC) reached 35 cmol (+) kg⁻¹. The permanent wilting point (PWP) was 18% and field capacity was 35.0%.

2.3.Irrigation treatments

Surface irrigation is common in Egypt [28]. Accordingly, two irrigation regimes were applied to generate environmental variation to evaluate the mean performance of bread wheat genotypes under both well-watered and water-stress conditions. The first regime represented well-watered conditions, in which irrigation was continued until physiological maturity (farmers practices), resulting in six irrigation events. The second environment represented limited-irrigation conditions, where irrigation was continued until the flowering

stage, resulting in four irrigation events. These contrasting environments served as selection pressure to select high-yielding under water-stress conditions.

2.4. Mutagen treatments

Three hundred dry grains per treatment per cultivar were used for irradiation. Seed moisture content was adjusted to 12.0% (w/w) prior to irradiation to decrease physical damage and enhanced mutation frequency. Grains of each cultivar were exposed to three doses of gamma rays (100, 200, and 300 Gy) using a Cobalt-60 source at the Egyptian Atomic Energy Authority, using a dose rate of 0.9 Gy s⁻¹. Exposure times were 111, 222, and 333 seconds for the respective radiation doses. Post irradiation, seeds were stored under cool and dry conditions (10°C) and 40% relative humidity to avoid physiological damage until sowing. An irradiation dose was selected based on previous studies [29, 30].

2.5. Field layout and experimental design

Each experimental plot consisted of three rows, each 3 m in length, spaced 60 cm apart, resulting a total plot area of 5.4 m². Sowing was manual and performed at a density of 350 grains m⁻². The mother cultivars (non-mutagenized grains) were used as controls. A 5 m spatial isolation distance was applied between plots to prevent any potential cross-contamination. The experiments were arranged in a split-plot design with four replications. Irrigation regimes were assigned to the main plot, while bread wheat genotypes were assigned to subplots. All recommended agronomic practices for wheat cultivation in the experimental region were uniformly applied during growing seasons.

2.6. Generations' advancement and selection density

The plant materials consisted of advanced mutant lines evaluated in the M3 and M4 generations, which were induced through gamma irradiation. The M1 and M2 generations were previously reported by Anter [31].

2.6.1. M3 generation (2022)

A total of 195 M2 bread wheat mutant lines (Supplementary, Table 1) were evaluated under two irrigation regimes. Selection was based on spike weight (SW, g), spike yield (SY, g), grains per spike (GS), 1000-grain weight (GW, g), spikes m⁻² (SN) and grain yield (t ha⁻¹). Eighteen superior mutant lines were selected, represented a selection density of approximately 10.0% from the M2 generation.

2.6.2. M4 generation (2023)

The selected eighteen mutant lines were re-evaluated as in the previous generation. Twelve superior and stable mutant lines demonstrated consistent performance under irrigation regimes represented a selection density of approximately 66.0% from the M3 generation. These lines were subsequently advanced for molecular characterization using SSR markers to validate genetic divergence and selection response.

2.7. Statistical analysis

Data were analyzed using a split-plot design [32], with four replications. Analysis of variance (ANOVA) was estimated to test the significance of genotypes, irrigation regimes effects and their interaction effects. The GGE biplot graphically computed to evaluate mean performance and stability across environments [33, 34]. Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated according to Singh and Choudhary [35]. Broad-sense heritability (hb%) was estimated, as described by Allard [36]. Genetic parameters were calculated from pooled data across environments separately for the M3 and M4 generations after testing homogeneity of error variance. Correlation analysis was calculated using variance and covariance components derived from replications. Path analysis was estimated using standardized correlation matrices, with grain yield considered as the dependent variable. All statistical analyses were computed using Gene Stat software [37].

2.8. Molecular studies

2.8.1. DNA extraction

Five cultivars and the twelve mutant lines of bread wheat were selected for molecular analysis for tolerance to water-stress conditions (1: Sakha-93, 2: Sids-13, 3: Maryut-5, 4: Giza-168, 5: Gemiza-9, 6: Sakha-93100, 7: Sids-13100, 8: Sids-13200, 9: Sids-13300, 10: Maryut-5100, 11: Maryut-5200, 12: Maryut-5300, 13: Giza-168100, 14: Giza-168200, 15: Giza-168300, 16: Gemiza-9100, 17: Gemiza-9200). Five seeds of each genotype were grown in the laboratory in earthen tubs containing soil collected from the nursery. Genomic DNA was extracted from young, fresh leaves of genotypes using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions,

and then genomic DNA was examined by loading it on a 0.8% agarose gel and separating it by electrophoresis. SSR markers amplification. Ten SSR primers (Table 3) were used for the amplification among seventeen wheat genotypes differing in drought tolerance. Each 20 μ L of PCR mixture consisted of 6.3 μ L (2X) of KAPA2G Fast Ready Mix 2, 0.5 μ L of forward primer, 0.5 μ L of reverse primer, a 1 μ L of DNA template, and nuclease-free water up to 11.7 μ L. In a Primus thermocycler, amplification was performed as follows: 95°C/3 min (one cycle) for initial denaturation, 94°C/1 min, 50–60°C (according to Ta of each primer)/45 s, 72°C/45 s (35 cycles), and 72°C/5 min (one cycle) for final extension. Then, holds were made at 4°C (infinite). The amplification product was separated by electrophoresis on agarose (3%) in 1 $\times$ TBE buffer for 2h at 90 V and stained with ethidium bromide, along with a DNA ladder. The gel was visualized under UV light.

Table 3 Names and sequences of SSR primers, forward sequence and reverse sequence			
No.	Primer	Forward Sequence	Reverse sequence
1	Cfd14	F: CCACCGGCCAGAGTAGTATT	R: TCCTGGTCTAACAACGAGAAGA
2	Cfa2049	F: ACGGCATCACAGGTAAAGG	R: GGTCTTTGCACTGCTAGCCT
3	gwm111	F: TCTGTAGGCTCTCTCCGACTG	R: ACCTGATCAGATCCCACTCG
4	Wmc396	F: TgcAcTgTTTTAccTTcAcggA	R: cAAAgcAAgAAccAgAgccAcT
5	Wmc517	F: ATccTgAcgTTAcAcgcAcc	R: AccTggAAcAccAcgAcAAA
6	Xgwm 11	F: GGATAGTCAGACAATTCTTGTG	R: GTGAATTGTGTCTTGTATGCTTCC
7	Xgwm 337	F: CCTCTTCCTCCCTCACTTAGC	R: TGCTAACTGGCCTTTGCC
8	Xgwm350	F: ACCTCATCCACATGTTCTACG	R: GCATGGATAGGACGCCC
9	Xwmc 78	F: AGTAAATCCTCCCTTCGGCTTC	R: AGCTTCTTTGCTAGTCCGTTGC
10	Xwmc 89	F: ATGTCCACGTGCTAGGGAGGTA	R: TTGCCTCCCAAGACGAAATAAC

2.8.2.SSR Data Analysis

The resulting gel images were examined using GelAnalyzer 3 software to determine the molecular sizes of the amplified fragments. The amplified fragments were scored as present (1) or absent (0). Polymorphic Information Content (PIC) and Expected Heterozygosity (He) were determined using the online software (<https://irscope.shinyapps.io/iMEC/>) according to Amiryousefi et al. [38]. Genetic similarity was calculated using the Dice coefficient, and the dendrogram was constructed based on the UPGMA algorithm using the NTSYSpc software.

2.8.3.Analysis of Molecular Variance (AMOVA)

To evaluate the distribution of genetic diversity, an AMOVA was performed using the binary SSR data. The 17 wheat genotypes were categorized into two groups: 5 original cultivars and 12 induced mutant lines. The analysis was executed using GenAlEx software (version 6.5) to partition the total genetic variance into components among groups and within groups.

3. Results

Analysis of variance (ANOVA) for the studied traits of bread wheat mutant lines in the M3 and M4 generations under irrigation regimes was presented in Table 4.

Table 4
Mean squares from the analysis of variance for the effects of irrigation regimes, genotypes and their interaction on the studied traits of bread wheat mutant lines in the M3 and M4 generations under both conditions

Sources of variation	SW(g)	SY (g)	GS	GW (g)	NS	GY(t ha ⁻¹)
M3 generation						
Replication	0.321	0.282	120.4	0.92	150.3	0.431
Irrigation treatment (E)	28.61*	35.40*	8200.2*	45.3*	18000.2*	145.81*
Error (a)	0.302	0.291	39.3	0.61	72.2	0.81
Genotypes (G)	4.81*	5.92*	1550.1*	99.22*	3700.4*	9.31*
G×E	1.23*	1.63*	420.2*	5.1*	900.5*	2.42*
Error (b)	0.38	0.451	122.3	0.92	247.2	0.66
M4 generation						
Replication	0.91	0.62	105	6.27	160.2	2.57
Irrigation treatment (E)	145.6*	120.4*	1850.0*	521.0*	2250.2*	412.86*
Error (a)	0.84	0.75	84.0	5.5	121.1	2.1
Genotypes (G)	4.3*	3.7*	930.2*	135.3*	956.4*	11.63*
G×E	1.8*	1.52*	244.3*	25.7*	256.3*	6.1*
Error (b)	0.18	0.17	20.9	4.14	26.7	0.47

Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m⁻² (SN), grain yield t ha⁻¹ (GY).

In the M₃ generation, irrigation treatments and genotypic showed significant effects ($p \leq 0.05$) on the studied traits. Furthermore, the significant genotypes × irrigation treatments interaction was significant for all traits. In the M₄ generation, irrigation regimes showed larger mean squares than those recorded in the M₃ generation. Genotypic effects remained highly significant for the studied traits. Likewise, The G × E interaction was significant for the studied traits.

3.1 .Mean performance

3.1.1. M3 generation

Table 5 showed mean performance and range of the studies traits for the mutant lines in the M3 generation and their mother cultivars under both conditions.

Table 5

Mean performance and range of the studied traits of bread wheat mutant lines in the M3 generation under both conditions

Genotype	SW(g)	SY(g)	GS	GW (g)	SN	GY (t ha ⁻¹)
Well-watered conditions						
Mother cultivars						
Sakha-93	7.9	5.5	102.1	54.3	108.1	6.1
Sids-13	6.7	5.9	97.3	46.0	145.2	6.5
Maryut-5	8.1	7.1	95.5	45.2	140.3	6.0
Giza-168	6.5	5.4	91.4	47.1	132.1	5.7
Gemiza-9	6.8	5.2	92.1	53.3	121.4	5.9
Mean (X̄)	7.2	5.8	95.4	49.1	129.2	6.0
Mutant lines						
Sakha-93 ₁₀₀	6.3	5.1	102.0	50.3	136.4	7.5
Sids-13 ₁₀₀	9.0	7.0	125.0	51.3	137.5	7.9
Sids-13 ₂₀₀	8.2	6.5	129.8	50.0	108.3	7.8
Sids-13 ₃₀₀	7.8	6.2	124.0	50.0	109.6	7.6
Maryut-5 ₁₀₀	8.7	7.1	138.3	50.0	108.8	7.3
Maryut-5 ₂₀₀	7.5	6.1	113.3	51.3	125.3	7.2
Maryut-5 ₃₀₀	7.9	6.3	125.5	50.0	118.6	7.0
Giza-168 ₁₀₀	6.5	5.2	103.5	50.0	130.9	6.9
Giza-168 ₂₀₀	9.3	7.5	129.5	51.5	101.2	6.7
Giza-168 ₃₀₀	7.7	6.3	109.0	51.8	117.8	6.7
Gemiza-9 ₁₀₀	7.8	6.1	116.3	50.3	111.4	6.6
Gemiza-9 ₂₀₀	7.8	6.4	108.0	51.3	123.8	6.5
G1	6.5	5.3	105.0	50.0	125.0	6.5
G2	6.8	5.2	104.0	50.0	124.4	6.5
G3	7.3	6.2	104.5	51.3	123.9	6.4
G4	8.4	6.7	133.0	50.0	95.2	6.4
G5	8.4	6.8	82.3	53.8	148.6	6.3
G6	7.0	6.0	120.5	50.0	102.2	6.2
Mean (X̄)	7.7	6.2	115.2	50.7	119.4	6.9
Range	6.3–9.3	5.1–7.5	82.3-138.3	50.0-53.8	95.2-148.6	6.2–7.9
CV%	19.3	12.3	15	16.7	9.2	8
Water- stress conditions						
Mother cultivars						
Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m ⁻² (SN), grain yield t ha ⁻¹ (GY). CV%: coefficient of variation, LSD _{0.05} : least significant difference						

Genotype	SW(g)	SY(g)	GS	GW (g)	SN	GY (t ha ⁻¹)
Sakha-93	5.9	4.7	86.9	50.2	94.7	4.3
Sids-13	5.4	4.5	90.1	49.7	93.9	4.2
Maryut-5	5.7	4.6	80.9	50.5	92.2	3.8
Giza-168	6	4.8	100.2	50.8	90.9	4.7
Gemiza-9	5.5	4.3	92.5	50.7	88.1	4.2
Mean ($\bar{X}$)	5.7	4.6	90.1	50.4	91.9	4.3
Mutant lines						
Sakha-93 ₁₀₀	7.0	6.0	96.8	49.8	102.9	5
Sids-13 ₁₀₀	6.0	5.0	97.2	50.1	100.4	4.9
Sids-13 ₂₀₀	5.7	4.7	96.0	51.0	97.8	4.8
Sids-13 ₃₀₀	7.0	5.9	102.0	49.8	96.7	4.9
Maryut-5 ₁₀₀	6.8	5.9	107.9	49.5	92.0	5
Maryut-5 ₂₀₀	5.5	4.3	77.8	49.8	90.8	3.5
Maryut-5 ₃₀₀	6.2	5.1	97.9	50.1	108.4	5.2
Giza-168 ₁₀₀	6.6	5.6	110.8	50.4	106.6	6.1
Giza-168 ₂₀₀	5.9	4.9	98.2	50.0	104	4.9
Giza-168 ₃₀₀	5.4	4.2	84.2	50.0	103.4	4.3
Gemiza-9 ₁₀₀	5.1	4.1	81.2	51.0	103.3	4.2
Gemiza-9 ₂₀₀	4.8	3.8	75.3	49.8	102.9	3.9
G1	5.8	4.8	95.5	49.8	102.3	5.0
G2	6.3	5.3	99.3	50.1	101.7	5.2
G3	5.0	4.1	81.4	50	98.7	4.0
G4	5.5	4.5	90.6	50.1	97.7	4.6
G5	4.8	3.9	77.9	49.6	96.8	3.8
G6	5.3	4.3	85.7	50.1	100.8	4.4
Mean ($\bar{X}$)	5.8	4.8	91.4	50	100.4	4.6
Rang	4.8-7.0	3.8-6.0	75.3-110.8	49.3-51.0	90.8-108.4	3.5-6.1
CV%	13.0	11.2	19.0	19.5	14.0	5.0
LSD _{0.05}	0.9	0.9	22.4	1.4	35.6	0.8
Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m ⁻² (SN), grain yield t ha ⁻¹ (GY). CV%: coefficient of variation, LSD _{0.05} : least significant difference						

Under well-watered conditions, four mutant lines (Sakha-93₁₀₀, Sids-13¹⁰⁰, Sids-13₂₀₀, and Sids-13₃₀₀) achieved higher grain yields (7.5–7.9 t ha⁻¹) compared to their respective mother cultivars (5.7–6.5 t ha⁻¹). Most mutant lines outperformed the highest-yielding cultivar, Sids-13, exhibited grain yield increases ranged from 15.0 to 36.0%. Most mutant lines showed 1.0–21.3% higher grain yield than higher cultivar. Mutant lines showed higher spikes m⁻² and heavier spike weight. In addition, the mutant lines exhibited wide range of variability

for all studied traits. Under water-stress conditions, all studied traits exhibited lower values compared with those under well-watered conditions. Nevertheless, 10 mutant lines outperformed the grain yield of the higher-yielding cultivar, Giza-168), with improvements ranged from 2.1% in Sids-13₂₀₀ to 29.7% in Giza 168₁₀₀. These promising mutant lines recorded higher grains per spike and spikes m⁻². Grains per spike and spikes M⁻² showed wide ranges of variation, whereas 1000-grain weight exhibited a narrow range. Based on agronomic performance, 12 promising mutant lines were selected to obtain the M4 generation for further evaluation.

3.2. M4 generation

Table 6 showed mean performance and range of mutant lines in the M4 generation with their mother cultivars under both well-watered and water-stress conditions.

Table 6

Mean performance and range of the studied traits of bread wheat mutant lines in the M4 generation under both well-watered and water-stress conditions

Genotype	SW(g)	SY(g)	GS	GW (g)	SN	GY (t ha ⁻¹)
Well-watered conditions						
Mother cultivars						
Sakha-93	8.7	6.6	105	56.5	110.2	6.5
Sids-13	7.0	6	100	48	155	7.4
Maryut-5	9.1	7.6	98	46	145	6.5
Giza-168	7.2	5.2	95	50	135	6.4
Gemiza-9	7.4	5.7	93	55	124	6.3
Mean (X̄)	7.8	6.2	98.2	51.1	133.8	6.6
Mutant lines						
Sakha-93 ₁₀₀	7.0	6.1	110.0	52.0	142.0	8.1
Sids-13 ₁₀₀	9.5	8.2	128.0	50.2	138.5	8.8
Sids-13 ₂₀₀	9.1	7.5	135.0	52.5	116.0	8.2
Sids-13 ₃₀₀	8.3	7.4	135.0	52.5	113.0	8.0
Maryut-5 ₁₀₀	8.1	6.9	118.0	52.6	128.0	7.9
Maryut-5 ₂₀₀	8.0	6.7	127.3	48.8	124.0	7.7
Maryut-5 ₃₀₀	7.4	6.2	109.0	49.8	135.0	7.3
Giza-168 ₁₀₀	10.1	8.2	136.0	48.7	106.0	7.0
Giza-168 ₂₀₀	8.1	6.8	115.0	52.3	121.0	7.3
Giza-168 ₃₀₀	8.0	6.8	121.0	48.2	115.0	6.7
Gemiza-9 ₁₀₀	8.3	7.0	114.0	50.1	127.0	7.3
Gemiza-9 ₂₀₀	7.5	8.4	110.0	48.9	130.0	6.9
Mean (X̄)	8.3	7.2	121.5	50.6	124.6	7.6
Range	7.0-10.1	6.1-8.4	109.0-136.0	48.2-52.6	106.0-142.0	6.7-8.8
CV%	7.7	19.5	19.3	11.4	18.4	19.0
Water- stress conditions						
Mother cultivars						
Sakha-93	6.1	4.9	91.0	52.0	91.0	4.5
Sids-13	5.6	4.6	100.0	53.0	86.8	4.6
Maryut-5	6.0	5.0	75.0	52.0	101.0	3.9
Giza-168	6.2	5.4	108.0	49.5	92.0	4.9
Gemiza-9	6.3	5.2	88.0	55.0	99.0	4.7
Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m ⁻² (SN), grain yield t ha ⁻¹ (GY). CV%: coefficient of variation, LSD _{0.05} : least significant difference						

Genotype	SW(g)	SY(g)	GS	GW (g)	SN	GY (t ha ⁻¹)
Mean ($\bar{X}$)	6.0	5.0	92.4	52.3	94.0	4.5
Mutant lines						
Sakha-93 ₁₀₀	6.9	5.1	109.0	51.0	107.0	5.9
Sids-13 ₁₀₀	6.4	5.0	109.0	52.0	105.0	5.9
Sids-13 ₂₀₀	6.4	5.1	99.0	53.0	109.0	5.7
Sids-13 ₃₀₀	6.7	4.9	95.0	54.0	114.0	5.8
Maryut-5 ₁₀₀	6.8	5.6	94.0	55.0	111.0	5.7
Maryut-5 ₂₀₀	6.5	5.5	95.0	49.0	106.0	4.9
Maryut-5 ₃₀₀	7.2	6.1	92.0	57.0	109.0	5.7
Giza-168 ₁₀₀	6.4	5.2	98.0	57.2	105.0	5.8
Giza-168 ₂₀₀	6.4	4.9	110.0	48.0	100.0	5.3
Giza-168 ₃₀₀	6.3	5.4	98.0	58.0	95.0	5.4
Gemiza-9 ₁₀₀	6.3	5.1	95.0	59.0	95.0	5.3
Gemiza-9 ₂₀₀	6.5	5.2	97.0	54.0	95.0	4.9
Mean ($\bar{X}$)	6.6	5.3	99.3	53.9	104.3	5.5
Range	6.0-7.2	4.9-6.1	92.0-110.0	48.0-59.0	95.0-114.0	4.9-5.9
CV%	9.0	12.5	14.4	14.5	19.1	17.9
LSD 0.05	0.55	1.0	20.0	9.5	72.2	0.85
Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m ⁻² (SN), grain yield t ha ⁻¹ (GY). CV%: coefficient of variation, LSD _{0.05} : least significant difference						

Under well-watered conditions, mutant lines exhibited outperformed their mother cultivars for most studied traits. The highest grain yield was recorded by mutant line Sids-13₁₀₀ (8.8 t ha⁻¹), followed by mutant line Sids-13₂₀₀ (8.2 t ha⁻¹), while all mutant lines consistently outperformed the check cultivar Giza-168. These mutant lines were higher grains per spike and spikes m⁻². Under water-stress conditions, all traits showed a reduction compared to well-watered conditions. However, mutant lines recorded higher mean performance than the mother cultivars. Mutant line Giza-168₁₀₀ recorded the highest grain yield (6.4 t ha⁻¹), followed by several lines exceeding the performance of the check cultivar Giza-168. Mutant lines exhibited high performance for the studied traits compared to their mother cultivars.

3.3.Genetic parameters

The estimates of genetic parameters for wheat genotypes in the M3 and M4 generations presented in Table 7. Higher values were recorded for traits recorded under well-watered compared with water-stress conditions.

Table 7
Heritability (hb%), phenotypic (PCV) and genotypic (GCV) coefficients of variability, and genetic advance (GA) of the studied traits for bread wheat mutant lines in the M3 and M4 generations under both conditions.

Para.	M3 generation						M4 generation					
	SW(g)	SY(g)	GS	GW(g)	NS	GYt ha ⁻¹	SW(g)	SY(g)	GS	GW(g)	SN	GYt ha ⁻¹
hp%	69.2	73.3	69.8	85.1	73.9	70.8	75.0	71.4	89.1	87.0	86.8	73.7
PCV%	16.7	22.5	19.5	10.8	28.0	26.8	12.5	14.2	11.6	11.0	10.7	17.2
GCV%	14.0	18.9	16.3	10.0	24.1	22.8	11.0	12.4	10.9	10.3	10.0	14.9
GA%	24.2	32.6	28.0	18.9	42.6	40.1	19.9	22.3	21.3	19.7	19.2	26.5

Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m⁻² (SN), grain yield t ha⁻¹ (GY).

High heritability estimates ($\geq 60\%$) coupled with moderate (10–20%) to high ($\geq 20\%$) genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were recorded for most traits under both conditions. Genetic advance (GA) was generally high ($\geq 20\%$) for the studied traits. In the M4 generation, genetic advance was reduced compared to the M3 generation.

3.4. Correlation coefficient and path analysis

Table 8 showed phenotypic (rp) and genotypic (rg) correlation between grain yield and the studied traits, along path analysis of mutant lines in the M4 generation. Grain yield was positively and significant correlated with all studied trait. Spike yield showed the highest correlation with grain yield, followed by spike weight and spikes m⁻².

Table 8
Phenotypic (rp) and genotypic (rg) correlation between grain yield and the studied traits and path analysis of bread wheat mutant lines in the M4 generation

Trait	Correlation coefficients		Path analysis		
	rp	rg	Direct	Indirect	Total
SW	0.87**	0.93**	0.28	0.59	0.87
SY	0.92**	0.97**	0.52	0.40	0.92
GS	0.79**	0.88**	0.22	0.57	0.79
GW	0.55**	0.68**	0.14	0.41	0.55
NS	0.83**	0.90**	0.31	0.52	0.83
R ²	0.969				

**; Significant level at 0.01, R²: coefficient of determination (R²) was 0.969. Spike weight (SW), spike yield (SY), grains per spike (GS), 1000-grain weight (GW), spikes m⁻² (SN), grain yield t ha⁻¹ (GY).

All studied traits showed high genotypic correlation values compared to phenotypic correlation. Path analysis further illustrated the nature of relationships. Spike yield showed the highest direct effect on grain yield, followed by spikes m⁻² and spike weight. Both grains per spike and 1000-grain weight showed exhibited moderate effect.

3.5. Genetic studies

3.5.1. SSR Marker Analysis and Polymorphism

The SSR markers created a total of fifty-three bands, of which forty-seven were polymorphic, resulting a high polymorphism percentage of 86.8%. Primer Xgwm350 was the most prolific and informative, conducting thirteen amplified bands, followed by Cfa2049 with eight bands (Table 9). In contrast, primers Cfd14 and Wmc396 were the least reactive, each producing only two bands. The average Polymorphism Information Content (PIC) was 0.3420 and an average Expected Heterozygosity (He) of 0.4460.

Table 9
Numerical data as obtained from PCR amplification by SSR primers among wheat genotypes

No.	Primer	AB	U B	PB	P %	PIC	He
1	Cfd14	2	0	2	100	0.1550	0.1690
2	Cfa2049	8	1	5	75	0.3740	0.4980
3	gwm111	5	0	3	60	0.3430	0.4390
4	Wmc396	2	0	2	100	0.3740	0.4980
5	Wmc517	5	0	4	80	0.3190	0.3990
6	Xgwm 11	4	0	4	100	0.3750	0.5000
7	Xgwm 337	4	0	4	100	0.3690	0.4880
8	Xgwm350	13	0	13	100	0.3720	0.4930
9	Xwmc 78	5	0	5	100	0.3620	0.4750
10	Xwmc 89	5	1	4	100	0.3740	0.4990
	Total	53	2	46	-	-	-
	Average	5.3	0.2	4.6	91.5	0.3420	0.4460

AB: amplified bands, UB: unique bands, PB: polymorphic bands, P%: polymorphism percentage, PIC: Polymorphic information content, He: expected heterozygosity

3.5.2. Genetic Relationships Analysis

The analysis of the genetic similarity matrix for the seventeen bread wheat genotypes (five mother cultivars and 12 mutant lines) revealed significant genomic variability, with Dice similarity coefficients ranging from 0.52 to 0.88 (Table 10). A high degree of genetic affinity between certain lines, most notably between genotypes 8 and 17, which exhibited the highest similarity value of 0.88, followed closely by the relationship between Sids-13₃₀₀ and Gemiza-9₂₀₀. Conversely, the lowest similarity coefficient of 0.52 was recorded between genotypes Sids-13₂₀₀ and Maryut-5₂₀₀. Other notable divergent relationships include genotypes Sids-13, Sids-13₂₀₀, Sakha-93₁₀₀, Maryut-5₂₀₀, Sids-13₂₀₀ and 15 Giza-168₃₀₀.

Table 10
Showed genetic similarity matrix based on Dice coefficient among the bread wheat genotypes based on SSR markers.

G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1																
2	0.72	1															
3	0.64	0.60	1														
4	0.73	0.62	0.68	1													
5	0.68	0.68	0.63	0.85	1												
6	0.59	0.57	0.69	0.63	0.62	1											
7	0.60	0.71	0.70	0.64	0.63	0.75	1										
8	0.60	0.54	0.59	0.65	0.64	0.62	0.64	1									
9	0.59	0.60	0.62	0.68	0.63	0.69	0.63	0.78	1								
10	0.64	0.68	0.78	0.84	0.80	0.59	0.63	0.60	0.71	1							
11	0.61	0.65	0.74	0.71	0.63	0.55	0.67	0.52	0.59	0.83	1						
12	0.74	0.60	0.65	0.77	0.72	0.64	0.62	0.67	0.73	0.76	0.72	1					
13	0.70	0.63	0.69	0.83	0.75	0.75	0.66	0.67	0.77	0.85	0.75	0.84	1				
14	0.66	0.75	0.68	0.69	0.65	0.67	0.71	0.62	0.79	0.77	0.77	0.83	0.79	1			
15	0.64	0.64	0.67	0.64	0.59	0.58	0.56	0.55	0.71	0.74	0.67	0.69	0.69	0.71	1		
16	0.59	0.66	0.77	0.69	0.68	0.79	0.74	0.61	0.68	0.77	0.71	0.70	0.76	0.75	0.74	1	
17	0.65	0.58	0.68	0.69	0.68	0.71	0.64	0.88	0.86	0.71	0.60	0.75	0.78	0.73	0.64	0.72	1

G: Genotypes **1:** Sakha-93, **2:** Sids-13, **3:** Maryut-5, **4:** Giza-168, **5:** Gemiza-9, **6:** Sakha-93₁₀₀, **7:** Sids-13₁₀₀, **8:** Sids-13₂₀₀, **9:** Sids-13₃₀₀, **10:** Maryut-5₁₀₀, **11:** Maryut-5₂₀₀, **12:** Maryut-5₃₀₀, **13:** Giza-168₁₀₀, **14:** Giza-168₂₀₀, **15:** Giza-168₃₀₀, **16:** Gemiza-9₁₀₀, **17:** Gemiza-9₂₀₀

UPGMA dendrogram analysis effectively grouped the seventeen genotypes into three clusters and one distinct outlier at a similarity coefficient of approximately 0.65 (Fig. 2). Cluster I was characterized by genotypes Sakha-93 and Sids-13, which maintained a lineage that was clearly differentiated from the rest of the study group. Cluster II represents the most expansive and intricate group, exhibiting further stratification into three sub-clusters: the first containing genotypes Maryut-5 and Maryut-5₂₀₀; the second comprising a larger set of Giza-168, Gemiza-9, Maryut-5₁₀₀, Maryut-5₃₀₀, Giza-168₁₀₀, and Giza-168₂₀₀) and the third including akha-93₁₀₀, Sids-13₁₀₀, and Gemiza-9₁₀₀. Cluster III grouped mutant lines Sids-13₂₀₀, Gemiza-9₂₀₀, 9 Sids-13₃₀₀, Sids-13₂₀₀ and Gemiza-9₂₀₀ displaying particularly high internal similarity. The mutant line Giza-168300 was the most genetically divergent plant, branching at the lowest similarity level and acting as a distinct outgroup compared with other groups.

3.5.3. Analysis of Molecular Variance (AMOVA)

The AMOVA results revealed that the majority genetic variation (86%) was attributed to differences within groups (among the individual mutant line). Meanwhile, the variation among groups (mother cultivars vs. mutant lines) accounted for 14% of the total molecular variance (Table 11).

Table 11
Analysis of molecular variance (AMOVA) between mother cultivars and their progenies (mutant lines).

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	Est. Var.	(%)
Among genotypes	1	8.42	8.42	0.92	14%
Within genotypes	15	86.15	5.74	5.74	86%
Total	16	94.57	-	6.66	100%
df :Degrees of freedom, Est. Var. :Estimated variance					

The results obtained from the genetic similarity matrix, cluster analysis, and AMOVA provided a consistent overview of the genetic structure of the studied wheat genotypes. The high internal variation (86%) identified by AMOVA, with the diverse branching observed in the UPGMA dendrogram, where mutant lines occupied various clusters away from their parents. Furthermore, the genetic distance recorded in the Dice matrix (0.52) confirms that radiation-induced mutations were effective in expanding the genetic base of the parental cultivars, creating novel profiles suitable for selection in breeding programs.

4. Discussion

Crop breeders aim to induce and exploit genetic variability to select promising mutant lines for commercial deployment to face issues [39, 40]. In this study, mutagenesis induced significant genetic variability for bread wheat improvement under well-watered and water-stress conditions, as indicated by significant differences among wheat genotypes for all studied traits in the M4 generation under both conditions. These findings aligned with previous studies demonstrated that mutation breeding generated wide genetic variability, enabling selection of high-performing genotypes under adverse conditions [41–43]. Notable, the magnitude of mean squares was decreased relative to well-watered conditions, reflecting the negative effect of water-stress conditions on trait expression and consequent reduction in observable genetic variance. Nevertheless, two mutant lines, Maryut-5₁₀₀ and Sakha-93₁₀₀, consistently outperformed their mother cultivars under both conditions (Fig. 3), indicating enhanced drought tolerance without grain yield penalty under well-watered conditions.

The high grain yield of the selected M4 mutant lines was attributed to improvements in grains per spike and spikes m⁻², indicating that mutagenesis induced substantial genetic diversity and enabled the fixation of favorable alleles. The high water-holding capacity of the clay soils (Table 2) likely maintained soil moisture availability (17%), reduced the impact of water-stress conditions during growth stages. The favorable interaction of these mutant lines with soil water dynamics enabled them to combine high grain yield with lower water requirements, thereby representing ideal genotypes under different conditions. Putative mutant line Maryut-5₁₀₀ combined high productivity across irrigation regimes, matching the site's moderate water availability (17%) and supported its selection as promising line. By contrast, the mother cultivars showed limited interaction with soil water dynamics, which was attributed to their weaker performance under water-stress [18, 44]. According to Nyaupane et al.[7], water-stress conditions reduced chlorophyll content and impaired photosynthetic capacity. These findings highlighted the effectiveness of mutagenesis in inducing genetic variability and demonstrated strong potential for selection for genetic enhancement under water-stress conditions. Similar findings have been reported [12, 13].

Genetic parameters values provided comprehensive insights into the nature and magnitude of variability among bread wheat mutant lines (Table 7). The close correspondence between PCV and GCV for several traits across generations under both conditions indicated low environmental influence on trait expression, reinforcing the reliability of phenotypic selection. However, under water-stress, the observed reduction in genetic variance suggested that water-stress hindered physiological and metabolic processes, thereby holding part of the genetic potential. This was due to water stress suppressing physiological and metabolic processes. However, the persistence of remarkable genetic variation under water-stress indicated the potential for effective selection of high-yielding lines. Importantly, high heritability ($\geq 60\%$) coupled with high genetic advance ($\geq 20\%$) as % of mean trait for all studied traits in the M4 generation suggested predominance of additive gene action, which was fixable through selection. This, in turn, enhanced the efficiency of phenotypic selection in early generations (Fig. 4).

Furthermore, the decline in genetic parameters from M3 to M4 generation reflected fixation of favorable alleles in the 12 promising mutant lines, thereby enhancing the efficiency of selection in advanced generations. These stabilizations enhanced selection efficiency by decreasing within-lines heterozygosity while maintaining between-lines variation [45–47].

Integrating genetic parameters with the GGE biplot analysis strengthened the reliability of selection decisions across water regimes. Breeding for drought tolerance was limited by genotype-by-environment interaction [14]. In this study, the GGE biplot detected bread wheat mutant lines that combined high grain yield with stability performance under both water regimes. The biplot graphically described the genotypes' main effects (productivity; PC1) and their interactions (stability; PC2). Putative mutant line Maryut-5100 was positioned on the positive side of PC1 and near zero on PC2, indicating high productivity and stability across water regimes. Consequently, this line was classified as an ideal genotype. Three mutant lines, Sids-13₂₀₀, Sakha-93₁₀₀ and Sids-13₁₀₀, were positioned away from the horizontal axis (high PC2 values), indicating high productivity coupled with G X E interaction and reduced stability. Meanwhile, mutant lines Giza-168₂₀₀, Giza-168₃₀₀ and Marut-5200 were located near the center point, indicating that mean performance and moderate stability (Fig. 5).

These findings aligned with previous studies of GGE biplot analysis for yield stability assessment in bread wheat genotypes [26,33–34].

Correlations analysis revealed that genotypic correlations were higher than their corresponding phenotypic correlations. Moreover, all studied traits showed positive and significant correlations with grain yield, indicating these correlations were mainly controlled by genetic factors with low environmental influence, supporting phenotypic selection effective for detecting high-yielding bread wheat mutant lines under water-stress conditions. Path coefficient analysis further illustrated the causal underlying these correlations. Spike yield showed the strongest direct effect on final grain yield, detecting it as the primary selection for grain yield improvement. In addition, spikes m^{-2} and spike weight showed moderate direct effects, whereas grains per spike and 1000-grain weight showed relatively weak direct effects but substantial indirect effects via spike yield. The coefficient of determination (R) illustrated that 96.9% of the total variation in grain yield was explained by the studied traits under both conditions, highlighting the reliability of the path analysis. These findings suggested that a selection index containing these traits would effectively detect promising mutant lines adapted to water-stress [48, 49].

Integrating SSR markers with AMOVA and cluster analysis revealed that gamma radiation-induced mutations significantly broadened the wheat genetic base, providing a robust genomic foundation for selecting drought-tolerant genotypes. The high polymorphism rate observed in this study (86.8%) underscores the potency of gamma radiation in inducing significant genomic changes in wheat, aligning with the 86.67% reported by Al-Naggar et al. [29] and Azad et al. [50]. These results, characterized by an average PIC of 0.342 and expected heterozygosity ($H_e = 0.446$), are highly consistent with the genetic diversity indices reported by Turkoglu et al. [51] (PIC = 0.43; $H_e = 0.46$) and Kumar et al. [52] (PIC = 0.30) in bread wheat. Furthermore, the ability of SSR markers to differentiate between original and induced genotypes under stress conditions underscores their efficacy as a tool for assessing stress tolerance [20, 53]. Ultimately, these variations confirm that induced mutations provide a richer allelic reservoir than traditional breeding, offering a robust genetic basis for selecting genotypes with enhanced drought tolerances. The observed similarity coefficients (0.52–0.88) align with the ranges reported by Bohn et al. [54] and Drikvand et al. [55]. The lower similarity limit (0.52) underscores the creation of genetic changes, a phenomenon corroborated by Al-Naggar et al. [29], who recorded even greater parental divergence (40–42%) following gamma radiation. The clustering of mutants into groups distinct from their parental lines aligns with the findings of Zulfiqar et al. [56]. These genetic changes support the conclusions of Hussain et al. [57], confirming that physical mutagens effectively broaden the genetic base for agronomic traits. This provides a robust framework for selecting drought-tolerant genotypes in crop improvement and breeding programs. The AMOVA results revealed that 86% of the total genetic variation resided within the studied genotypes, whereas 14% was partitioned between groups (parents vs. mutants). This high level of within-group variation suggests that each mutant line followed a distinct evolutionary trajectory despite its common parental background, consistent with the findings of Hussain et al. [56]. Such high within-population variation is a critical indicator for breeding programs, as it reflects a diverse "genetic reservoir" essential for selecting stress-tolerant traits without compromising elite parental characteristics [56]. The selected mutant lines, especially Maryut-5100, could advance to multi-location yield trials under different water regimes to confirm their adaptability and stability.

5. Conclusion

This study illustrated that mutation breeding was a successful approach for inducing useful genetic variability in bread wheat to accelerate the development of promising mutant lines adapted to water available. Evaluation of the M4 mutant lines under well-watered and water-stress conditions revealed significant genetic variation, and detected high-yielding mutant lines that superiority over their mother cultivars. Genetic parameters, including high heritability and advanced genetic estimates for spike yield, spike weight and spikes m^{-2} , highlighted the effectiveness of phenotypic selection for these traits. GGE biplot analysis selected Maryut-5100 as an ideal genotype, combining high productivity (PC1) and stability (PC2) under both conditions. Correlation and path coefficient analyses revealed that spike yield, spike weight and spikes m^{-2} were reliable selection criteria for improvising productivity. SSR markers analysis

revealed a high polymorphism (86%) and substantial genetic divergence between mutant lines and their mother cultivars, confirming the successful induction of novel genetic resources through mutagenesis. AMOVA further illustrated that the majority of genetic variation resided within genotypes attributed to genetic factors. From a breeding perspective, these promising mutant lines, particularly Marut-5100, represent valuable potential genetic resources for cultivar development programs aimed at improving bread wheat productivity under water-limited environments in Egypt.

Declarations

Consent to participate

Not applicable.

Conflict of Interest Statement

The authors declare no conflict of interest.

Statement of Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

No custom code was developed in this study. All analyses were performed using standard statistical software.

Plant collection permissions

No specific permits, licences, or permissions were required for the plant materials used in this study. The parental bread wheat cultivars were officially released commercial cultivars obtained from the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt, where they are commercially available for purchase. No plant specimens or seeds were collected from natural habitats, government-owned land, protected areas, or private farmland. The M4 mutant lines were developed by the authors as part of their research program.

Authors' contributions

A.Anter. (corresponding author) was responsible for the development of the mutant lines, field evaluation, statistical analysis, and manuscript writing. G.S. contributed to the SSR molecular analysis and participated in manuscript writing. Both authors read and approved the final manuscript.

Declaration of competing interests

No

Ethics approval and consent to participate:

Not applicable. This study did not involve human participants, animals, or clinical trials. The plant materials used in this study were cultivated bread wheat (*Triticum aestivum* L.) cultivars obtained from the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt. These cultivars are officially released commercial cultivars and are commercially available for purchase. Their use complied with all applicable institutional and national guidelines. No plant materials were collected from wild populations, protected areas, government-owned land, or private farmland; therefore, no collection permits or licenses were required. The M4 mutant lines were developed by the authors as part of this research program.

Consent for publication

Not applicable.

Clinical trial registration

Not applicable.

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Figures

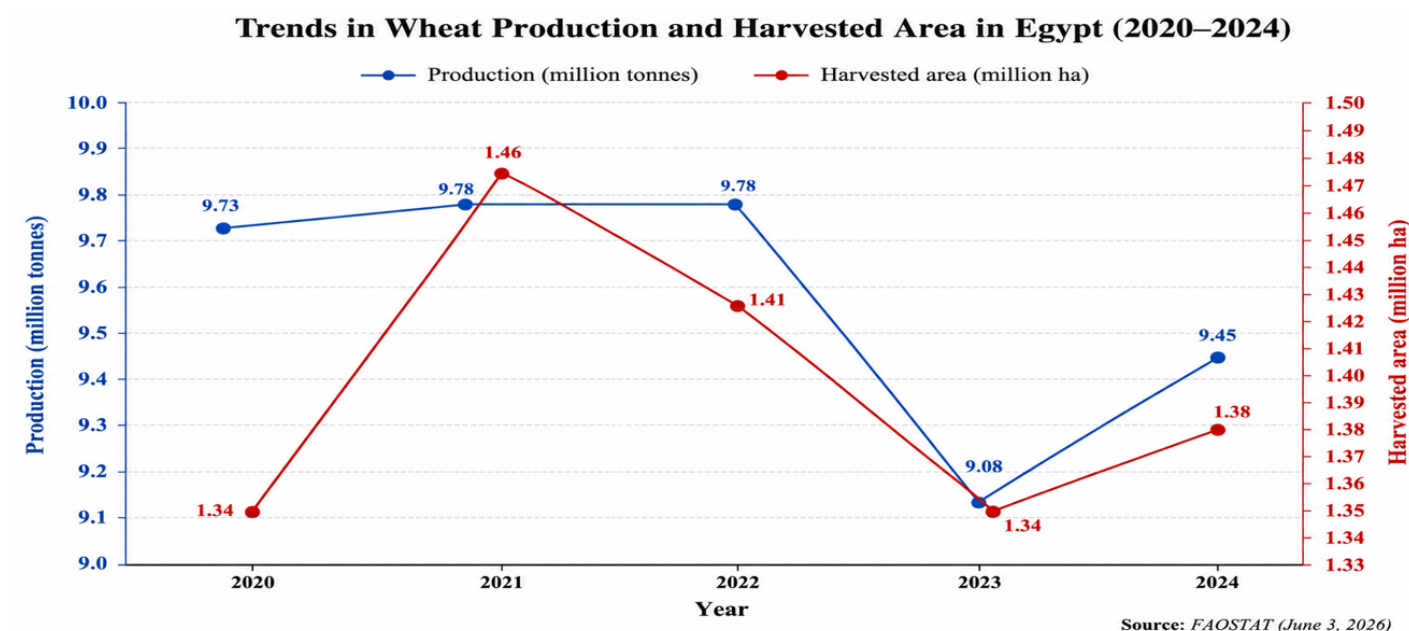


Figure 1

Bread wheat productivity and harvested area from 2020 to 2024 in Egypt (Source: FAOSTAT, 2026).

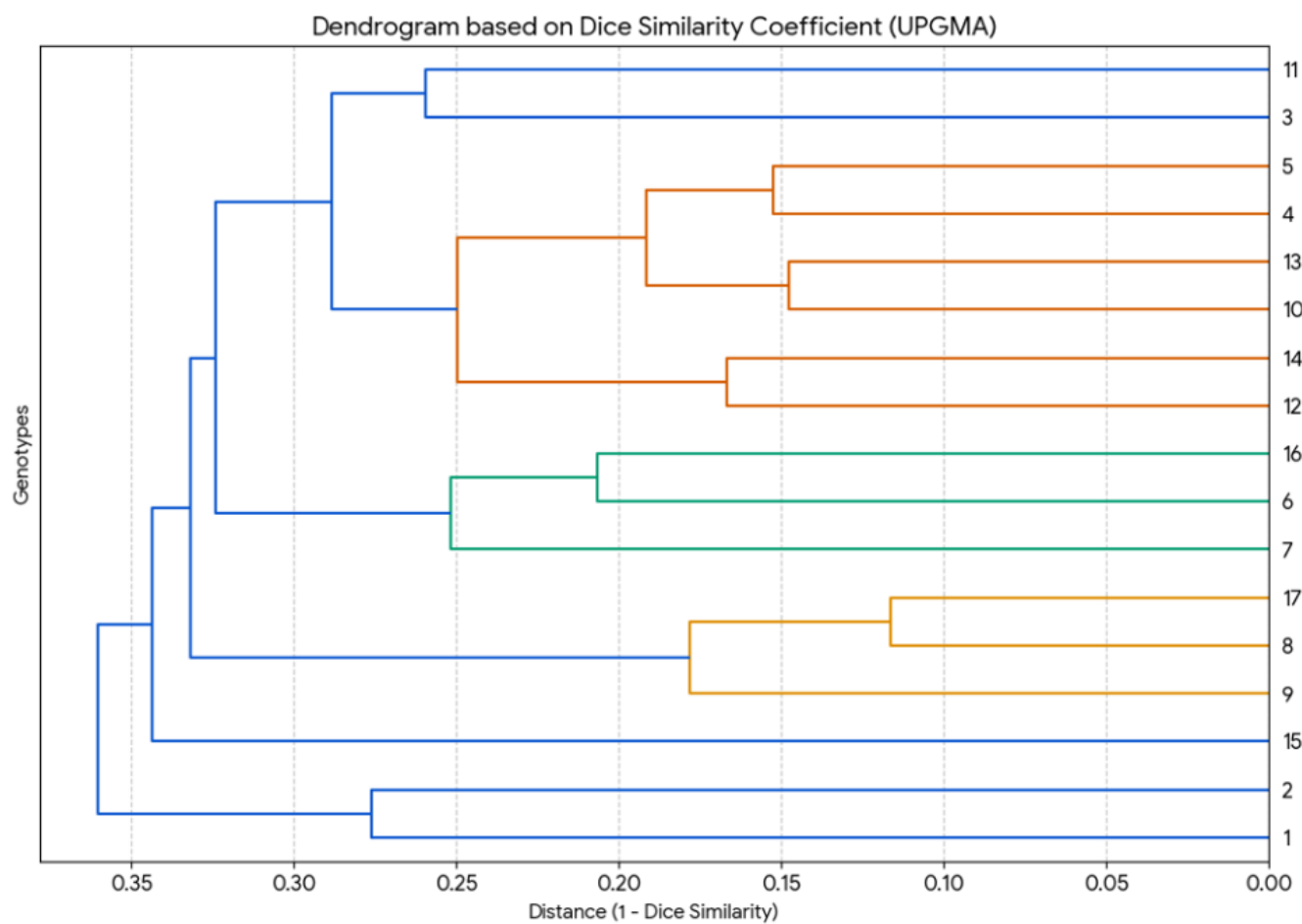


Figure 2

Dendrogram exhibited the genetic relationships among seventeen wheat genotypes based on Dice similarity coefficient and UPGMA clustering method.

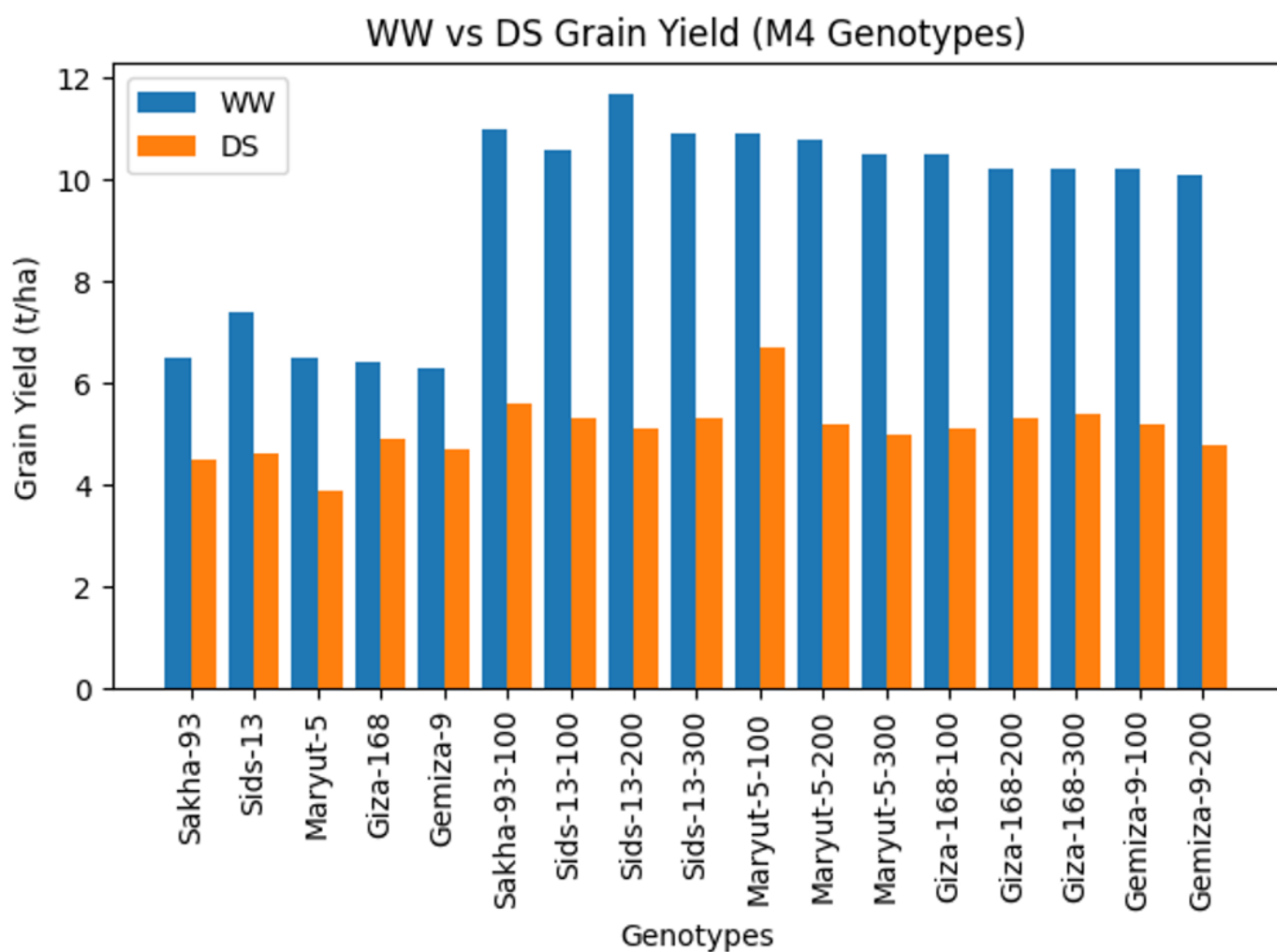


Figure 3

comparative between 12 mutant lines and their parents based on grain yield under well-watered (WW) and water- stress conditions (DS).

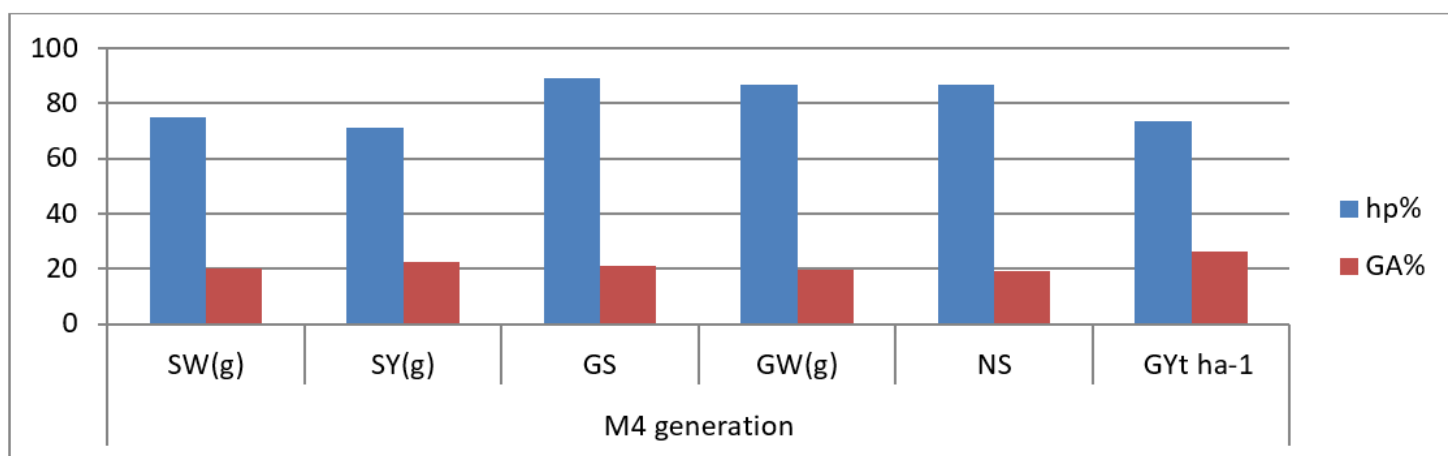


Figure 4

Heritability (hp%) and genetic advanced (GA%) for the studied traits of mutant lines in the M4 generation

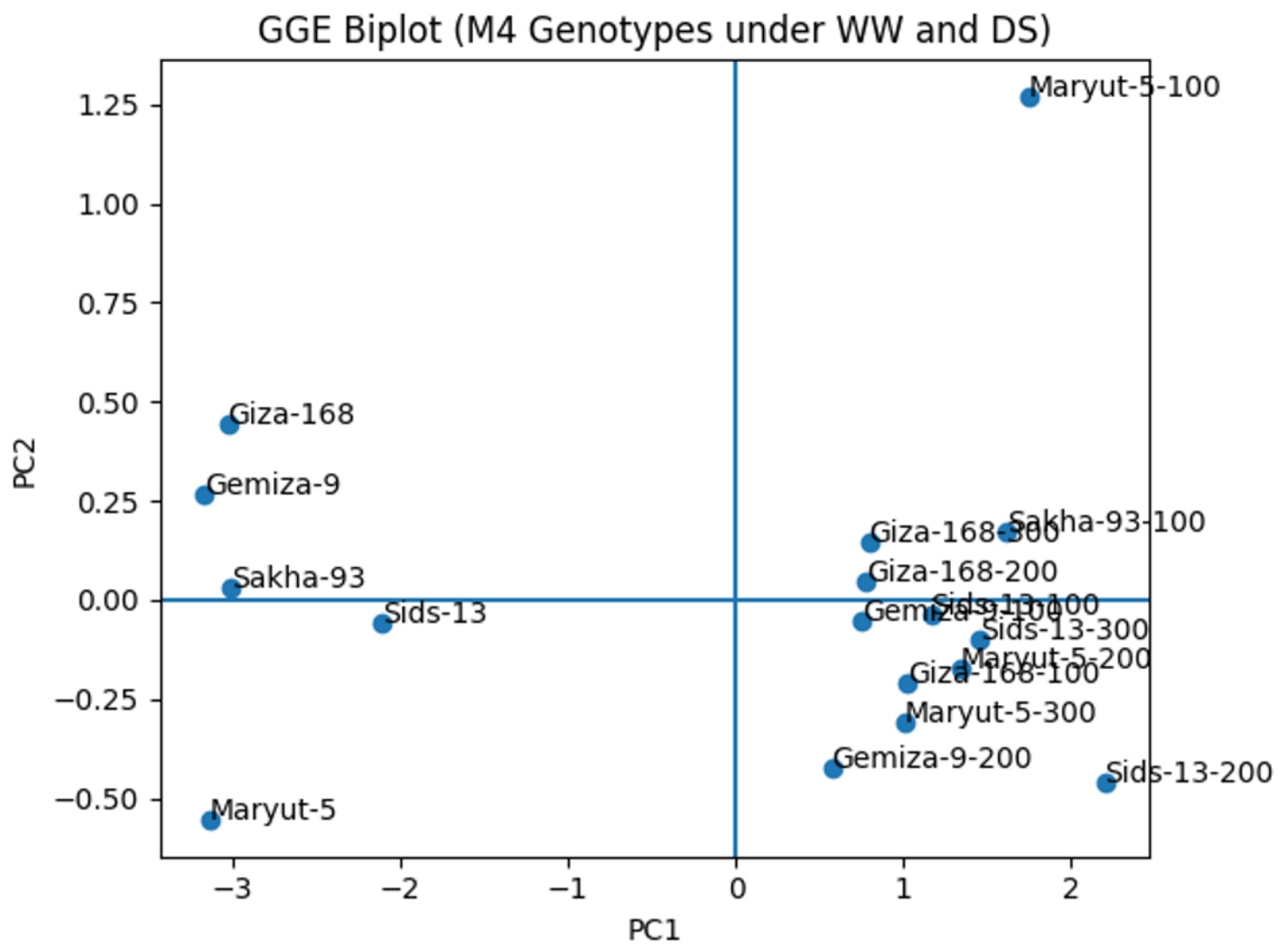


Figure 5

GGE biplot of M4 generation under well-watered (WW) and water-stress conditions (DS)

Supplementary Files

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- [Supplementarywheat5.xlsx](#)