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Phenotypic and Molecular Analyses Reveal Targets for Yield and Quality Improvement in Bread Wheat

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WHEAT breeding relies on genetic diversity and heritability to develop high-yielding, stress-tolerant varieties. Combining ability studies help optimize grain quality and climate resilience by analyzing additive and non-additive genetic effects. our study evaluates genetic variation in grain yield and quality traits among six bread wheat cultivars (Giza 171, Misr 1, Sids 13, Misr 2, Sakha 94, Gemmeiza 11) under Mediterranean conditions in Egypt's north Delta over three growing seasons (2015–2018). Using a line × tester mating design, nine F_1 crosses were generated. Data were recorded on agronomic traits (grain yield, 1000-kernel weight, protein content, gluten quality) and molecular traits (SSR markers: Dx5, Xpsp1, Xcn15) analyzed. Parents and their F_1 hybrids were assessed in a randomized complete block design with four replications, followed by evaluation of the F_2 generation (Giza 171 × Misr 2) and its parental lines. Results revealed significant heterosis, with Misr 1 × Sakha 94 achieving the highest grain yield (62.93 g/plant). Combining ability analysis indicated additive and non-additive gene effects for key traits, identifying Giza 171 and Misr 1 as superior general combiners for yield and protein content. The F_2 population exhibited moderate to high broad-sense heritability (51.7–99.1%) and genetic advance, with phenotypic variances significantly exceeding environmental variances. Molecular analysis confirmed genetic divergence between high- and low-protein F_2 individuals, clustering high-protein progeny with the parent Misr 2. Significant correlations emerged between yield, 1000-kernel weight, and gluten content. This approach highlights the potential of targeted crosses to enhance yield and quality in wheat breeding programs.

Keywords: Bread wheat, combining ability, heterosis, heritability, SSR markers.

1. Introduction

Wheat (*Triticum spp.*) remains a foundational crop in global agriculture, serving as a food source for billions, particularly in temperate regions. As a hexaploid species, bread wheat (*Triticum aestivum* L.) is distinguished by its allo-hexaploid (AABBDD) genetic makeup, with the D genome playing a pivotal role in imparting unique baking qualities that differentiate it from other *Triticum* species (Sleper & Poehlman, 2006). Its global significance extends beyond food production, as wheat is also employed in industries such as alcohol distillation, livestock feed, and textile manufacturing, where wheat starch is used for fabric sizing and glucose syrups (Tayyar, 2008).

Recent advances in wheat genetics have demonstrated crop adaptability to diverse environmental conditions, making it one of the most resilient cereals grown globally. This adaptability is primarily driven by its genetic diversity, enabling it to thrive in climates ranging from -35˚C in vegetative stages to over 40˚C during grain filling (Elahmadi, 1994; Haji & Hunt, 1999). A critical review by Reynolds *et al*. (2021) emphasized the role of genetic improvement in enhancing wheat's resilience to heat stress and other environmental challenges, pointing out the necessity of modern breeding efforts to maintain wheat's global prominence as a staple crop (Reynolds et al., 2021). Moreover, In the World wheat production in 2020 reached 765 million tons and increased to 808 million tons in 2022 (FAO, 2023). With an ever-growing global population, the demand for increased wheat production has become critical. As a result, thousands of new wheat varieties have been developed, with international breeding programs aiming to enhance genotypes to boost yield, food

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quality and environmental adaptability (Fernandes et al., 2000). In Egypt, wheat remains a top agricultural priority due to the need to support a population exceeding 110 million (Hamada et al., 2015; Sadek et al., 2013). However, breeding efforts in Egypt have traditionally focused on yield and disease resistance, with less attention given to grain quality traits, an opportunity for future research and genetic improvement. The role of genetic variation in breeding programs cannot be overstated, particularly when it comes to enhancing traits such as grain yield, protein content, and gluten strength (Ajmal et al., 2000). In recent years, large-scale genetic diversity assessments and high-throughput phenotyping platforms have facilitated the discovery of novel alleles associated with agronomically important traits in wheat (Bapela et al., 2022; Taranto et al., 2023).

These discoveries enable breeders to leverage genetic diversity more effectively in breeding programs. Moreover, genomic-assisted breeding tools have become increasingly crucial for evaluating genetic variability and improving the heritability of desirable traits (Guzmán et al., 2017). This modern approach accelerates the selection of superior genotypes, ensuring that wheat cultivars are better adapted to changing environmental conditions (Reynolds et al., 2021). Combining abilities evaluation have also been essential in recent wheat research, providing insights into additive and non-additive gene actions that determine the inheritance of crucial traits. In this trend, Mallikarjuna *et al.* (2011) demonstrated that combining ability significantly affects grain yield and protein content traits. Recent investigations have further underscored the importance of specific combining abilities in improving grain quality and stress tolerance (Guzmán et al., 2017; Mallikarjuna et al., 2011). As climate change continues to challenge global wheat production, combining ability studies will remain a crucial aspect of breeding programs, particularly in efforts to mitigate the impacts of heat and drought stress (Langridge & Reynolds, 2021).

Post-2019 research emphasizes the critical importance of genetic variability and heritability in wheat breeding. Genetic variation forms the foundation for identifying superior genotypes with desirable traits such as higher grain yield, improved protein content, and gluten strength (Ajmal et al., 2000). Kumar *et al.* (2018) underscored the importance of gene action, including additive and non-additive effects, to improving wheat traits, such as grain quality and yield components. Recent studies have further substantiated this, showing that selecting parents with superior combining abilities can significantly enhance yield and quality traits in wheat breeding programs (Kumar et al., 2018).

Heritability estimates for traits like biological yield, protein content, and plant height in wheat are high, underscoring their utility in selection (Mahdy et al., 2015; Sadeghi et al., 2012). Recent studies highlight the importance of additive and non-additive gene effects in enhancing wheat performance under optimal and stress conditions, aiding adaptation to environmental challenges like water scarcity and temperature shifts (Aglan et al., 2020; Nassar, 2019). Robust heritability of yield traits across diverse agro-climatic zones further supports breeding program resilience (El-Din et al., 2005; Fouad et al., 2020). Heterosis, driven by dominance effects, has proven critical for improving yield and grain quality in hybrid wheat, offering pathways to boost productivity under variable environments (Aljubory & Al–Tamim, 2019; Bilgin et al., 2022). This study aimed to enable the development of high-yielding, high-quality wheat germplasm resilient to climate-related stress and food-security pressures. We quantified genetic variation for yield and quality attributes, estimated key genetic parameters, including broad- and narrow-sense heritability, general and specific combining ability, and additive versus non-additive effects and analyzed phenotypic correlations among traits to guide selection.

2. Materials and Methods

2.1 . Location and climatic conditions

This study was conducted over three growing seasons (2015/16, 2016/17, and 2017/18) at the Sakha Agricultural Research Station Experimental Farm in Kafrelsheikh Governorate, Egypt. A Mediterranean climate with mild winters and warm summers characterizes the site. The experimental farm's soil is a clay loam, typical of the Nile Delta region, and it was prepped with standard agricultural practices, including plowing, leveling, and irrigation before sowing. All experiments were conducted under typical wheat-growing conditions, with no water or nutrient stress imposed to simulate optimal growth conditions. Monthly temperature and rainfall data were collected from a nearby meteorological station to monitor the environmental conditions during the growing seasons. This information was considered during data analysis to assess potential environmental effects on the phenotypic traits measured.

2.2 . Plant materials and experimental design

Six bread wheat cultivars, commonly grown in Egypt, were selected based on their known agronomic performance and genetic diversity. These cultivars were Giza 171, Misr 1, Sids 13 Misr 2, Sakha 94, and Gemmeiza 11. The cultivars used in this study were released and selected by the Wheat Research Department, with their superiority validated in prior research. The cultivars included Sakha 94 (El-Din et al., 2005), Sids 13 (Moustafa et al., 2010), Gemmeiza 11 (Sadek et al., 2013), Giza 171 (Hamada et al., 2015), Misr 1 and Misr 2 (Hamada et al., 2017).

In the first season (2015/16), a line × tester mating design was employed in this study to partition the genetic variance of bread wheat into its components, facilitating the selection of favorable parents for breeding. The methodology followed the procedures outlined by (Kempthorne, 1957). Therefore, three spring bread wheat cultivars were used as lines and an additional three as testers, in line × tester, resulting in nine F_1 hybrids. Furthermore, the Giza 171 x Misr 2 cross was advanced to the F_2 generation, where additional genetic parameters were valid.

In the second season (2016/17), the F_1 hybrids and their six parental lines were grown in a randomized complete block design (RCBD) with four replicates. Each experimental plot consisted of 4 m rows, with row-to-row spacing of 30 cm and plant-to-plant spacing within rows at 15 cm. Standard agronomic practices followed national recommendations, including irrigation, fertilizer application (120 kg N/ha in three splits), and pest control. In the third season (2017/18), F_1 of line x tester crosses, in addition to F_2 generation of the Giza 171 × Misr 2 combination were separately evaluated using a similar RCBD layout with three replicates in the case F_1 hybrids. While, in the case of F_2 generation, the plots consisted of ten rows, 4 meters in length, with rows spaced 25 cm apart and plants spaced 20 cm apart within the row. This design allowed for robust analysis of subsequent genetic variation and inheritance patterns.

2.3 . Trait measurements and data collection

Multiple phenotypic traits were evaluated in both F_1 and F_2 generations. In the F_1 generation, data were collected from 10 randomly selected plants per plot. For the F_2 generation, measurements were taken from 300 randomly selected competitive plants across three replicates.

2.3.1 . Field trait measurements

Germination percentage was calculated after 8 days, following ISTA rules (1999), by germinating seeds in moist sand under controlled conditions and counting the number of normal seedlings. The seedling shoot and root lengths were measured using a ruler on 10 normal seedlings per replicate. After germination, the seedlings were dried at 65°C for 24 hours, and their dry weight (mg) was determined using a precision balance. Random samples of 1000 kernels were taken from each plot to determine the 1000-kernel weight (g) using an electronic balance, and the measurements were recorded to two decimal places for accuracy. Grain yield per plant (g) was assessed by harvesting and weighing all the grains produced by each plant individually. In addition, the data were recorded on, plant height (cm) from the soil surface to the tip of the spike (excluding awns), spike length (cm) from the base to the tip of the main spike (excluding awns), the total number of spikes per plant, and the average number of spikelets per spike.

2.3.2 . Grain protein content

Grain protein content was determined by measuring the total nitrogen content and converting it to protein, following the method described by (A.O.A.C, 2000). Grains were first ground into a fine powder, and nitrogen content was measured using the micro-Kjeldahl. In the Kjeldahl method, the sample was heated with sulfuric acid and a catalyst to convert organic nitrogen into ammonium sulfate, which was distilled and quantified by titration. In the Dumas method, the sample was combusted in an oxygen-rich environment to produce nitrogen gas, measured using a thermal conductivity detector. The total nitrogen was then converted to protein using a conversion factor 5.85. The protein content was reported as a percentage of the grain sample.

2.3.3 . Wet and dry gluten content

Gluten content in wheat flour was determined using the method described by the American Association of Cereal Chemists (Chemists & Committee, 1983). Gluten content was determined by mixing 25 g of flour with water (60% absorption) to form a dough, kneaded to a firm consistency. The dough was soaked in water for 20–60 minutes, then kneaded and washed to remove starch. Once starch-free, the gluten mass was rolled into a ball, excess water was removed, and the wet gluten was weighed. The gluten was then dried to a constant weight, cooled, and weighed again. Wet and dry gluten percentages were calculated using the following formulas:

Wet gluten (%) = (wet gluten weight/sample weight) × 100.

Dry gluten (%) = (dry gluten weight/sample weight) × 100.

2.3.4 . Relative Density of Seeds (g/mm^3) and Hectoliter Weight (kg/hl)

The physical properties of wheat seeds, including seed volume and bulk density, were determined using the method of (Kramer & Twigg, 1966). The volume of 1000 seeds was measured in cm^3, and bulk density was calculated in g/cm^3. Seed density was also estimated by dividing the mass of the seeds by their volume, expressed in g/mm^3, following the same method. Hectoliter weight (HLW), expressed in kilograms per hectoliter (kg/hl), was determined using the method described by (Lee, 2013). A 500g sample of wheat was poured into the top hopper of a Dickey-John GAC2100 grain analyzer. The grain was then released into the cell, where moisture content and weight were measured. The HLW was recorded as kg/hl, providing critical data on the wheat's density and quality.

2.4 . Biometrical Analysis

All collected data were statistically analyzed using ANOVA for RCBD, following the procedures outlined by Snedecor and Cochran (1980). The genotypic variance was partitioned into parent, cross, and parent vs. cross (heterosis) components. Mean separation was performed using the least significant difference (LSD) test at both 5% and 1% levels of significance.

2.4.1. Variances and Genetic parameters

Combining ability analysis was conducted following the line × tester analysis developed by (Kempthorne, 1957) to investigate genetic interactions further. The effects of general combining ability (GCA) and specific combining ability (SCA) were estimated according to the procedures described by (Singh & Chaudhary, 1981). The statistical significance of combining ability effects was evaluated through ANOVA, and the proportional contributions of lines, testers, and their interactions to the total genetic variance were calculated. Further genetic parameters were estimated, as following: heritability in the broad sense was estimated as $H\% = \sigma^2g / \sigma^2p \times 100$, following the classification by Robinson *et al*. (1949) where heritability values were categorized as low (0-30%), medium (30-60%), and high (>60%). The expected genetic gain, which was calculated as $\Delta g = k\sigma pH$, where k is the selection differential (2.06 for 5% selection intensity). Genetic advance as a percentage of the F_2 mean was also determined. Potency ratio was calculated following the method described by Smith (1952) to determine the degree of dominance. The formula used was $P = (F_1 - M.P.) / 0.5(P_2 - P_1)$, where P represents the relative potency of the gene set, P_1 is the mean of the lower parent, P2 is the mean of the higher parent, and M.P. is the mid-parent value, calculated as $(P_1 + P_2)/2$. Complete dominance was indicated when P = +1, while partial dominance was identified when P ranged between -1 and +1, excluding zero, which indicated no dominance. Overdominance was observed when the potency ratio exceeded ±1, with the sign indicating the dominance direction of either parent. The amounts of heterosis as the percentage deviation of F_1 mean performance from the mid-parent and better parent were estimated according to Mather and Jinks (2013) as follows:

Heterosis over mid-parent (%) = $(\bar{F}_1 - M\bar{P}) / M\bar{P}) \times 100$

Heterosis over better parent (%) = $(\bar{F}_1 - B\bar{P}) / B\bar{P}) \times 100$

A t-test was used to test the significance of heterosis and calculated as:

$$\pm\sqrt{2MSe / r}$$

Where MSe is the mean square of experimental error from the analysis of variance, and r is the number of replications.

2.4.2 . Phenotypic and genotypic coefficient of variation

To estimate the genotypic and phenotypic correlations between any pairs of traits, a covariance analysis between all pairs of studied traits was made according to the procedure outlined by (Burton, 1952). As Steel *et al.* (1960) described, correlation coefficients were computed to assess the relationships between the pairs of studied traits (Steel & Torrie, 1960).

2.5 . Molecular Analysis

DNA was isolated using CTAB method from fresh leaves of 12 bread wheat genotypes according to Doyle and Doyle (1990).The PCR reactions using three SSR primers were used in this study as shown in (Table 4). The SSR primers were selected as a result from research conducted by (D'ovidio & Anderson, 1994; Manifesto et al., 1998). The PCR reactions were carried out in a final volume of 20 µL, containing DNase-free water, Buffer 1X, 2.5 mM magnesium chloride, a set of 200 µM Buffer 1X, 2.5 mM magnesium chloride, a set of 200 µM dNTPs, 10 pmol of each of the primers, Taq polymerase 0.5 units (Fermentas) and 30 ng/µl of the extracted DNA. The PCR thermal cycling

program was as following: the initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 40 sec and extension at 72°C for 1 min. The amplified products were analyzed by a horizontal electrophoresis from Bio-Rad with a 2% agarose gel in 1 × TBE buffer. The gel documented by the Gel Doc XR documentation system (Bio-Rad). The presence of the specific part of a gene was verified using standard DNA ladder, Sizer TM 50plus.The genetic relationships were analyzed using PowerMarker v3.25 software (Liu & Muse, 2005). Genetic distances were estimated based on Jaccard genetic distance metric. Subsequently, a clustering dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA 5.0 software (Kumar et al., 2004), enabling the visualization of genetic relatedness.

3. Results and discussion

The obtained results could be concentrated in two parts, the first one is related to the line x tester combinations (F_1) and the other one is related to evaluation of the F_2 generation of one hybrid.

3.1 . Mean performance Line x tester in F_1

3.1.1. Grain yield plant^{-1} and l000-kernel weight

The grain yield per plant showed notable variation among lines, testers, and F_1 hybrids. The highest cultivars for grain yield were the tester Giza 171 (35.96, 1.36-fold of the tester's mean) and the line Gemmeiza 11 (34.54 g, 1.19-fold of the line's mean), while the tester Sids 13 (16.62 g) and the line Sakha 94 (20.37 g) were the lowest. Complete overdominance was observed in all hybrids for high grain yield, except for Giza 171 x Gemmeiza 11, which showed dominance for low grain yield. Among the hybrids, Sakha 94 x Misr1 achieved the highest yield (62.93 g, 1.79-fold of the crosses mean), while Sakha 94 x Sids 13 yielded the lowest (21.77 g). Significant differences were observed, as shown in Figure 1(a).

The 1000-kernel weight among the lines, testers, and F_1 hybrids displayed significant variation. The heaviest 1000-kernel weight was recorded by the line Gemmeiza 11 (47.98 g, 116% of the lines mean) and the tester Giza 171 (44.60 g, 106% of the lines mean), while the lightest weight was obtained by the lines Misr 2 (37.9g) and the tester Sids 13 (38.43g). Among hybrids, complete dominance was observed for heavier kernel weights, except for Sakha 94 x Giza171 and Gemmeiza 11 x Giza171, which showed dominance or overdominance for lighter kernel weights. In the F_1 hybrids, Sakha 94 x Giza171 produced the heaviest kernel weight (54.45 g, 12.1-fold of the crosses mean), while the lightest was observed in was Sakha 94 x Sids 13 (38.18 g, 85%). The overall mean kernel weight for the crosses was 45.14 g. These results emphasize the superior performance of certain crosses, particularly Sakha 94 x Giza171, which exceeded the mean by 21%, as shown in Figure 1(b).

3.1.2. Grain protein content % and wet and dry gluten %

The grain protein content varied significantly among the lines, testers, and hybrids. High protein levels were observed in Sids 13 (10.91 %) and Giza 171 (10.87 %), while Misr 2 (7.95%) and Misr 1 (8.16%) showed the lowest. The F_1 hybrids exhibited complete to over-dominance for higher protein content, with Sakha 94 x Giza171 having the highest at 12.12%, a 1.14-fold increase over the crosses mean. However, Gemmeiza 11 x Sids 13 and Gemmeiza 11 x Giza171 showed dominance for lower protein levels, with the lowest hybrid being Gemmeiza 11 x Sids 13 at 7.78 %, as shown in Figure 2 (a).

The wet gluten content varied significantly among the lines, testers, and hybrids. The line Gemmeiza 11 (4.86%) and the tester Giza 171 (5.6 %) obtained the highest percentage of wet gluten, while the lowest contents were recorded in the tester Sids 13 (2.79 %) and the line Sakha 94 (3.96 %). The mean wet gluten content for the lines was 4.32%, and for the testers, it was 4.12%. In the hybrids, complete to over-dominance for higher wet gluten contents was observed in all crosses, except for Sakha 94 x Sids 13 and Gemmeiza 11 x Sids 13, which showed dominance for lower contents. The highest wet gluten content was recorded in Misr 2 x Giza171 (6.23 %), a 1.31-fold increase over the crosses mean, while the lowest was in Sakha 94 x Sids 13 (3.67%). The hybrids' mean was 4.77%, and the overall mean for all genotypes was 4.55%, as shown in Figure 2(b).

Dry gluten content varied significantly across lines, testers, and hybrids. The highest levels were observed in Giza 171 (2.12 %) and Gemmeiza 11 (1.92 %), while the lowest was in the tester Sids 13 (1.15 %) and the line Misr 2 (1.52 %). Among hybrids, Gemmeiza 11x Giza171 had the highest content (2.23%), a 1.31-fold increase over the crosses mean, while Sakha 94 x Sids 13 had the lowest (1.36%. This result indicates that certain hybrids, particularly those involving Giza 171, have the potential to enhance dry gluten content compared to their parental testers, as shown in Figure 2(c).

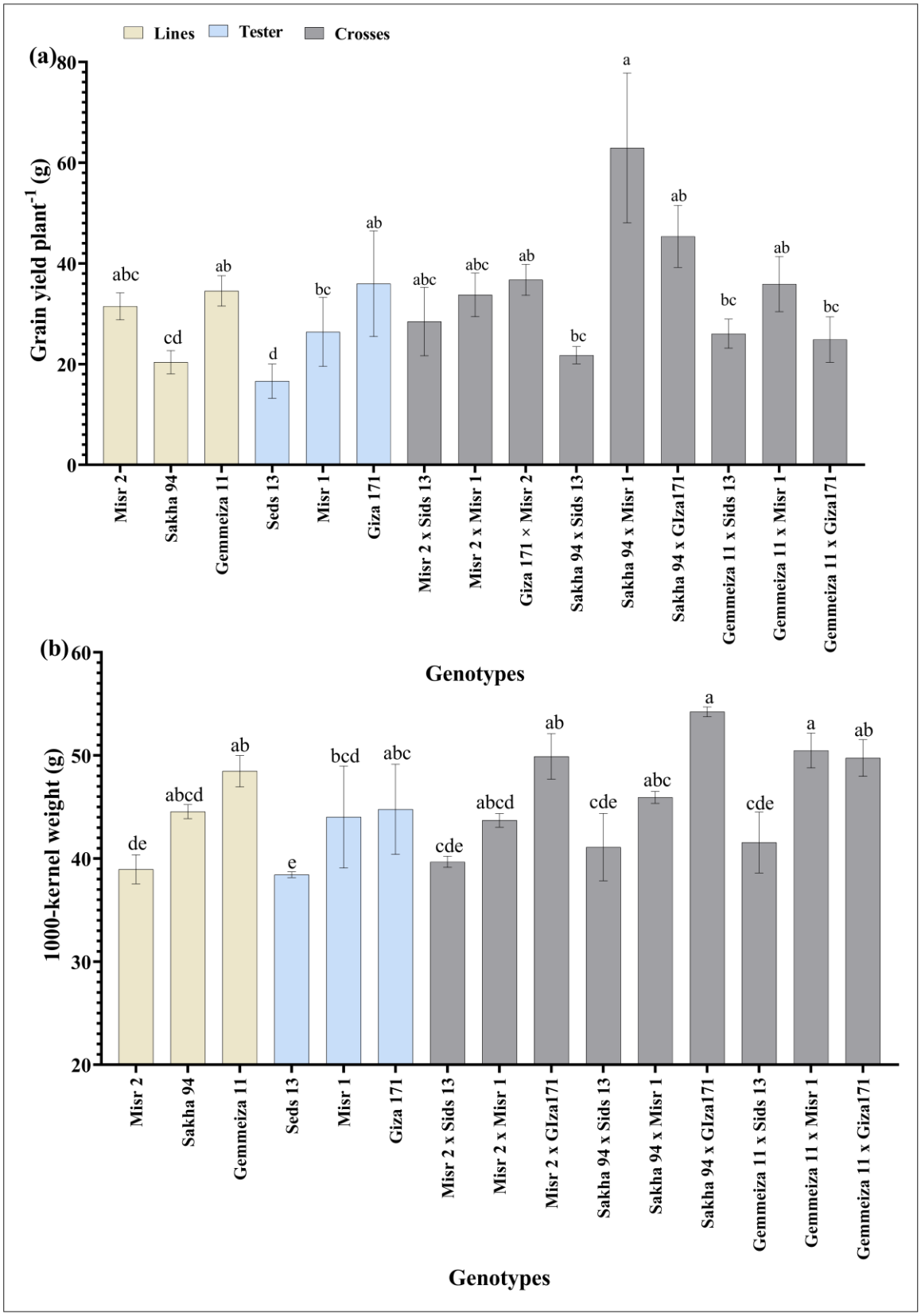

Fig. 1. Mean performance for grain yield plant-1 and 1000-kernel weight of 6 parental lines (three lines and three testers) and their nine wheat crosses. Different letters indicated significant variations among the treatments using LSD 0.05.

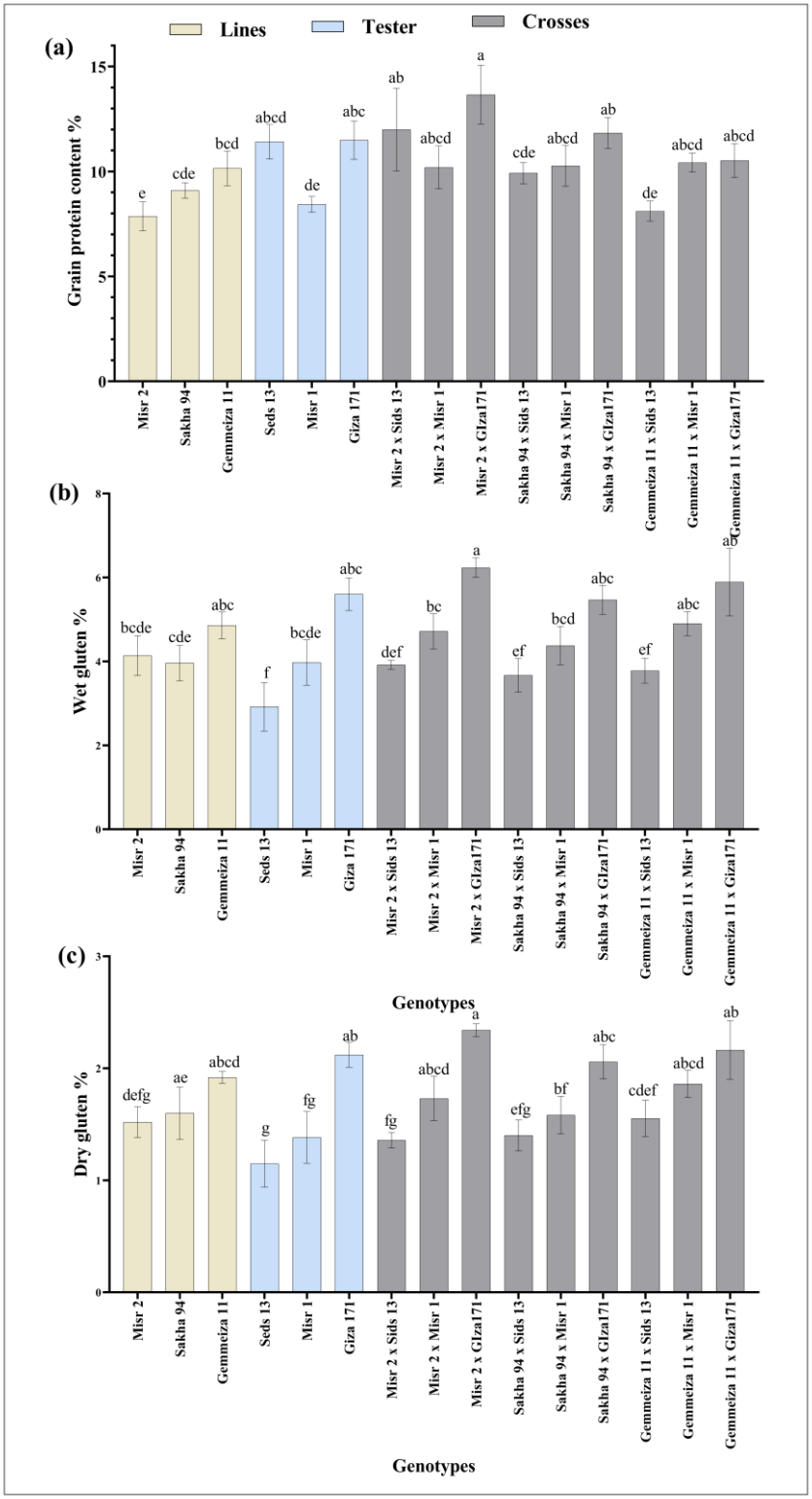

Fig. 2. Mean performance for grain protein content% % (a), wet gluten% % (b), and dry gluten% % (c) of 6 parental lines (three lines and three testers) and their nine wheat crosses. Different letters indicated significant variations among the treatments using LSD 0.05.

3.1.3. Shoot, root length, and dry weight

The shoot length of lines, testers, and their F_1 hybrids showed clear variation. Giza 171 had the highest shoot length among the testers at 15.13 cm, 13.4% above the mean (13.89 cm), while Misr 1 had the lowest, 11% below the mean. Among lines, Misr 2 showed the highest shoot length at 13.74 cm, 7% above the lines mean (12.85 cm), and Gemmeiza 11 the lowest, 7% below the mean. In crosses, Gemmeiza 11 x Misr 1 reached 11.97 cm, 1.26-fold above the overall mean (14.11 cm), while Misr 2 x Giza 171 showed the lowest at 12.73 cm, 14% below the crosses mean (14.80 cm). Crosses mean had 15% and 11% higher shoot lengths than lines and testers means, as shown in Figure 3 (a).

The root length varied significantly among the genotypes. Giza 171 had the longest root length among the lines at 17.11 cm, 13% above the testers mean (15.67 cm). Misr 1 showed the lowest at 5.4% below the testers mean. Misr 2 recorded the longest root length in lines at 17.64 cm, 9.1% above the testers' mean (16.07 cm), while Gemmeiza 11 had the shortest at 12.5% below the lines mean. Among the crosses, Sakha 94 x Sids 13 achieved the highest root length of 19.69 cm, 1.4-fold greater than the overall mean (16.90 cm), while Misr 2 x Sids 13 had the lowest at 19.1% below the crosses mean (16.23 cm), as shown in Figure 3 (b).

The study evaluated dry weight across lines, testers, and crosses. Among the lines, Misr 2 had the highest dry weight (23.00 mg, 4.4% above the lines mean). For testers, Giza 171 showed the highest (24 mg, 11% above), and Misr 1 showed the lowest (20 mg, 6.9% below). In crosses, Gemmeiza 11 x Giza 171 recorded the highest (81 mg, 171% above the crosses mean), and Misr 2 x Giza 171 the lowest (20 mg, 15.1% below). The overall mean was 30 mg, as shown in Figure 4 (d).

3.1.4. Relative density and hectoliter weight

The study evaluated the relative density of lines, testers, and F_1 hybrids. Among the testers, Giza 171 had the highest relative density (1.40 g mm^{-3}), about 6.4% higher than the overall mean (1.32 g mm^{-3}), while Sids 13 had the lowest (1.3 g mm^{-3}), 1.4% lower. For lines, Sakha 94 recorded the highest value (1.31 g mm^{-3}), 3% above the mean. Misr 2 and Gemmeiza 11 were the lowest lines (1.25 g mm^{-3}), 4.8% below. In crosses, Gemmeiza 11 x Sids 13 had the highest (1.44 g mm^{-3}, 9.6% above the mean), and Misr 2 x Misr 1 had the lowest (1.2 g mm^{-3}, 8.8% below the overall mean (1.32), as shown in Figure 4 (a).

The highest hectoliter weights were detected in line Sakha 94 (95.07 kg hl^{-1}) and the tester Sids 13 (94.97 kg hl^{-1}), with Sakha 94 being 6.4% above and Sids 13 being 5.1% above the overall mean (90.33 kg hl^{-1}). The lowest weights were recorded for the tester Giza 171 (87.23 kg hl^{-1}, 3.4% below the overall mean) and the tester Misr 2 (87.03 kg hl^{-1}, 3.7% below the overall mean). In crosses, Misr 2 x Misr 1 has the highest value (92.03 kg hl^{-1}, 2.8% above the crosses' mean). In comparison, the lowest was Gemmeiza 11 x Giza 171 (87.37 kg hl^{-1}, 1.8% below the overall mean), as shown in Figure 4 (b).

The combined ANOVA across environments showed highly significant genotypic mean squares for all traits (Table 1), confirming ample genetic variability among entries. Comparable wheat studies report significant among-genotype variation for grain yield, kernel weight, protein and gluten traits when parents and their crosses are evaluated together, validating the interpretation that real genetic differences underlie these phenotypes (Abdel Nour et al., 2011; Al-Mafarji et al., 2024). Parents (P) and Crosses (C). Both groups varied significantly for grain yield, 1000-kernel weight, grain protein, wet gluten and dry gluten, indicating that useful diversity exists in both the parental set and their hybrids. This pattern, significant P and C mean squares for yield and quality traits, is routinely observed in wheat combining-ability experiments (Abdel Nour et al., 2011; Khodadadi et al., 2012). Parents vs Crosses (P vs C). In your data, P vs C was significant only for wet gluten %, which implies average heterosis specifically for wet gluten across the set of crosses. In line with standard interpretation, the P vs C mean square tests average heterosis; significance has been reported for various traits in cereals and directly linked to heterotic responses (Al-Daej, 2022; Ismail, 2015).

Lines, Testers (GCA) and Line×Tester (SCA). The partitioning of crosses showed that testers were significant for 1000-kernel weight, wet- and dry-gluten, while Line×Tester (SCA) was significant for many traits (Table 1). By definition, GCA effects reflect additive variance, whereas SCA captures non-additive components (dominance/epistasis). Thus, significance of tester (GCA) terms for kernel weight and gluten points to an additive basis in part for these traits in your material, while the broad SCA significance indicates important non-additive effects for several traits (Griffing, 1956). These patterns are consistent with wheat studies using line×tester or diallel designs, where both GCA and SCA are often significant for yield and

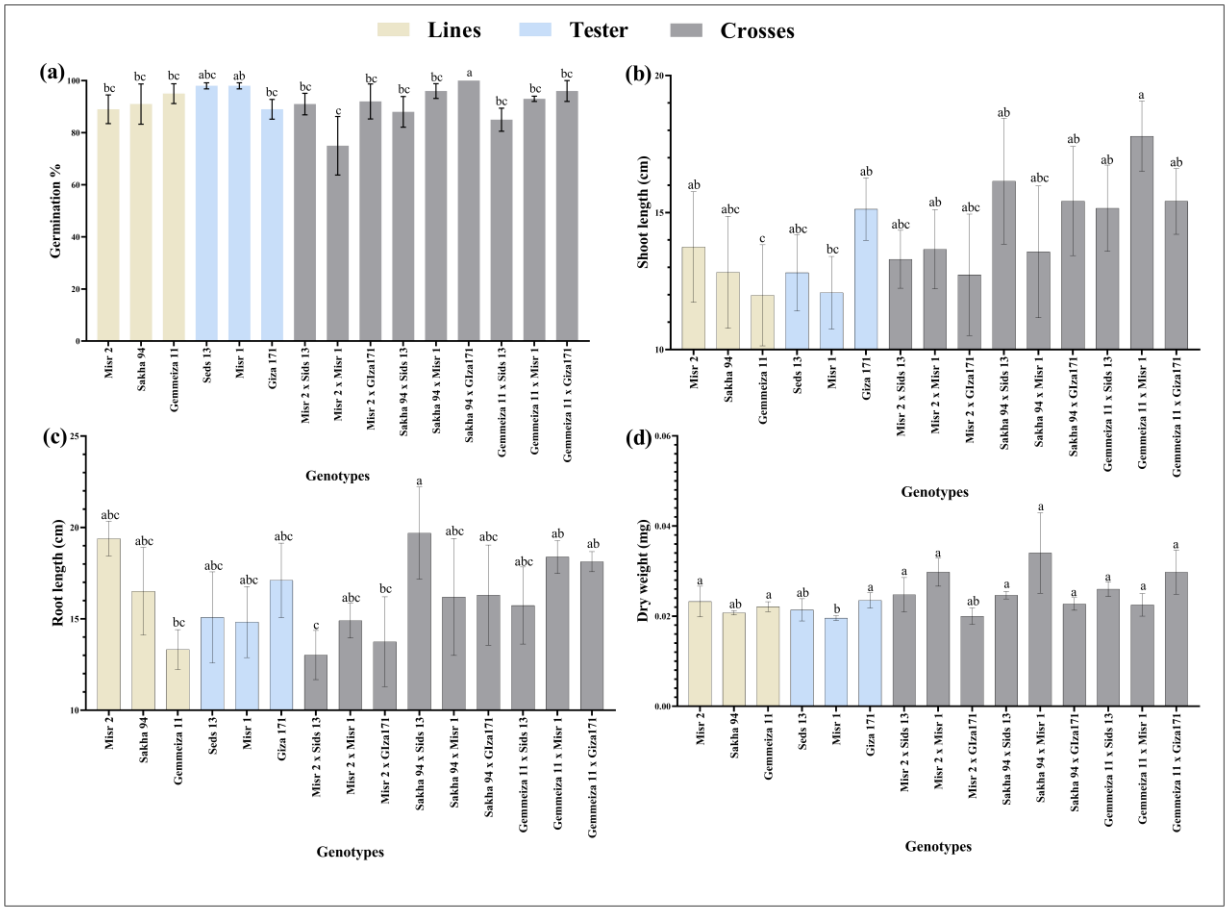

Fig. 3. Mean performance for grain protein content% % (a), wet gluten% % (b), and dry gluten% % (c) of 6 parental lines (three lines and three testers) and their nine wheat crosses. Different letters indicated significant variations among the treatments using LS

quality traits; some reports even find SCA > GCA for many agronomic traits, emphasizing the role of non-additive gene action alongside additive effects (Abdel Nour et al., 2011; Istıplıler et al., 2015).

Breeding implications. When GCA is significant (e.g., tester effects for 1000-kernel weight and gluten), parent selection based on GCA should be effective; where SCA predominates, emphasis on specific cross combinations and evaluation of later-generation segregants is prudent, a recommendation echoed for wheat when non-additive effects are substantial. Using the Baker predictability ratio (2·GCA/(2·GCA+SCA)) can formally gauge whether additive or non-additive effects dominate for each trait before fixing selection strategies (Murtadha et al., 2018; Rauf et al., 2023).

Methodological note. Your ANOVA layout (Parents, Crosses, P vs C; Lines, Testers, Line×Tester) follows the established line×tester framework (Kempthorne's formulation) used to estimate GCA/SCA and to test average heterosis via P vs C, reinforcing that the inferences above rest on standard quantitative-genetic partitions (Singh & Chaudhary, 1981).

3.1.5. General combining ability effects

General combining ability effects (GCA) of the three lines and three testers were tested for the studied traits and the results are presented in Table 2. Positive and significant general combining abilities for grain yield were obtained by the tester Misr 1(9.1) and the line Sakha 94 (8.27). On the other hand, negative and significant general combining abilities for grain yield were detected by the line Sids 13 (-9.66). For 1000-kernel weight, desirable positive and significant general combining abilities were recorded by the tester Giza 171 (5.28), while the tester Sids 13 (-6.39) had negative and significant values. These results agreed with those of Farhat and Mohamed (2018), in their study, Giza 171 was a good combiner for kernel weight and grain yield and had significant positive GCA.

Positive and significant general combining abilities for protein content were observed by the tester Giza 171 (1.52) and the line Misr 2 (1.23),
while the line Gemmeiza 11 (-1.33) had negative and significant values. Among the lines and the

. Specific combining ability effects

Estimates of the specific effects of the hybrids for the studied traits are given in Table 3. No significant specific combining abilities were detected for the studied traits. In addition, there were nonsignificant but desirable specific combining ability were detected by Misr 2xGiza171 for wet gluten %, Sakha 94 x Misr 1 for grain yield, Giza 171 x Sakha 94 for grain yield and l000-kemel weight, Misr 1 x Gemmeiza 11 for l000-kemel weight, protein content, wet gluten, Sids 13 x Misr 2 for grain yield, l000-kemel weight and protein content, Sids 13 x Gemmeiza 11 for grain yield, Giza 171 x Misr 2 for relative density and hectoliter weight and Sids 13 x Sakha 94 for relative density and hectoliter weight. El-Nahas and Ali (2021) evaluated six Egyptian bread wheat varieties—Giza 171, Misr 1, Gemmeiza 12, Shandawel 1, Sids 13, and Sakha 95—and their 15 F_1 hybrids under normal, mid, and severe water stress conditions. Significant differences were found among genotypes for all yield-related traits. Crosses like Giza 171 × Misr 1, Giza 171 × Gemmeiza 12, and Gemmeiza 12 × Sids 13 showed high grain yield under all conditions. Both general (GCA) and specific (SCA) combining abilities were significant, with GCA/SCA ratios greater than one for most traits, indicating additive gene effects. Giza 171 showed strong GCA effects for number of kernels per spike, grain yield per spike, and grain yield per plant. Crosses such as Gemmeiza 12 × Sids 13 and Shandawel 1 × Sids 13 had desirable SCA effects under stress. Positive and significant heterosis was observed in crosses like Giza 171 × Gemmeiza 12, Misr 1 × Gemmeiza 12, Gemmeiza 12 × Sids 13, Shandawel 1 × Sids 13, and Sids 13 × Sakha 95 for key yield traits under all irrigation conditions.

testers, the line Giza 171 (1.09) was the only one with positive and significant general combining ability for wet and dry gluten content, while the tester Sids 13 (-0.98) showed negative and significant values. For hectoliter weight, no significant general combining abilities were detected, but a positive and nonsignificant value (1.67) was shown by the tester Misr 1. Farhat and Mohamed (2018) found that Giza 171 was the best parent with significant and positive GCA for wet and dry gluten.

Farhat and Mohamed (2018) reported that the preferred significant SCA for grain yield was observed in Giza 171 × Gemmeiza 12, Giza 171 × Sids 12, and Giza 171 × Misr 3. In addition, Giza 171 × Line 3 and Gemmeiza 12 × Line 2 had the highest SCA values and differed significantly from other crosses for wet gluten. In addition, Giza 171 × Sids 12 showed the highest SCA for dry gluten.

3.1.7. Estimates of genetic variance and contribution to the total variance

Table 4 presents the estimates of genetic parameters for the studied traits. A line x tester mating design was developed to partition genetic variance into its components. The genetic variance was divided into lines, testers, and Line x tester interaction components. The line and tester variance estimates the additive genetic variance, while the Line x tester interaction indicates the non-additive genetic variance, including dominance. Dominance variances were higher than additive ones for all the studied traits, except for kernel weight, kernel wet and dry gluten%, indicating the presence of non-additive gene effects. This highlights the need to proceed with hybrid breeding for possible transgressive offspring.

The Testers were more prominent and essential for grain yield, l000-kemel weight, protein content, wet gluten%, and dry gluten% traits, indicating a predominant maternal influence which should be used in further breeding programs to allow crop improvement. These results showed that testers and the interaction lines x testers brought much variation in the expression of the studied traits, which is similar to those of Fellahi *et al.* (2013) with respect to interaction lines x testers (Fellahi et al., 2013).

3.1.8. Heterosis

Heterosis is expressed as an increment percentage of the F_1 hybrids more than the mid-parent or better parent. Table 5 presents the estimates of better and mid-parent heterosis for the studied traits. For grain yield, desirable positive heterosis was obtained by Misr 1 x Gemmeiza 11 (138.34, 169.08) and Giza 171 x Gemmeiza 11 (26.17, 61.09) for both better and mid parent, respectively. More than negative better parent heterosis were attained by Misr 2 x Sids 13 (-9.58), Gemmeiza 11x Sids 13 (-24.58), and Gemmeiza 11x Giza 171 (-30.84).

In terms of l000-kemel weight, preferable positive better parent heterosis were obtained by Misr 2 x Giza 171 (19.21), Giza 171 x Sakha 94 (22.22), and Gemmeiza 11 x Giza 171 (0.89).

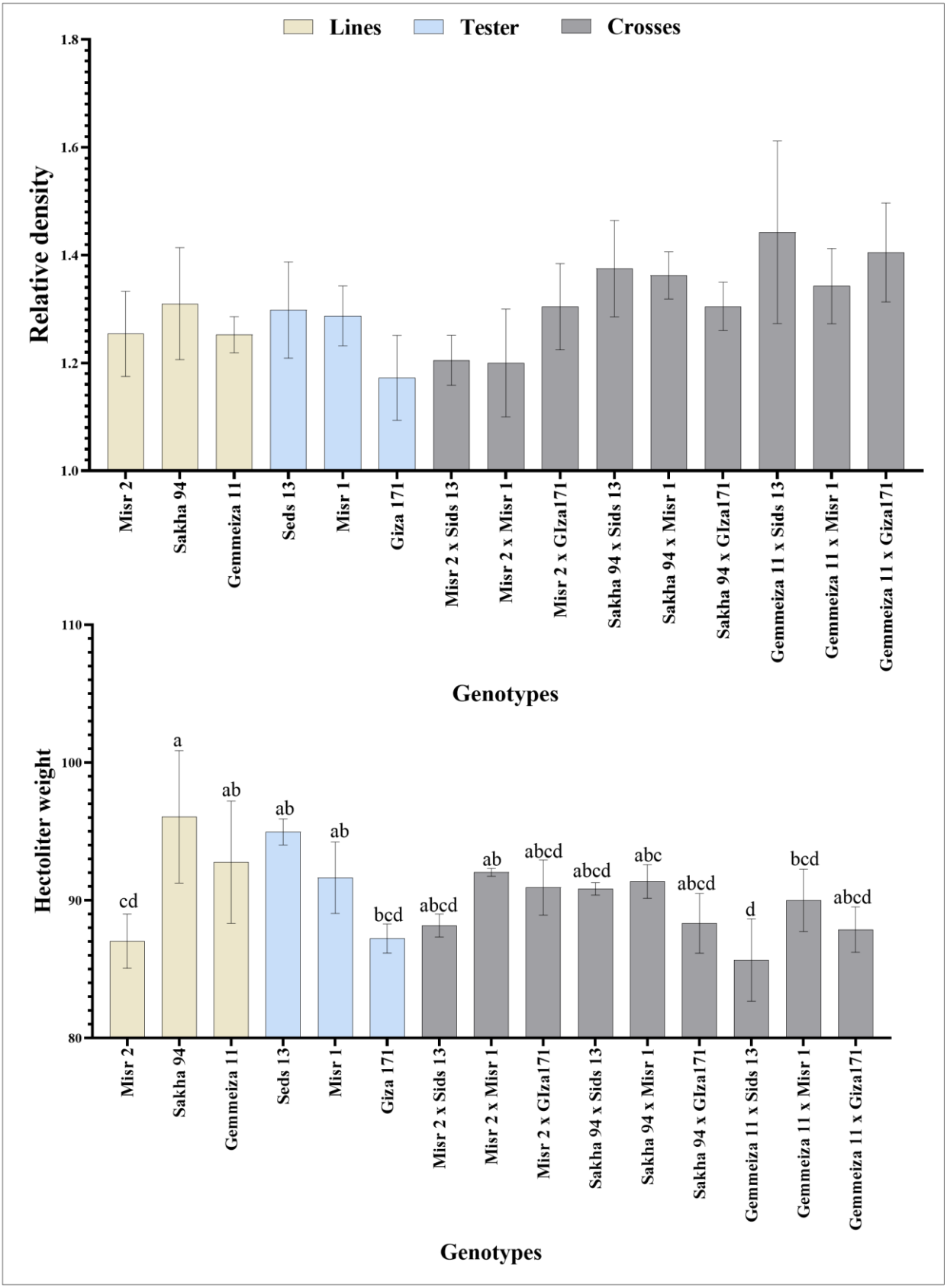

Fig. 4. Mean performance for relative density (a) and hectoliter weight (b) of 6 parental lines (three lines and three testers) and their nine wheat crosses. Different letters indicated significant variations among the treatments using LSD 0.05.

Table 1. Mean squares of the studied traits under normal, water and combined treatment conditions.

SOV	Df	Grain yield plant^{-1} (g)	l000-kernel weight (g)	Grain protein content %	Wet gluten %	Dry gluten %	Germination %	Shoot length (cm)	Root length (cm)	Dry weight (mg)	Relative density (g mm^{-3})	Hectoliter weight (kg hl^{-1})
Replication	3	64.99	23.65*	0.79	0.2	0.01	2.94**	12.59**	10.24**	7.2**	0.01	4.96
Genotypes (G)	14	458.51**	101.66**	9.86**	3.02**	0.51**	5.14**	12.9**	17.78**	17.16**	0.02**	28.74**
Parents (P)	5	230.65**	65.84**	9.42**	2.46**	0.26**	5.04**	9.72**	16.76**	31.42**	0.01*	48.84**
Crosses (C)	8	529.84**	125.56**	8.94**	3.45**	0.72**	5.53**	12.22**	20.61**	10.06**	0.03**	13.75*
P vs C	1	1027.19**	89.6	19.4*	2.48**	0.08	2.43	34.22*	0.2	2.704	0.001	48.14*
Lines (GCA)	2	1024.11	419.33*	7.89	13.1**	2.72**	12.11	2.32	0.03	12.84	0.03	25.05
Testers (GCA)	2	456.45	17.04	21.07	0.54	0.17**	4.16	30.77	60.79	9.31	0.04	9.95
Lines x Testers (SCA)	3	319.41**	32.93**	3.41**	0.08	0.01	2.93**	7.91**	10.82**	9.04**	0.02*	10.0
Error	4	24.30	6.3	0.4	0.1	0.0	0.7	1.3	1.7	1.33	0.001	6.5
Total	42											
CV	59	15.3	5.7	5.9	7.8	7.9	3.4	7.5	7.7	4.9	5.6	2.8

* and ** and ns = significant at 0.05, 0.01 levels of probability (ns) = no significant, respectively.

Table 2. General combining ability for lines and testers for the studied traits.

Lines	Grain yield plant^{-1} (g)	l000-kernel weight (g)	Grain protein content %	Wet gluten %	Dry gluten %	Germination %	Shoot length (cm)	Root length (cm)	Dry weight (mg)	Relative density	Hectoliter weight
Lines											
Misr 2	-2.11	-1.32	1.23	0.18	0.03	-1.17	-1.57	-2.34	-0.01	-0.09	0.91
Sakha 94	8.27	1	0.1	-0.27	-0.1	1	0.25	1.16	0.02	0.02	0.71
Gemmeiza 11	-6.15	0.32	-1.33	0.08	0.08	0.17	1.32	1.18	-0.01	0.07	-1.62
Testers											
Sids 13	-9.66	-6.39*	-1.03	-0.98	-0.35	-0.67	0.07	-0.09	-0.01	0.01	-1.25
Misr 1	9.1	1.11	-0.5	-0.11	-0.06	-0.67	0.21	0.26	0	-0.03	1.67
Giza 171	0.57	5.28*	1.52	1.09*	0.4	1.33	-0.28	-0.18	0.01	0.01	-0.42
Line $LSD_{0.05}$ (gi-gj)	9.81	3.25	1.16	0.69	0.27	1.89	2.05	2.81	0.03	0.15	3.91
Line $LSD_{0.01}$ (gi-gj)	13.12	4.35	1.55	0.92	0.36	2.53	2.74	3.76	0.04	0.19	5.22
Tester $LSD_{0.05}$ (gi-gj)	9.81	3.25	1.16	0.69	0.27	1.89	2.05	2.81	0.03	0.15	3.91
Tester $LSD_{0.01}$ (gi-gj)	13.12	4.35	1.55	0.92	0.36	2.53	2.74	3.76	0.04	0.19	5.22

* and ** and ns = significant at 0.05, 0.01 levels of probability (ns) = no significant, respectively.

Table 3. Specific combining ability for the crosses shown by the studied traits.

Lines	Grain yield plant^{-1}	l000-kernel weight	Grain protein content %	Wet gluten %	Dry gluten %	Germination %	Shoot length	Root length	Dry weight	Relative density	Hectoliter weight
Misr 2 x Sids 13	5.15	1.97	0.23	-0.05	-0.11	1.92	0	-0.78	0.01	-0.05	-0.97
Misr 2 x Misr 1	-8.33	-1.27	-1.3	-0.13	-0.02	-2.08	0.23	0.75	0	-0.01	-0.01
Misr 2 x GIza171	3.19	-0.7	1.07	0.19	0.13	0.17	-0.22	0.03	-0.01	0.06	0.98
Sakha 94 x Sids 13	-11.92	-1.57	0.3	0.15	0.07	-1	1.03	2.38	-0.02	0.01	1.9
Sakha 94 xMisr 1	10.47	-1.46	-0.12	-0.02	-0.04	1	-1.69	-1.46	0	0.04	-0.48
Sakha 94 x GIza171	1.45	3.04	-0.17	-0.13	-0.03	0	0.65	-0.92	0.02	-0.05	-1.43
Gemmeiza 11 x Sids 13	6.77	-0.4	-0.53	-0.09	0.04	-0.92	-1.03	-1.6	0.01	0.03	-0.93
Gemmeiza 11 x Misr 1	-2.14	2.74	1.43	0.15	0.06	1.08	1.46	0.71	-0.01	-0.03	0.49
Gemmeiza 11 x Giza171	-4.64	-2.34	-0.9	-0.06	-0.1	-0.17	-0.43	0.89	0	0	0.45
$LSD_{0.05}$ (Sij-Sik)	17.00	5.64	2.01	1.20	0.47	3.28	3.55	4.87	0.05	0.25	6.77
$LSD_{0.01}$ (Sij-Sik)	22.72	7.54	2.69	1.60	0.63	4.38	4.75	6.51	0.07	0.34	9.05

* and ** and ns = significant at 0.05, 0.01 levels of probability (ns) = no significant, respectively.

Table 4. Dominance and additive genetic components for the studied traits.

Character	Dominance	Additive	Contribution of lines	Contribution of testers	Contribution of lines x testers
Grain yield plant^{-1}	72.53	17.90	25.66	40.94	33.40
l000-kernel weight	4.33	7.72	3.39	83.49	13.11
Grain protein content %	1.05	0.61	36.76	40.35	22.89
Wet gluten %	-0.14	0.28	4.85	93.31	1.84
Dry gluten %	-0.02	0.04	5.33	89.51	5.16
Germination %	1.70	0.13	26.30	29.36	44.34
Shoot length	0.40	0.22	61.02	1.78	37.20
Root length	0.51	0.47	63.86	0.83	35.31
Dry weight	0.00	0.00	50.64	15.38	33.99
Relative density	-0.005	0.001	73.20	5.19	21.61
Hectoliter weight	-3.35	0.68	34.50	39.16	26.34

In terms of l000-kemel weight, preferable positive better parent heterosis were obtained by Misr 2 x Giza 171 (19.21), Giza 171 x Sakha 94 (22.22), and Gemmeiza 11 x Giza 171 (0.89). Additionally, positive mid-parent heterosis was shown by Misr 2 x Misr 1 (5.75), Misr 2 x Giza 171 (23.23), Sakha 94 x Giza 171 (27.89), and Gemmeiza 11 x Giza 171 (9.29). On the other hand, grain protein content % showed positive heterosis in Misr 2 x Sids 13 (1.73 and 17.71), Misr 2 x Misr 1 (23.63 and 25.29), Misr 2 x Giza 171 (33.26 and 53.99), Sakha 94 x Giza 171 (11.43 and 19.79) and Misr 1x Gemmeiza 11 (5.98 and 15) over better and mid parent, respectively.

Preferably positive better parent heterosis were found for wet gluten% % by Misr 2 x Giza 171 (11.24 and 27.99), over better and mid parent, respectively. Also, Misr 2 x Giza 171 (27.99) and Sakha 94 x Sids 13 (14.37) detected positive heterosis over mid-parent. Moreover, Giza 171 x Sakha 94 (-2.44) achieved negative and significantly better parent heterosis. Desirable positive better parent heterosis were obtained for dry gluten% by Misr 2 x Giza 171 (10.38). In addition, positive mid-parent heterosis was found by Misr 2 x Giza 171 (12.73). Farhat and Mohamed (2018) found that significant positive heterotic effects for dry gluten were shown by Giza 171 × Sids 12 (Farhat & Mohamed, 2018).

Conversely, Germination % suffered negative and significant heterosis in Misr 2x Misr 1 (15.73 and 23.47) over the better and mid parent, respectively. Also, relative density showed negative and significant heterosis, which was observed by Misr 2 x Misr 1 (-23.47 and 23.47-19.79) over better and mid parent, respectively. For Hectoliter weight, preferably positive heterosis was obtained by Gemmeiza 11 x Giza 171 (47.29 and 47.87) over the better and mid parent, respectively.

Table 5. Heterosis is above the better and mid-parent value for the studied traits.

Lines	Grain yield plant^{-1}	l000-kernel weight	Grain protein content %	Wet gluten %	Germination %	Shoot length	Root length	Dry gluten %	Relative density	Hectoliter weight
Better parent heterosis										
Misr 2 x Sids 13	-9.58	2.54	1.73**	-5.32	-7.14	-3.26	-26.2	-10.75	-7.14	-3.26
Misr 2 x Misr 1	7.2	-2.13*	23.63*	14.02	-23.47*	-0.6	-15.5	13.82	-23.47*	-0.6
Misr 2 x GIza171	2.14	19.21**	33.26**	11.24**	3.37	-15.83	-22.1	10.38**	3.37	-15.83
Sakha 94 x Sids 13	6.88	-14.31	-8.01	-7.33	-10.2	25.94	19.26	-12.5	-10.2	25.94
Sakha 94 x Misr 1	138.34**	2.63	8.43	10.15	-2.04	5.81	-1.88	-1.04	-2.04	5.81
Sakha 94 x GIza171	26.17**	22.22**	11.43**	-2.44*	9.89	1.95	-4.73	-2.99	9.89	1.95
Gemmeiza 11 x Sids 13	-24.58	-19.39	-28.71	-22.29	-13.27	18.39	4.31	-18.96	-13.27	18.39
Gemmeiza 11 x Misr 1	3.93	2.76	5.98*	0.82	-5.1	47.29**	24.01	-2.96*	-5.1	47.29**
Gemmeiza 11 x Giza171	-30.84	0.89**	-8.48	5.12	1.05	1.88	5.96	2.04	1.05	1.88
Mid parent heterosis										
Misr 2 x Sids 13	18.35	3.18	17.71**	13.14	-2.67	0.16	-20.4	1.75	-2.67	0.16
Misr 2 x Misr 1	16.6	5.75*	25.29*	16.32	-19.79*	5.82	-8.18	19.17	-19.79*	5.82
Misr 2 x GIza171	8.93	23.23**	53.99**	27.99**	3.37	-11.8	-20.9	28.57**	3.37	-11.8
Sakha 94 x Sids 13	17.72	-7.98	-0.97	8.75	-6.88	26.01	24.66	1.94	-6.88	26.01
Sakha 94 x Misr 1	169.08**	2.69	15.82	10.38	1.59	8.98	3.4	6.15	1.59	8.98
Sakha 94 x GIza171	61.09**	27.89**	19.79**	14.37*	11.11	10.36	-3.03	10.57	11.11	10.36
Gemmeiza 11 x Sids 13	1.84	-10.47	-24.46	-1.22	-11.92	22.33	7.92	1.41	-11.92	22.33
Gemmeiza 11 x Misr 1	17.81	6.51	15*	10.94	-3.63	47.87**	27.25	12.73*	-3.63	47.87**
Gemmeiza 11 x Giza171	-29.45	9.29**	-3.16	12.58	4.35	13.71	16.29	7.18	4.35	13.71

* and ** and ns = significant at 0.05, 0.01 levels of probability (ns) = no significant, respectively.

3.1.9. Correlation analysis of yield and quality traits in wheat

The correlation heatmap (Fig. 5) shows that grain yield increased with dry weight, indicating that genotypes accumulating more biomass tended to yield more. This is consistent with reports that above-ground biomass and yield are positively associated in wheat across environments, reflecting source capacity and dry-matter partitioning to grain (Li et al., 2022; Liu et al., 2018; Rahimi Eichi et al., 2019). Kernel size/weight and quality. We observed positive correlations between 1000-kernel weight (TKW) and both wet and dry gluten. Although literature notes genotype- and environment-dependent signs, there are contexts where TKW is positively related to protein (and thus gluten) (e.g., with balanced N–K fertilization); together with the well-established protein and wet-gluten linkage, this supports our finding that larger kernels can accompany higher gluten (Daaloul Bouacha et al., 2014; Kulkarni et al., 1987). In addition, several studies use protein +TKW to predict wet gluten, reinforcing the practical connection among these traits (Pasynkov et al., 2021). From a breeding standpoint, this suggests scope to improve yield and end-use quality simultaneously when selection environments favor aligned responses of kernel weight and protein/gluten. Seleiman and Abdel Aal (2018) reported that drought stress caused by skipping two irrigations (I3 regime) significantly reduced grain quality traits, including protein and carbohydrate yields per feddan and carbohydrate percentage, when compared to the full irrigation treatment (I1). However, they observed that the protein percentage actually increased significantly under the severe drought stress of the I3 regime. This suggests a trade-off between quantity and concentration of grain protein under water-limited conditions.

As expected, wet and dry gluten were tightly and positively correlated in our data, reflecting that both track the same protein fraction; strong WG–DG associations are widely documented (Kulkarni et al., 1987; Tian et al., 2025). Given gluten's central role in dough viscoelasticity and baking performance, the positive protein and gluten relationships observed here support their continued use as screening metrics for quality (Pizzi, 2024). Seedling vigor and early growth. TKW correlated with dry weight, aligning with evidence that larger seeds enhance early vigor by providing more reserves for seedling growth (Soltani et al., 2006). We also found shoot length correlated positively with root length, consistent with multi-genotype seedling studies where shoot length associates with root traits, and with broader analyses linking root length to shoot biomass. Such coupling suggests coordinated allocation that benefits water and nutrient capture (Chen et al., 2020; Sallam et al., 2024). Furthermore, vigor metrics (e.g., density/seedling indices) tended to associate with both root and shoot length in our panel, in line with studies reporting positive links between seed/seedling density measures and seedling lengths and with standard vigor indices (Kiran et al., 2021).

Implications. Collectively, these patterns indicate that yield, grain quality, and vigor are not independent in this material. Breeding indices that give moderate weight to biomass/TKW alongside gluten/protein could enrich for high-yield, high-quality types, while simple seedling vigor traits serve as rapid, early-generation filters. Given known environment × genotype effects and trait collinearity, we recommend confirming these associations across years/locations and partitioning direct/indirect effects via path analysis or multiple regression before final index weighting (Desheva & Deshev, 2022). Zubair et al. (2021) reported that among 18 wheat genotypes evaluated under irrigated and rainfed conditions, significant variation was found in key grain quality traits such as thousand grain weight (TGW), protein content, gluten, starch, and test weight. Genotype BWP-122559 showed the highest TGW (39.04g) in both conditions. Cluster analysis grouped the genotypes into three clusters, with Cluster-1 (including NR-443, IV-II, WBG-14, and CT-12176) showing superior performance in TGW, protein, and gluten content. These genotypes were identified as promising for wheat breeding programs aimed at improving grain quality under different environmental conditions.

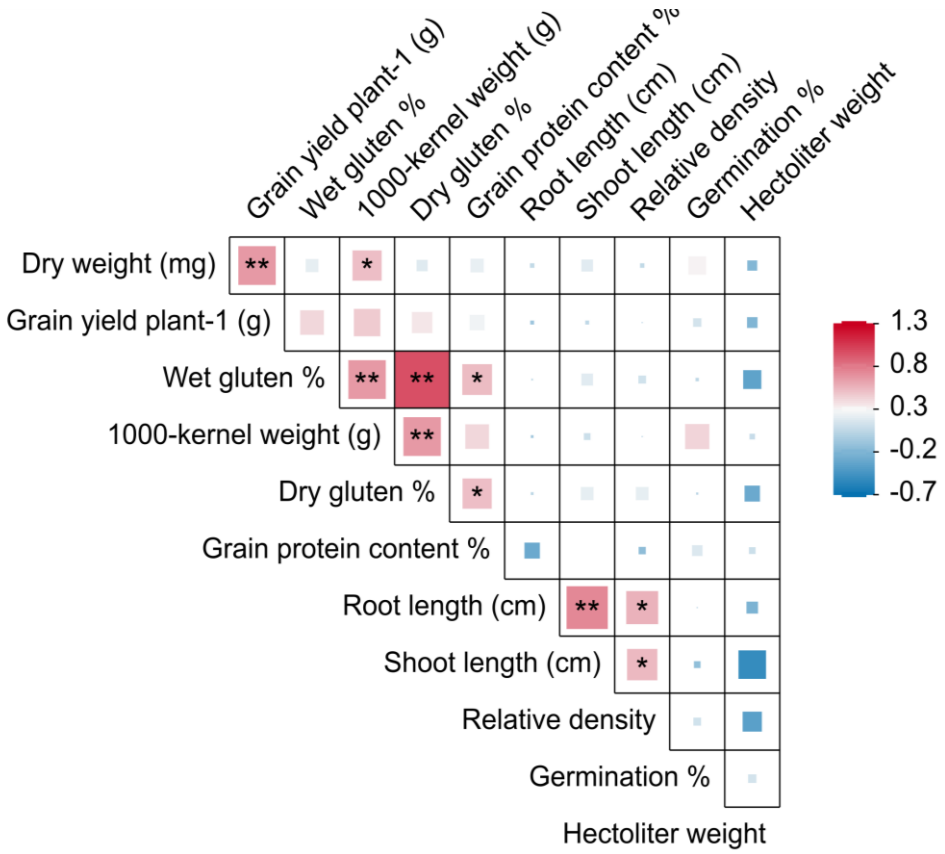

Fig. 5. The correlation heatmap Correlation among the studied characters

3.2 Genetic Studies in the F_2 Generation

The inheritance of bread-making quality traits in the F_2 generation of wheat was evaluated, revealing the complex polygenic control of most characteristics. Descriptive statistics demonstrated significant differences between the two cultivars, Giza 171 and Misr 2 ($P < 0.01$ or 0.05), for all traits except for germination percentage (Table 7). The cultivar Misr 2 outperformed Giza 171 in several traits, including plant height (4.29% higher), number of spikes per plant (28.87% higher), germination percentage (1.57% higher), dry weight (10.34% higher), root length (5.70% higher), and specific density (8.85% higher). On the other hand, Giza 171 showed superior values in traits such as spike length (29.50% higher), number of spikelets per spike (13.83% higher), 1000-kernel weight (19.01% higher), grain yield per plant (11.58% higher), shoot length (5.52% higher), and grain protein content (12.53% higher).

The F_1 hybrid performance exceeded or approached the high parents' values for several key traits. For example, plant height in the F_1 hybrid increased by 1.51%, the number of spikes per plant was nearly the same as the higher parent (0.17% difference), 1000-kernel weight increased by 16.61%, and grain yield per plant increased by 7.46%. Germination percentage (1.68% higher), grain protein content (18.09% higher), and specific density (close to the parents, with a slight reduction of 2.44% compared to Misr 2) also showed improvement in the F1 hybrid. However, the F_1 hybrid had lower values for traits like spike length (-17.07%), number of spikelets per spike (-28.62%), dry weight (-9.70%), shoot length (-9.52%), and root length (-21.01%) compared to the parents.

In the F_2 generation, the mean values exceeded both parents for the number of spikes per plant (14.46% higher), grain protein content (21.21% higher), and specific density (8.85% higher). However, for traits such as spike length (-7.71%), number of spikelets per spike (-8.86%), 1000-kernel weight (-8.73%), grain yield per plant (-14.17%), germination percentage (-17.73%), dry weight (-8.35%), shoot length (-20.97%), and root length (-24.72%), the F2 values were lower or close to the lowest parent values. Plant height in the F_2 generation was intermediate, showing a slight decrease of 0.89% compared to the parental mean. Additionally, the F_2 generation displayed a broader range of values than both parents for all traits except shoot length, highlighting significant variability in the F_2 population. The results indicate that the F_2 generation exhibited a wide range of trait inheritance patterns, with transgressive segregation occurring in some attributes, allowing for the potential selection of desirable traits. The number of spikes per plant and grain protein content showed promising increases, essential for improving yield and bread-making quality in wheat breeding programs.

Table 7. Descriptive statistics of the studied characters for the two cultivars, Giza 171 and Misr 2, and their F_1 and F_2 populations in the 2017/18 season.

Generation	Parameters	Plant height	No. of spikes plant^{-1}	Spike length	No. of spikelets spike^{-1}	1000-kernel weight (g)	Grain yield plant^{-1}
Giza 171	Mean	108.67	15.00	20.50	24.67	47.28	38.26
	SE	0.78	0.24	0.19	0.16	0.57	1.41
	Variance	30.33	3.00	1.75	1.33	16.26	99.03
Misr 2	Mean	113.33	19.33	15.83	21.67	38.30	34.29
	SE	0.58	0.28	0.11	0.21	0.35	0.92
	Variance	16.67	3.87	0.57	2.27	6.17	42.24
Parents means		111.00	17.17	18.17	23.17	42.79	36.28
T-test		**	**	**	**	**	*
F_1	Mean	114.00	17.20	17.00	17.60	49.90	38.99
	SE	0.59	0.23	0.22	0.24	0.62	1.24
	Variance	17.50	2.70	2.50	2.80	19.52	77.27
F_2	Mean	111.02	19.65	16.77	21.11	34.73	29.41
	SE	0.64	0.50	0.14	0.23	0.41	0.91
	Min	80.00	10.00	15.00	16.00	22.00	10.40
	Max	142.00	35.00	26.00	25.00	47.70	54.00
Generation	Parameters	Germination %	Dry weight	Shoot length	Root length	Grain protein content	Specific density
Giza 171	Mean	22.25	0.0232	15.65	18.09	11.50	1.13
	SE	0.27	0.0002	0.19	0.31	0.22	0.00
	Variance	3.58	0.0002	1.77	4.76	2.34	0.00
Misr 2	Mean	22.60	0.0256	14.83	19.12	10.22	1.23
	SE	0.35	0.0001	0.19	0.30	0.14	0.01
	Variance	6.30	0.0001	1.75	4.50	1.03	0.003
Parents means		22.43	0.02	15.24	18.60	10.86	1.18
T-test		ns	**	**	*	**	**
F_1	Mean	23.00	0.0212	14.16	15.10	13.58	1.20
	SE	0.48	0.0001	0.22	0.39	0.19	0.01
	Variance	11.33	0.0001	2.38	7.76	1.71	0.0025
F_2	Mean	18.40	0.0206	11.80	14.39	13.94	1.23
	SE	0.39	0.0003	0.17	0.27	0.17	0.02
	Min	10.00	0.011	8.00	9.07	9.73	1.01

* and ** and ns = significant at 0.05, 0.01 levels of probability (ns) = no significant, respectively.

3.3 Variance components

The variance components for the Giza 171 x Misr 2 F_2 population indicate a significant genetic influence on the traits studied (Table 8). Phenotypic variances significantly differed from environmental variances for all traits ($P < 0.01$ or 0.05), highlighting the importance of genetic factors in trait expression. Genotypic variances exceeded environmental variances across all characters, suggesting strong genetic control. The genotypic variance for key traits like plant height was 60.7, compared to an environmental variance of 21.5. Spike length showed a genotypic variance of 47.46, with an environmental variance of 3.19. Grain yield per plant had a genotypic variance 19.63, while the environmental variance was 13.98. Similarly, the number of spikelets per spike had a high genotypic variance of 91.74, compared to an environmental variance of 72.85. These results demonstrate that genetic variance is dominant in the variation observed for these traits, indicating a strong potential for selection and improvement in breeding programs.

3.4 Heritability and genetic advance

The broad heritability, genetic advance, and genetic advance as a percentage of the F_2 mean are presented in Table 9. All the studied characters showed moderate to high broad-sense heritability values, ranging from 51.73 % for root length to 99.09% for dry weight. When the characters have high estimates of heritability, this means that these

characters are under the control of polygenes, which is useful for breeders. The important role of the genetic variances compared to the phenotypic ones and the moderate to high broad-sense heritability for most studied characters indicate the effectiveness of selection in the early generations and that modified pedigree/bulk and selected bulk are recommended methods in this cross (Abdelkhalik, 2019). These results generally align with (Aglan et al., 2020; Darwish et al., 2018).

In this respect, El-Rawy and Hassan (2014) obtained low to moderately narrow-sense heritability for root length, shoot length, and seedling dry weight. They also observed moderate to high genetic advance for root and shoot lengths (El-Rawy & Hassan, 2014). Knowledge of the expected response to selection and the consequent expected genetic gain is essential to identify the appropriate selection criteria. The selection intensity was 5 % of the base population in each F_2 cross for the studied characters. The expected genetic gain from selection ranged from 0.09 for dry weight to 23.81 for the number of spikelets per spike. Heritability alone does not indicate the degree of genetic improvement that would result from selecting an individual genotype. The selection's effectiveness depends on heritability and genetic advance. Hence, knowledge of heredity coupled with genetic advances is more useful. In addition, it is essential to predict the expected genetic gain from a selection cycle. In the present study, a moderate genetic advance was observed for plant height (13.79), no. of spikes per plant (13.74), and grain yield per plant (14.73), while the other traits showed low genetic advance. Overall, the progress of genetic progression and genetic advance as a percentage of the mean was moderate to high for all the studied traits.

A relative comparison of heritability and genetic advance as a percentage of the means over the traits showed that all the studied traits had high to moderate heritability estimates combined with moderate to high genetic advance as a percentage of the mean, indicating genotypic variations for such a trait. These results confirmed that the additive nature of the genes primarily controlled these characters and that direct selection for these traits could be effective. In this regard, Elmassry *et al* (2020) used the cross Giza 171 × Shandaweel 1. They found high heritability estimates in the broad and narrow sense and moderate to high genetic advance for most studied characters (Elmassry et al., 2020).

In addition, Aglan *et al*. (2020) studied the Giza171 × Sids12 hybrid and found that Giza171 was preferable for yield and its components. All characters showed moderate to high broad-sense heritability values in F_2 and F_3 generations (Aglan et al., 2020)

3.5. Heterosis, inbreeding depression, and potency ratio

The estimates of better and mid-parent heterosis, potency ratio, and inbreeding depression for the studied traits are presented in Table 10. The estimates of better and mid-parent heterosis, potency ratio, and inbreeding depression for the Giza 171 x Misr 2 F_2 population revealed several important findings. Positive better parent heterosis was observed for desirable traits such as plant height (4.9%) and protein content (18.1%). However, certain traits showed undesirable negative heterosis, including spike length (-11%), number of spikes per plant (-17.1%), and 1000-kernel weight (-28.6%). Mid-parent heterosis also reflected a mix of desirable and undesirable traits. While positive heterosis was detected for plant height (2.7%) and grain yield per plant (16.6%), undesirable negative values were recorded for spike length (-6.4%), number of spikelets per spike (-24%), and dry weight (-13.1%). Inbreeding depression results indicated both positive and negative effects. Significant and desirable negative inbreeding depression was observed for traits like the number of spikes per plant (-14.23%) and the number of spikelets per spike (-19.95%),

Table 8. Phenotypic (σ_P^2), genotypic (σ_g^2) and environmental (σ_e^2) variance components for the studied characters in Giza 171 x Misr 2 F_2 population.

Character	σ^2_P	σ^2_e	σ^2_g
Plant height	82.2**	21.5	60.7
Spike length	50.65**	3.19	47.46
No. of spikes plant^{-1}	3.71**	1.61	2.1
I000-kernel weight (g)	10.41**	2.13	8.28
Grain yield plant^{-1}	33.62**	13.98	19.63
No. of spikelets spike^{-1}	164.59**	72.85	91.74
Germination percentage	30.11**	7.07	23.03
Dry weight	0.00002**	0	0.00002
Shoot length	5.57**	1.97	3.61
Root length	14.18**	5.67	8.51
Grain protein content	5.7**	1.69	4.01
Specific density	0.05**	0.002	0.05

* and ** = significant at 0.05 and 0.01 probability levels, respectively.

suggesting potential for these traits in breeding programs. However, undesirable inbreeding depression was found in traits like 1000-kernel weight (-19.95 %), grain yield per plant (30.41%), and germination percentage (20.01%), which may present challenges for further breeding efforts.

The potency ratio estimates reflected partial dominance for traits such as plant height (0.03), grain yield per plant (0.17%), and protein content (0.25%), indicating a degree of favorable inheritance. On the other hand, negative potency ratios, as seen in spike length (-0.06), number of spikelets per spike (-0.07), and root length (-0.19%), indicated varying degrees of under-recessiveness for these traits. These results highlight traits with desirable heterosis and negative inbreeding depression that can be targeted for improved wheat breeding outcomes.

3.5 Phenotypic and genetic coefficient of variation

Table 11 presents the estimates of the phenotypic (PCV) and genotypic (GCV) coefficients of variation for various traits in the Giza 171 × Misr 2 F_2 population, PCV exceeded GCV, indicating that observed variation included an environmental component in addition to genetic differences. Similar PCV > GCV patterns and their interpretation as environmental influence, are widely reported for wheat panels evaluated across regimes and sites (Divya Chaudhary et al., 2023; Sewore & Abe, 2024). For specific density, the GCV was closest to the PCV, implying minimal environmental masking and a larger transmissible (genetic) share of variance, hence better prospects for selection. In wheat, a small PCV–GCV gap is commonly taken to mean lower environmental influence and higher heritability (Divya Chaudhary et al., 2023; Taherian et al., 2024). The magnitude of variability we observed (e.g., PCV from ~8% for plant height to ~44% for spikelets spike^{-1}; GCV from ~7% to ~35%) is in line with reports that height and maturity traits tend to show low–moderate PCV/GCV, whereas spike-/yield components often show moderate–high values. Several wheat studies document comparable ranges and emphasize that higher PCV/GCV for spike, kernel, and yield traits reflects ample scope for gain under selection (Poudel et al., 2021; Prasad et al., 2021).

Consistent with your table, plant height showed low variability (PCV≈8%, GCV≈7%), a pattern frequently noted for stature and phenology traits in wheat; by contrast, spikelets spike^{-1} displayed higher variability, a trait often reported with moderate–high PCV/GCV and good response to selection in wheat panels (Aiman et al., 2024; Fufa et al., 2024). Taken together, the high-to-moderate PCV and GCV for most traits indicate adequate genetic variability within this germplasm, supporting the feasibility of improvement through selection. As standard practice, traits can be categorized as low (0–10%), moderate (10–20%), and high (>20%) for both PCV and GCV when prioritizing breeding targets and gauging expected gain (Azimi et al., 2017; Dutamo et al., 2015). Implication for a merged Results–Discussion. Report the PCV and GCV for each trait, note where PCV ≈ GCV (candidate traits for early-generation selection), and flag traits with high PCV but noticeably lower GCV as more environment-sensitive, warranting multi-environment validation before heavy selection pressure (Divya Chaudhary et al., 2023; Taherian et al., 2024).

Table 9. Broad sense heritability ($H^{2)}$, genetic advance (ΔG) and genetic advance as a percentage of F_2 mean (ΔG%) for the studied characters in the Giza 171 x Misr 2 F_2 population.

Characters	H^2	ΔG	ΔG%
Plant height	73.84	13.79	12.42
Spike length	93.7	13.74	69.92
No. of spikes plant^{-1}	56.67	2.25	13.4
I000-kernel weight (g)	79.51	5.29	25.04
Grain yield plant^{-1}	58.4	6.98	20.09
No. of spikelets spike^{-1}	55.74	14.73	50.09
Germination percentage	76.51	8.65	47
Dry weight	83.1	0.008	39.59
Shoot length	64.72	3.15	26.68
Root length	59.99	4.65	32.33
Grain protein content	70.3	3.46	24.8
Specific density	95.94	0.45	36.81

Table 10. Best and mid-parent heterosis, potency ratio, and inbreeding depression for the Giza 171 x Misr 2 F_2 population studied characters.

Character	Best parent heterosis	Mid-parent heterosis	Potency ratio	Inbreeding depression
Plant height	4.9*	2.7**	0.03	2.61**
Spike length	-11.0*	0.2	0.002	-14.23**
No. of spikes plant^{-1}	-17.1**	-6.4**	-0.06	1.35
1000-kernel weight (g)	-28.6**	-24**	-0.24	-19.95**
Grain yield plant^{-1}	5.6	16.6**	0.17	30.41**
No. of spikelets spike^{-1}	1.9	7.5	0.07	24.58**
Germination percentage	1.8	2.6	0.03	20.01**
Dry weight	-17.2	-13.1**	-0.13	2.96
Shoot length	-9.6	-7.1**	-0.07	16.64**
Root length	-21.1**	-18.9**	-0.19	4.64
Grain protein content	18.1**	25.0**	0.25	-2.66
Specific density	-2.3	1.9*	0.02	-2.38

* and ** = significant at 0.05 and 0.01 levels of probability, respectively

Table 11. Phenotypic (PCV% %) and genotypic (GCV% %) coefficient of variation for the studied characters in Giza 171 x Misr 2 F2 population.

Character	PCV %	GCV %
Plant height	8.166	7.018
Spike length	36.221	35.062
No. of spikes plant^{-1}	11.479	8.641
I000-kernel weight (g)	15.285	13.63
Grain yield plant^{-1}	16.696	12.76
No. of spikelets spike^{-1}	43.626	32.571
Germination percentage	29.822	26.085
Dry weight	23.126	21.082
Shoot length	20.01	16.097
Root length	26.157	20.26
Grain protein content	17.125	14.359
Specific density	18.626	18.243

6 Molecular diversity assessment

In the study of genetic differences between F_2 high and low protein populations, DNA samples were subjected to gel electrophoresis using Xpsp1, Dx5, and Xcn15 primers. The analysis revealed that the F_2 high protein population displayed strong and consistent banding patterns across all primers. Specifically, band intensities for the high protein group were approximately 50% greater than those observed in the low protein group, indicating robust amplification and uniform genetic similarity within this group. This pattern was consistent across different primers, with the F_2 high protein population showing about 1.5 times stronger amplification efficiency than the low protein group when analyzed with the Xcn15 primer. Further insights were gained through cluster analysis using genetic similarity data, highlighting distinct clustering patterns. The high protein population clustered closely with parent P2, demonstrating a strong genetic linkage with intra-group similarity exceeding 95%. This indicates a predominant inheritance of high protein traits from parent P2. Conversely, the low protein population showed a closer genetic relationship with parent P1, with notable internal variability. Specifically, subsets like F_2low1 and F_2low2 were closely associated with P1, showing about 90% similarity, while other subsets, such as F_2low3, F_2low4, and F_2low5, exhibited more divergence, with approximately 85% similarity within these groups.

These results underscore the genetic distinctions between the F_2 population individuals, with the high protein group demonstrating higher amplification efficiency, greater uniformity, and genetic closeness to parent P2, indicative of the inheritance patterns affecting protein expression. The detailed analysis of band intensities and clustering patterns provides a robust framework for understanding the genetic basis of protein traits in these populations, which is crucial for advancing targeted breeding programs to enhance desirable traits such as high protein content. Three SSR primers (Dx5, Xpsp1, and Xcn15) were used to evaluate genetic differences between the Giza 171 (P1, low protein), Misr 2 (P_2, high protein), and their F_2 high and low protein individuals (Table 12). The molecular size of the amplified bands ranged from 67 bp to 144 bp. Seven amplified fragments (loci) were obtained, and the polymorphism percentage was 100%.

DNA samples were amplified using the three SSR primers to evaluate genetic differences between the F_2 high and low protein populations (Table 13). The data revealed distinct amplification patterns across the parental Giza 171 (P_1), Misr 2 (P_2) and F_2 populations. P_1 and P_2 samples displayed clear amplified bands, indicating the presence of the target DNA. The F_2 high protein individuals exhibited strong and consistent banding patterns, suggesting robust amplification and uniform genetic similarity within this group. In contrast, the F_2 low-protein individuals showed less intense bands, reflecting either lower amplification efficiency or genetic differences than the high-protein population. These results highlight clear differences in band intensity and patterning between the high- and low-protein individuals, suggesting underlying genetic variation.

A dendrogram was constructed using genetic similarity data to investigate further the genetic relationships among the samples (Figure 6). The dendrogram revealed two distinct clusters: one containing the F_2 high protein population and P_2, and the other grouping the F_2 low protein population with P_1. Within the F_2 high protein population, F_2high1, F_2high2, and F_2high3 formed a tight sub-cluster, indicating strong genetic similarity among these samples. Similarly, within the F_2 low protein population, F_2low1 and F_2low2 clustered closely with P1, while F_2low3, F_2low4, and F_2low5 formed a separate subgroup, indicating minor genetic variation within the low protein group. The clustering patterns observed in the dendrogram are consistent with the banding patterns seen in gel electrophoresis, providing further evidence for the genetic distinction between high- and low-protein populations.

These findings align with previous studies that have demonstrated significant genetic divergence within biparental F_2 populations due to recombination and segregation of traits (Odell et al., 2022; Xian-Liang et al., 2006). The clustering of the high protein population with P2 and the low protein population with P1 further indicates that the high protein trait is predominantly inherited from P2. In contrast, the low protein trait is associated with P1.

The observed genetic divergence has significant implications for crop improvement and breeding programs. By identifying markers such as those amplified by the Xpsp1, Dx5, and Xcn15 primers, breeders can more effectively select desired traits, such as high protein content Marker-assisted selection (MAS) has been widely recognized as a powerful tool for accelerating genetic gains in crop improvement (Collard & Mackill, 2008). The markers identified in this study could serve as key tools in developing high-protein lines with greater genetic uniformity, thereby enhancing breeding efficiency.

4- Conclusion

The cross Giza 171 × Misr 2 demonstrated a unique and valuable combination of high grain protein content, elevated wet gluten levels, and superior vegetative traits such as long shoot and root lengths. These characteristics, along with its stable performance in the F_2 generation, position it as a highly promising candidate for breeding programs aimed at developing nutritionally enriched and agronomically resilient wheat cultivars. This finding underscores the potential of targeted hybrid breeding to enhance both yield quality and plant vigor in bread wheat.

Table 12. Numbers and types of the amplified DNA bands and the polymorphism percentage generated by the three SSR primers.

Primer name	Sequence	Molecular size Range (bp)	Fragments		Polymorphism %
			Monomorphic	Polymorphic	
Dx5	F: GCC TAG CAA CCT TCA CAA TC R: GAA ACC TGC TGC GGA CAA G	67	0	1	100%
Xpsp1	F: TCC CGC CAT GAG TCA ATC R: TTG GGA GAC ACA TTG GCC	77 - 96	0	3	100%
Xcn15	F: GGT GAT GAG TGG CAC AGG R: CCC AAC AGT TGC AGA AAA TTA G	97 - 144	0	3	100%
Total			0	7	100%

Table 13. The presence (1) or absence (0) of the used primers.

Primer name	bands w.	F2high 1	F2high 2	F_2high 3	F2high 4	F2high 5	P 1	P 2	F2low 1	F2low 2	F2low 3	F2low 4	F2low 5
Dx5	67	1	1	1	1	1	0	1	0	0	0	0	0
Xpsp1	96	1	1	1	1	1	0	1	0	0	0	0	0
	82	0	0	0	0	0	1	0	1	1	1	1	1
	77	0	0	0	0	1	0	0	0	0	0	0	0
Xcn15	144	0	0	0	1	1	1	1	1	1	1	1	0
	123	1	1	1	0	0	0	0	0	0	0	0	0
	97	0	0	0	0	0	0	0	0	0	0	0	1

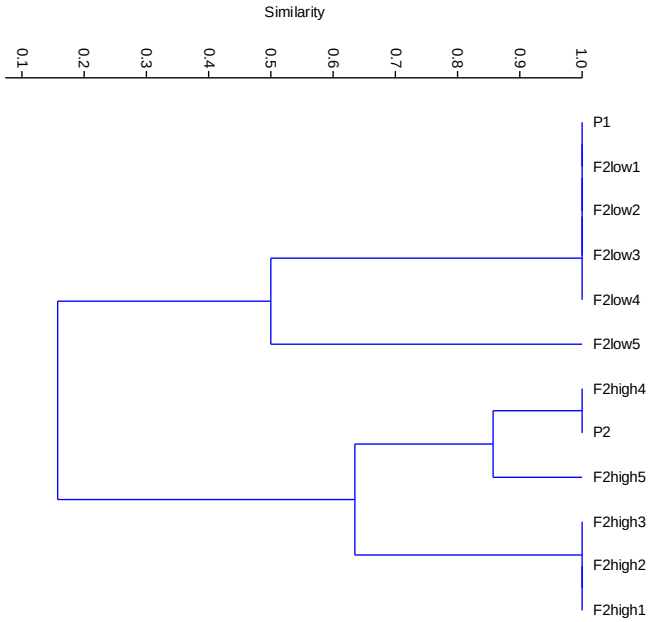

Fig. 6. Dendrogram showing genetic similarity relationships among Giza 171 (P1), Misr 2 (P2), F_2 high protein population, and F_2 low protein population.

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All authors declare their consent for publication.

Author contribution statement:
Antar N. El-Banna: Formal analysis, writing original draft and correspondence. **Ismael A. Khatab:** Experimental work, Data curation, Writing, review, and editing. **Rasha Ramadan:** performed molecular validation, wrote review & edited.

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References

A.O.A.C. (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (Vol. 11). The Association.

Abdel Nour, N. A., El–Fateh, H. S., Mostafa, A. K. (2011). Line x Tester analysis for yield and its traits in bread wheat. *Egyptian Journal of Agricultural Research*, *89*(3), 979-992.

Abdelkhalik, S. (2019). Assessment of some genetic parameters for yield and its components in four bread wheat crosses using six parameter model. *Egypt. J. Plant Breed*, *23*(5), 719-736.

Aglan, M. A., Mohamed, E. N., Shahin, A. (2020). Selection for yield, rust resistance and quality traits in early generations of Giza 171 x Sids 12 cross of bread wheat. *Journal of Plant Production*, *11*(3), 259-266.

Aiman, S., Muhammad, S., Arsalan, K., Khan, N. U., Muhammad Yousaf Shah, H., Sundas, B. (2024). Evaluation of Genetic Potential and Heritability Estimates in Wheat Genotypes. *International Journal of Agriculture Innovations and Cutting-Edge Research (HEC Recognised)*, *2*(4), 01-09. https://jai.bwo-researches.com/index.php/jwr/article/view/70

Ajmal, S. U., Khanam, B., Khanum, S., Akram, Z. (2000). Gene action studies for some biometric traits in a diallel crosses of wheat. *Pakistan Journal of Biological Sciences (Pakistan)*, *3*(11).

Al-Daej, M. I. (2022). Estimation of heterosis, heritability and genetic parameters for some agronomic traits of rice using the line × tester method. *Journal of King Saud University - Science*, *34*(4), 101906. https://doi.org/https://doi.org/10.1016/j.jksus.2022.101906

Al-Mafarji, T., Al-Jubouri, D. J. M. A., Kanbar, A. (2024). Estimate Combining Ability and Gene Action of Yield, and Some Qualitative Traits of Genotypes Bread Wheat.(*Triticum aestivum* L.) of Half-Diallel Crosses. *Tikrit Journal for Agricultural Sciences*, *24*(3), 182-196.

Aljubory, J., Al–Tamim, O. (2019). Estimating the Heterosis, Combining Ability and Gene Action for the Quality of Wheat Grains. *Mesopotamia Journal of Agriculture*, *47*(Supplement I), 2-3.

Azimi, A. M., Marker, S., Bhattacharjee, I. (2017). Genotypic and phenotypic variability and correlation analysis for yield and its components in late sown wheat (*Triticum aestivum* L.). *Journal of Pharmacognosy and Phytochemistry*, *6*(4), 167-173.

Bapela, T., Shimelis, H., Tsilo, T. J., Mathew, I. (2022). Genetic improvement of wheat for drought tolerance: Progress, challenges and opportunities. *Plants*, *11*(10), 1331.

Bilgin, O., Balkan, A., Başer, İ. (2022). Selection for high yield and quality in half-diallel bread wheat F2 populations (*Triticum aestivum* L.) through heterosis and combining ability analysis. *International Journal of Agriculture Environment and Food Sciences*, *6*(2), 285-293.

Burton, G. (1952). Qualitative inheritance in grasses. Vol. 1. Proceedings of the 6th International Grassland Congress, Pennsylvania State College,

Chemists, A. A. o. C., Committee, A. A. o. C. C. A. M. (1983). Approved methods of the American association of cereal chemists.

Chen, Y., Palta, J., Prasad, P. V. V., Siddique, K. H. M. (2020). Phenotypic variability in bread wheat root systems at the early vegetative stage. *BMC Plant Biology*, *20*(1), 185. https://doi.org/10.1186/s12870-020-02390-8

Collard, B. C., Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 557-572.

D'ovidio, R., Anderson, O. (1994). PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. *Theoretical and Applied Genetics*, *88*(6), 759-763.

Daaloul Bouacha, O., Nouaigui, S., & Rezgui, S. (2014). Effects of N and K fertilizers on durum wheat quality in different environments. *Journal of Cereal Science*, *59*(1), 9-14. https://doi.org/https://doi.org/10.1016/j.jcs.2013.11.003

Darwish, M., Abd El-Kreem, T., Farhat, W. (2018). Selection studies in some bread wheat F_3 crosses at Sakha and Nubaria locations. *Journal of Plant Production*, *9*(1), 81-89.

Desheva, G., Deshev, M. (2022). Correlation and regression relationships between main grain quality characteristics in bread winter wheat. *Agriculturae Conspectus Scientificus*, *87*(2), 135-143.

Divya Chaudhary, S., Kumar, A., Reddy, B. S. (2023). Genetic variability and character association studies in bread wheat (*Triticum aestivum* L.) under two different water regimes. *Electronic Journal of Plant Breeding*, *14*(4), 1330-1336.

Dutamo, D., Alamerew, S., Eticha, F., Assefa, E. (2015). Genetic variability in bread wheat (*Triticum aestivum* L.) germplasm for yield and yield component traits. *Journal of Biology, Agriculture and Healthcare*, *5*(17), 140-147.

El-Din, S., El-Shami, M., Abdel-Latif, A., Mostafa, M., Abdel-Aleem, M., Sharshar, M., Menshawy, A., El-Borhamy, H., Hammad, S., Mahrous, M. (2005). An introduction to Sakha 94, the new bread wheat cultivar. *Journal of Plant Production*, *30*(1), 91-101.

El-Nahas, M. M., Ali, O. A. M. (2021). Estimation of combining ability and heterosis for wheat yield and its components under water stress conditions. *Egyptian Journal of Agronomy*, *43*(2), 227–293. https://doi.org/10.21608/agro.2021.71677.1312

El-Rawy, M. A., Hassan, M. I. (2014). A diallel analysis of drought tolerance indices at seedling stage in bread wheat (Triticum aestivum L.). *Plant Breeding and Biotechnology*, *2*(3), 276-288.

Elahmadi, A. (1994). Development of wheat germplasm tolerant to heat stress in Sudan. *Wheat in heat–stressed environment, Irrigated, Dry Areas and Rice–Wheat Farming System, Mexico, DF: CIMMYT*, 48-54.

Elmassry, E., Darwish, M., Elkot, A. (2020). Inheritance of stripe rust resistance in adult plants and some economic traits of some bread wheat crosses. *Journal of Plant Production*, *11*(12), 1587-1596.

FAO. (2023). *Suite of Food Security Indicators. In: FAOSTAT. Rome. https://www.fao.org/faostat/en/#data/FS*. https://www.fao.org/faostat/en/#data/FS.

Farhat, W., Mohamed, E. (2018). Breeding for some agronomic and quality characters in bread wheat. *Journal of Plant Production*, *9*(3), 215-231.

Fellahi, Z. E. A., Hannachi, A., Bouzerzour, H., Boutekrabt, A. (2013). Line× tester mating design analysis for grain yield and yield related traits in bread wheat (*Triticum aestivum* L.). *International Journal of Agronomy*, *2013*(1), 201851.

Fernandes, M. I. B. d. M., Zanatta, A. C. A., Prestes, A. M., Caetano, V. d. R., Barcellos, A. L., Angra, D. C., Pandolfi, V. (2000). Cytogenetics and immature embryo culture at Embrapa Trigo breeding program: transfer of disease resistance from related species by artificial resynthesis of hexaploid wheat (*Triticum aestivum* L. *em. Thell*). *Genetics and Molecular Biology*, *23*, 1051-1062.

Fouad, H., El-Ashmoony, M., El-Karamity, A., Sarhan, M. K. (2020). Direct and indirect selection for grain yield in Bread Wheat (*Triticum aesitivum* L.). *Journal of Plant Production*, *11*(3), 241-249.

Fufa, M., Gedebo, A., Leta, T., Lule, D. (2024). Genetic variation, genetic advance, heritability and correlation analysis of phenotypic traits in tetraploid wheat (*Triticum turgidum* spp.) landraces and some improved cultivars of Ethiopia. *Plant Genetic Resources: Characterization and Utilization*, *22*(1), 8-16. https://doi.org/10.1017/S1479262123001089

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Australian journal of biological sciences*, *9*(4), 463-493.

Guzmán, C., Autrique, E., Mondal, S., Huerta-Espino, J., Singh, R. P., Vargas, M., Crossa, J., Amaya, A., Peña, R. J. (2017). Genetic improvement of grain quality traits for CIMMYT semi-dwarf spring bread wheat varieties developed during 1965–2015: 50 years of breeding. *Field Crops Research*, *210*, 192-196.

Haji, H., Hunt, L. (1999). Genotype× environment interactions and underlying environmental factors for winter wheat in Ontario. *Canadian Journal of Plant Science*, *79*(4), 497-505.

Hamada, A., Abd El-Majeed, S., El-Saied, E., Tawfeles, M., El-Borhamy, H. (2017). Misr 1 and Misr 2: Two new, high yielding and rust resistant bread wheat cultivars. *Menoufia Journal of Plant Production*, *2*(2), 177-192.

Hamada, A., Abo-Warda, A., Abd El-Majeed, S., El-Sayed, E., Meshref, M. (2015). Giza 171: A new high

yielding bread wheat cultivar. Egypt J. *Plant Breed*, *19*(7), 2063-2079.

Ismail, S. K. (2015). Heterosis and combining ability analysis for yield and its components in bread wheat (*Triticum aestivum* L.).

Istıplılıer, D., Ilker, E., Tonk, F. A., Cıvı, G., Tosun, M. (2015). Line× tester analysis and estimating combining abilities for yield and some yield components in bread wheat. *Turkish Journal of Field Crops*, *20*(1), 72-77.

Kempthorne, O. (1957). *An introduction to genetic statistics*. John Wiley and Sons, Inc., New York. 545 **pp**

Khodadadi, E., Aharizad, S., Sabzi, M., Shahbazi, H., Khodadadi, E. (2012). Combining ability analysis of bread quality in wheat. *Annals of Biological Research*, *3*(5), 2464-2468.

Kiran, S. Y., Singh, V., Mor, V., Dey, S., Kumar, D. (2021). Multivariate analysis of seed vigour parameters in late sown wheat (*Triticum aestivum* L. *em. Thell*). *Int J Chem Stud*, *9*, 275-278.

Kramer, A., Twigg, B. A. (1966). Fundamentals of quality control for the food industry. *(No Title)*.

Kulkarni, R., Ponte, J., Kulp, K. (1987). Significance of gluten content as an index of flour quality. *Cereal Chem*, *64*(1), 1-3.

Kumar, A., Razdan, A., Sharma, V., Kumar, N., Kumar, D. (2018). Study of heterosis and inbreeding depression for economic and biochemical traits in bread wheat (*Triticum aestivum* L.). *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 558-564.

Kumar, S., Tamura, K., Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in bioinformatics*, *5*(2), 150-163.

Langridge, P., Reynolds, M. (2021). Breeding for drought and heat tolerance in wheat. *Theoretical and Applied Genetics*, *134*(6), 1753-1769.

Lee, G. D. (2013). Determining reference test weight per bushel value of grains. *Weights and Measures Connection*, *4*(6), 1-4.

Li, H., Li, L., Liu, N., Liu, Z., Lu, Y., Shao, L. (2022). Balanced below- and above-ground growth improved yield and water productivity by cultivar renewal for winter wheat [Original Research]. *Frontiers in Plant Science, Volume 13 - 2022*. https://doi.org/10.3389/fpls.2022.1022023

Liu, K., Muse, S. V. (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, *21*(9), 2128-2129.

Liu, L., Wang, Z.-h., Diao, C.-p., Wang, S., Li, S. (2018). Grain yields of different wheat cultivars and their relations to dry matter and NPK requirements in dryland. *Journal of Plant Nutrition and Fertilizers*, *24*(3), 599-608. https://doi.org/10.11674/zwyf.17168

Mahdy, E., El-Karamity, A., Mokadem, S. A., Abd-Elmawgood, A., Fouad, H. (2015). Selection for grain yield in bread wheat under normal irrigation and drought stress conditions. *Journal of Plant Production*, *6*(4), 547-562.

Mallikarjuna, N., Chandrashekhar Haradari, C. H., Nagabhushan, N., Pavan, R., Prahalada, G. (2011). Gene action and combining ability studies for grain yield and quality characters in Bread Wheat (Triticum aestivum L.).

Manifesto, M., Feingold, S., Hopp, H., Schlatter, A., Dubcovsky, J. (1998). Molecular markers associated with differences in bread-making quality in a cross between bread wheat cultivars with the same high Mr glutenins. *Journal of Cereal Science*, *27*(3), 217-227.

Moustafa, M., Shrshar, M., Shehab El-Din, T., Abo Shereef, M., Abdel-Majeed, S., Abdel-Aleem, M., Sabry, S., Sadek, I. M., Hamada, A., Abo-Warda, A. (2010). Sids13: A new bread wheat cultivar. *Journal of Plant Production*, *1*(1), 125-135.

Murtadha, M. A., Ariyo, O. J., Alghamdi, S. S. (2018). Analysis of combining ability over environments in diallel crosses of maize (*Zea mays*). *Journal of the Saudi Society of Agricultural Sciences*, *17*(1), 69-78. https://doi.org/https://doi.org/10.1016/j.jssas.2016.01.004

Nassar, S. (2019). Pedigree selection in bread wheat under water deficit for yield in Toshka conditions. *Egyptian Journal of Desert Research*, *69*(2), 1-18.

Odell, S. G., Hudson, A. I., Praud, S., Dubreuil, P., Tixier, M.-H., Ross-Ibarra, J., Runcie, D. E. (2022). Modeling allelic diversity of multiparent mapping populations affects detection of quantitative trait loci. *G3*, *12*(3), jkac011.

Pasynkov, A. V., Zavalin, A. A., Pasynkova, E. N., Andreev, V. L. (2021). Comparative Assessment of Various Methods for Predicting Protein Content in Wheat Grain. *Russian Agricultural Sciences*, *47*(4), 363-369. https://doi.org/10.3103/S1068367421040133

Pizzi, K. (2024). *Assessing Wheat Quality Using Protein and Wet Gluten Contents* Retrieved from chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://cerealscanada.ca/wp-content/uploads/2024/11/2024-Assessing-Wheat-Quality-Using-Protein-and-Wet-Gluten-Contents-FINAL-1.pdf?utm

Poudel, M. R., Poudel, P. B., Puri, R. R., Paudel, H. K. (2021). Variability, correlation and path coefficient analysis for agro-morphological traits in wheat genotypes (*Triticum aestivum* L.) under normal and heat stress conditions. *International Journal of Applied Sciences and Biotechnology*, *9*(1), 65-74.

Prasad, J., Dasora, A., Chauhan, D., Rizzardi, D., Bangarwa, S., Nesara, K. (2021). Genetic variability, heritability and genetic advance in bread wheat (*Triticum aestivum* L.) genotypes. *Genetics and Molecular Research*, *20*(2), 1-6.

Rahimi Eichi, V., Okamato, M., Haefele, S. M., Jewell, N., Brien, C., Garnett, T., Langridge, P. (2019). Understanding the Interactions between Biomass, Grain Production and Grain Protein Content in High and Low Protein Wheat Genotypes under Controlled Environments. *Agronomy*, *9*(11), 706. https://www.mdpi.com/2073-4395/9/11/706

Rauf, A., Khan, M., Jan, F., Gul, S., Afridi, K., Khan, I., Bibi, H., Khan, R., Khan, W., Kumar, T. (2023). Genetic analysis for production traits in wheat using line x tester combining ability analysis.

Reynolds, M. P., Lewis, J. M., Ammar, K., Basnet, B. R., Crespo-Herrera, L., Crossa, J., Dhugga, K. S., Dreisigacker, S., Juliana, P., & Karwat, H. (2021). Harnessing translational research in wheat for climate resilience. *Journal of Experimental Botany*, *72*(14), 5134-5157.

Sadeghi, F., Dehghani, H., Najafian, G., Aghaee, M. (2012). Genetic analysis of bread-making quality attributes in hexaploid wheat (*Triticum aestivum* L.). *Annals of Biological Research*, *3*(7), 3740-3749.

Sadek, E. M., Ageez, A., El-Menofy, M., Abo Shereef, M., Hamada, A., Moussa, A., Kumber, R., Sleem, S., El-Shaarawy, G., Morad, A. (2013). Gemmeiza 11–A new Egyptian high yielding bread wheat (*Triticum aestivum* L.) cultivar. *Journal of Plant Production*, *4*(2), 183-204.

Sallam, A., Awadalla, R. A., Elshamy, M. M., Börner, A., Heikal, Y. M. (2024). Genome-wide analysis for root and leaf architecture traits associated with drought tolerance at the seedling stage in a highly ecologically diverse wheat population. *Computational and Structural Biotechnology Journal*, *23*, 870-882. https://doi.org/https://doi.org/10.1016/j.csbj.2024.01.020

Seleiman, M. F., Abdel-Aal, M. S. M. (2018). Response of growth, productivity and quality of some Egyptian wheat cultivars to different irrigation regimes. *Egyptian Journal of Agronomy*, 40(3), 313–330.

Sewore, B. M., Abe, A. (2024). Genetic variability and trait associations in bread wheat (*Triticum aestivum* L.) genotypes under drought-stressed and well-watered conditions. *CABI Agriculture and Bioscience*, *5*(1), 64. https://doi.org/10.1186/s43170-024-00259-6

Singh, R. K., Chaudhary, B. D. (1981). Biometrical methods in quantitative genetic analysis.

Sleper, D. A., Poehlman, J. M. (2006). *Breeding field crops*.

Soltani, A., Gholipoor, M., Zeinali, E. (2006). Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental and Experimental Botany*, *55*(1), 195-200. https://doi.org/https://doi.org/10.1016/j.envexpbot.2004.10.012

Steel, R. G. D., Torrie, J. H. (1960). Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw Hill, New York, 187-287.

Taherian, M., Saeidnia, F., Hamid, R., Nazeri, S. M. (2024). Identification of high-yielding and stable cultivars of wheat under different sowing dates: Comparison of AMMI and GGE-biplot analyses. *Heliyon*, *10*(20), e39599. https://doi.org/https://doi.org/10.1016/j.heliyon.2024.e39599

Taranto, F., Esposito, S., De Vita, P. (2023). Genomics for Yield and Yield Components in Durum Wheat. *Plants*, *12*(13), 2571. https://www.mdpi.com/2223-7747/12/13/2571

Tayyar, S. (2008). Grain yield and agronomic characteristics of Romanian bread wheat varieties under the conditions of Northwestern Turkey. *African Journal of Biotechnology*, *7*(10).

Tian, Y., Liu, P., Kong, D., Nie, Y., Xu, H., Han, X., Sang, W., Li, W. (2025). Genome-wide association analysis and KASP markers development for protein quality traits in winter wheat. *BMC Plant Biology*, *25*(1), 149. https://doi.org/10.1186/s12870-025-06171-z

Xian-Liang, S., Xue-Zhen, S., Tian-Zhen, Z. (2006). Segregation distortion and its effect on genetic mapping in plants. *Chinese Journal of Agricultural Biotechnology*, *3*(3), 163-169.

Zubair, M., Akhtar, L. H., Minhas, R., Bukhari, M. S., Hussain, S., Ali, I., Mahmood, M. T., Rehman, M., Akram, M. I., Ullah, R. (2021). Assessment of wheat genotypes for quality attributes grown under irrigated and rainfed conditions. *Egyptian Journal of Agronomy*, *43*(1), 97–104.