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EXPLORING FITNESS OF BREAD WHEAT F₃ POPULATIONS TO DEVELOP CLIMATE-RESILIENT CULTIVARS UNDER AGROCLIMATIC CONDITIONS OF SARGODHA, PAKISTAN

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SUMMARY

Wheat (*Triticum aestivum* L.) production has had increasing challenges with rising temperatures due to climate change. This study aimed to evaluate the genetic potential of bread wheat F₃ populations and their parents under terminal heat stress using physio-morphic traits via a randomized complete block design. Five F₃ populations, derived from diverse crosses, entailed assessment in climatic conditions of Sargodha, Pakistan. Significant variation among populations emerged for all traits. The cross Punjab 76 × E109 outperformed in grain yield per plant (17.20 g), the number of tillers per plant (8.59), the number of seeds per spike (75.83), and cell membrane thermostability (CMS, 95.65%). Akbar 2019 × E145 and E116 × C228 also exhibited high-yield potentials. High heritability (0.99), genetic advance (9.12), and strong positive correlations appeared for grain yield with spike length, tiller number, and CMS. Principal component analysis (PCA) revealed that PC1 and PC2 explained 84.61% of the total variation, confirming trait clustering. Among parental lines, C271 (98.19 cm) and E121 (11.90 g) showed a good performance for plant height and yield, respectively. These results suggest that crosses like Punjab 76 × E109 and Akbar 2019 × E145 can serve as potential genetic sources for improving heat resilience and productivity in wheat.

Keywords: wheat (*T. aestivum* L.), F₃ populations, PCA, climate resilient, CMS, biplot, yield-related traits, grain yield

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Key findings: Heat stress affects a lot of grain yield production of wheat (*T. aestivum* L.). A reduction of 3%–10% of grain yield occurred in wheat caused by an increase of each one °C ambient temperature. Out of five populations, three F₃ populations, such as Punjab 76 × E109 (17.20 g), Akbar 2019 × E145 (12.07 g), and E116 × C228 (11.53 g), yielded better for grain yield per plant than the parents under terminal heat environment. Therefore, these populations are candidate-breeding lines for developing heat-resilient wheat cultivars.

INTRODUCTION

Filial generations of wheat (*Triticum aestivum* L.) are vital for selecting desired plants to develop a cultivar, particularly in self-pollinated crops (Begna, 2021). Filial generations referred to the successful populations of offspring due to a cross between contrasting parents in plant breeding studies (Jin *et al.*, 2013). In the context of climate change and its impact on crops, it is necessary to have varieties that can bear the climatic effects and ensure food security. In achieving this task, the presented study evaluated five F₃ generations of wheat in the climate conditions of Sargodha, Pakistan.

Bread wheat (*Triticum aestivum* L.) is a globally significant staple crop, known for its high nutritional value, providing essential fibers, protein, carbohydrates, and fats (Wieser *et al.*, 2023). It is the primary source of energy for over one-third of the population globally (Shahzadi *et al.*, 2024). Pakistan ranked eighth in the world for producing wheat and averaged 29.69 to 31.4 million tons, with most of it coming from Punjab, Sindh, and Khyber Pakhtunkhwa (Sikandar *et al.*, 2024). Climate change causes significant impacts on wheat cultivation in arid and semi-arid areas (Hussain *et al.*, 2021). A rise in temperature during the lifecycle of wheat plants, especially at the reproductive phase, causes huge yield losses, which is a risk for food security, as many people could face a lack of food soon. Moreover, Pakistan mainly depends on irrigated farming, and water shortage leads to a great concern toward eco-friendly farming (Imran *et al.*, 2022).

Sargodha, Pakistan (32°4'N, 72°40'E), experiences a subtropical steppe climate, which is generally dry. This region witnesses a wide range of temperatures throughout the year, with an average low around 8.4 °C in

January and a high reaching 43.8 °C by June and typically low rainfall (Climate-Data.org, 2024; Visual Crossing, 2025). Shifting temperatures due to climate change are directly affecting crop growth. Scientists have observed this change clearly during 2019 to 2023 (Climate-Data.org, 2024). In just four years, the average lowest temperature rose by about 2.5 °C (from 18.79 °C to 21.29 °C). Similarly, the average highest temperature for the year increased from 30.58 °C (2019) to 33.15 °C (2023), as illustrated in Figures 1 and 2 (Visual Crossing, 2025). The stability of wheat yields will increasingly face threats from the impacts of climate change. The need to find more genetic resources resilient to heat and water deficit conditions and their efficient use in wheat hybridization is essential. The primary objective is to develop new wheat cultivars that are resilient to adverse climates in Pakistan, thereby ensuring food security. In the reported study, wheat F₃ populations incurred evaluation under prevailing natural environmental conditions for the development of climate-resilient wheat cultures that can respond to the current and projected impacts of climate change.

MATERIALS AND METHODS

Experimental site and plant material

The conduct of this study began during wheat (*T. aestivum* L.) growing on November 15, 2023, at the research farm of the College of Agriculture, University of Sargodha, Pakistan. The experimental material consisted of 10 wheat genotypes as parents and five segregating F₂ populations as offspring. The plant material came from the Department of Plant Breeding and Genetics, College of

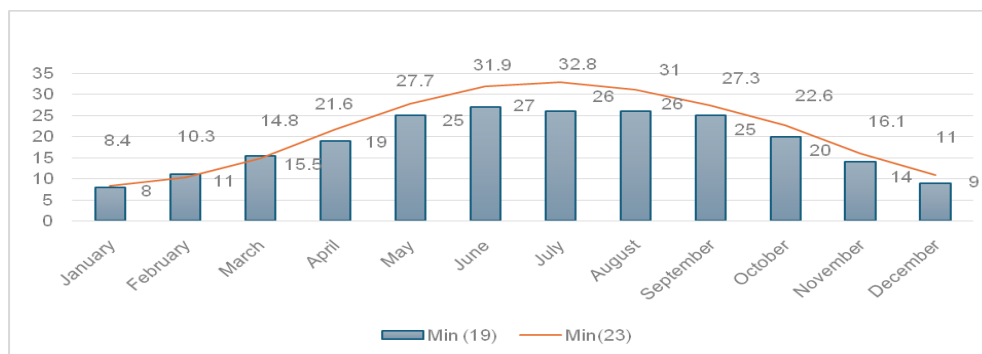


Figure 1. Minimum temperature difference between 2019 and 2023 in Sargodha, Pakistan.

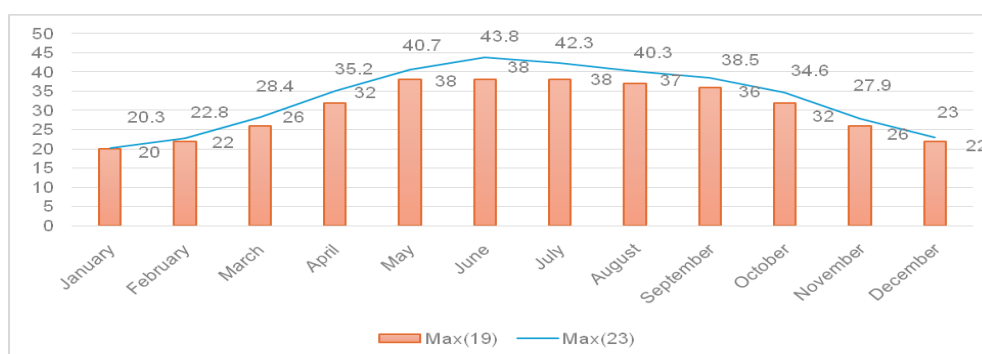


Figure 2. Maximum temperature difference between 2019 and 2023 in Sargodha, Pakistan.

Table 1. List of parents and F₃ populations of wheat.

No.	Parents	No.	Parents	No.	F ₃ Populations
1	Akbar 2019	6	E121	1	Akbar 2019 × E145
2	C228	7	E133	2	E116 × C228
3	C271	8	E145	3	E121 × C271
4	E109	9	Fsd-08	4	Fsd-08 × E133
5	E116	10	Punjab 76	5	Punjab 76 × E109

Agriculture, University of Sargodha. The F₃ populations reached development by sowing F₂ seeds of the selected plants along with their parental lines (Table 1). The experiment layout was in a randomized complete block design with three replications. All cultural practices proceeded following the recommended nitrogen and phosphorus fertilizers, thinning to ensure plant-to-plant distance and plant protection measures, and canal irrigations. At proper growth stages, data recording ensued for plant height (cm), spike length (cm), the number of tillers per plant, the number of

seeds per spike, grain yield per plant (g), and cell membrane thermostability (%) as recommended by Bibi *et al.*, (2020). The calculation of cell membrane thermostability (%) assessed heat stress tolerance using the formula below:

$$\text{CMS (\%)} = (1 - [T_1 / T_2]) \times 100$$

Where T₁ = conductivity of heat-treated samples before autoclave and T₂ = conductivity of heat-treated samples after autoclave.

Statistical analysis

All recorded data underwent statistical analysis to determine the extent of genetic variations among the genotypes. Analysis of variance (ANOVA) succeeded in following the method, as described by Steel *et al.*, (1997). Means' comparison used the least significant difference (LSD) test at a 5% probability level, performed by the open-source R-language software version 3.0.1 using the 'agricolae' package per the standard method given by Kempthorne (1957). Broad-sense heritability (H^2), genetic advance (GA), and genotypic and phenotypic coefficients of variation (GCV and PCV) obtained measurements for the studied characters following Griffiths *et al.*, (2000). Pearson's correlation coefficients entailed calculation to explore association among grain yield and yield-related traits. Performing the principal component analysis (PCA) identified patterns of variation and key traits contributing to heat tolerance.

RESULTS AND DISCUSSION

Components of variations

Plant height plays an important role in determining wheat (*T. aestivum* L.) yield and lodging resistance. The plant height of studied wheat genotypes (10 parents and five F_3 populations) ranged from 84.15 to 111.88 cm, with an overall mean value of 100.63 cm. The semi-dwarf trend of plant height in wheat

genotypes created lodging resistance and heat tolerance in newly selected plants of F_3 populations to develop climate-resilient wheat cultivars. Genotypic mean square values (200.75) of plant height showed a highly significant response toward the presence of ample genetic variability among studied wheat genotypes. This revealed that plants' selection in F_3 populations based on height would be effective in improving the grain yield if selection depended on semi-dwarf wheat plants only. However, the phenotypic variance (83.51) was higher than the genetic variance (58.62), which indicated environmental factors also contributed to plant height variation. The broad sense heritability estimate (0.7019) of this trait suggested approximately 70% of the observed variation was heritable, implying that selection could be effective through environmental stability (Table 2). The estimates of genetic advance (13.21) based on selected plants in F_3 populations revealed the expected gain in the next generation could be beneficial to improve yield. This study's results are in line with the findings of Bazai *et al.*, (2020), who reported a similar type of heritability and genetic advance values for plant height in wheat breeding experiments to develop high-yielding cultivars.

The wheat spike is not just a structure that contains grains but also an organ that performs a pivotal role in photosynthesis due to its large green zone and position on the plant stem. The spike length of studied wheat genotypes ranged from 8.35 to 14.79 cm, with an overall mean spike length of studied wheat

Table 2. Components of variation for studied plant traits in wheat genotypes.

Plant Traits	R	\bar{x}	MSG	MSE	δ^2g	δ^2p	$h^2(b.s)$	G.A
Plant height	111.9 - 84.15	100.63	200.75**	24.89	58.62	83.51	0.7019	13.21
Spike length	14.79 - 8.35	11.96	8.556*	0.342	2.74	3.08	0.8889	3.21
Number of tillers per plant	8.59 - 1.36	3.95	12.548**	0.139	4.14	4.27	0.9674	4.12
Number of seeds per spike	75.84 - 49.53	62.5	236.04**	14.26	73.93	88.17	0.8383	16.21
Grain yield per plant	17.2 - 1.53	7.65	59.35**	0.14	19.74	19.87	0.9929	9.12
Cell membrane thermostability	95.65 - 1.53	56.63	2565**	9.9	851.7	861.6	0.9885	59.77

R = Range (Max–Min), \bar{x} = Grand mean, MSG = Mean sum of square of genotypes, MSE = Mean sum of square of error, δ^2g = Genotypic variance, δ^2p = Phenotypic variance, $h^2(b.s)$ = Broad-sense Heritability, and G.A = Genetic advance.

genotypes of 11.96 cm. Genotypic mean square value (8.556) indicated significant genetic variation among wheat genotypes for spike length (Table 2). The heritability value of spike length (0.8889) was relatively high, suggesting this trait is under genetic control and has enough genetic variation. The selection of plants with a large spike length in F₃ populations could be useful to develop high-yielding and heat-tolerant wheat cultivars. The genetic advance value of 3.21 confirmed that considerable improvement in F₄ populations is attainable through selection. Spike length also showed strong association with grain yield per plant and most of the other studied plant traits, which favored selection of plants based on spike length. Previous studies have found that spike length has a positive correlation with grain yield, reinforcing the importance of selecting for this trait in breeding programs (Wang *et al.*, 2023). However, environmental conditions, such as soil fertility and water availability, may still influence spike elongation (Teng *et al.*, 2023).

The number of tillers per plant ranged from 1.36 to 8.59, with a grand mean of 3.95 tillers exhibiting a high heritability (0.9674), indicating minimal environmental influence and strong genetic control (Table 2). The significant mean sum of squares of genotypes (MSG) value (12.548) and the genetic variance (4.14) further support this conclusion. The genetic advance of 4.12 implies that selection would be highly efficient in increasing tiller production. Studies in wheat breeding have also reported similar findings, where tiller number is a key determinant of biomass accumulation and yield potential (Al-Tabbal and Al-Fraihat, 2012).

The number of seeds per spike varied from 49.53 to 75.84, with an overall recorded mean value of 62.50 per spike, a highly significant MSG (236.04), and a heritability estimate of 0.8383 (Table 2). The genetic advance (16.21) showed that selection could effectively enhance this trait. Since the genetic variance (73.93) is relatively close to the phenotypic variance (88.17), it indicates a strong genetic control with a minor environmental influence. This outcome aligned with previous research, which highlights that seed number per spike is a major contributor

to grain yield and can attain improvement through targeted selection (Wang *et al.*, 2019; Kumar *et al.*, 2023).

The grain yield per plant of parents and F₃ populations had a range from 1.53 to 17.2 g, with an average mean of 7.65 g, indicating that sufficient genetic variability was present in wheat genotypes under study for seed yield improvement. The genotypic mean square value (59.35) of analysis of variance demonstrated a highly significant genetic variation among wheat genotypes (Table 2). The broad-sense heritability estimate (0.9929) signified nearly all the observed variability for grain yield per plant was due to genetic factors, and the direct selection of desirable plants for grain yield improvement could be effective. Thus, a simple plant selection in F₃ populations would be favorable to achieve maximum grain yield potential under a terminal heat-stress environment. The genetic advance estimate (9.12) also provided the feasibility of grain yield improvement in the next generations. In literature, previous studies have emphasized grain yield as a complex polygenic trait influenced by multiple yield components, including tiller number, spike length, grains per spike, grain weight, etc. (Li *et al.*, 2023; Tillett *et al.*, 2022; Cao *et al.*, 2020).

Cell membrane thermostability (CMT) is an essential physiological plant parameter, which reflects the ability of a genotype for better heat tolerance. In the reported study of CMT of selected plants, the range was from 1.53% to 95.65%, with an overall mean of genotypes recorded as 56.63%. The results of ANOVA indicated that the genotypes' mean square value (2565) displayed highly significant differences among themselves (Table 2). The genetic variance (851.7) closely matched with the phenotypic variance (861.6), which resulted in an extremely high heritability (0.9885). The genetic advance (59.77) suggested that the CMT trait was highly responsive to plants' selection and would be desirable for developing heat-resilient wheat cultivars due to its essential physiological association with heat tolerance. Our results are in accordance with the findings of Yadav *et al.*, (2022), who studied the genetic basis of CMT

in stress adaptation research. The high genetic control of this trait makes it a promising candidate for breeding heat-tolerant cultivars, particularly under climate change scenarios.

Genetic variability and correlation

The height of plants varied relevantly among the wheat genotypes. The parental line E116 (111.88), followed by Fsd-08 (110.62) and the F₃ population Akbar 2019 × E145 (108.48), showed a tall plant height. Meanwhile, the F₃ population E121 × C271 (99.68), the parent Akbar 2019 (96.84), the F₃ population E116 × C228 (96.31), Punjab 76 × E109 (93.76), Fsd-08 × E133 (88.34), and the parent E145 (84.15) showed a moderate plant height, which was suitable for lodging resistance (Figure 3). This determined that the plant height resulted from a combination of factors, such as genes and its surroundings. In this study, plant height and grain yield per plant had a notably negative relationship (-0.46), as presented in Figure 4. The increase in vegetative growth may prevent tall plants from reaching their grain-growth potential. A slight decrease in cell membrane thermostability matched with increased plant height (-0.27), meaning that taller plants might be slightly less able to cope with heat stress. This implied that plant architecture in rice and wheat has a linkage with their ability to resist stress (Singh *et al.*, 2024).

Regarding spike length, it was evident that three F₃ populations, Fsd-08 × E133 (14.79), Punjab 76 × E109 (14.42), and Akbar 2019 × E145 (12.38), and two parents, E121 (13.69) and Punjab 76 (13.59), attained a longer spike length than others. Although the F₃ population E121 × C271 and parents Fsd-08 and C-228 showed smaller spikes (10.92, 10.32, and 8.35, respectively) (Figure 3). A positive correlation between spike length and grain yield per plant (0.52) revealed a slight association existed between longer spikes and higher yields (Figure 4). Previous research has shown that longer spikes can boost crop yields, though incorporating tiller counts is even more important, and longer spikes displayed an association with more tillers per plant (Tilley *et al.*, 2019).

The number of tillers per plant exhibited significant differences among wheat genotypes. Wheat F₃ populations Punjab 76 × E109 (8.59), E121 × C271 (6.14), Fsd-08 × E133 (6.05), Akbar 2019 × E145 (5.67), and E116 × C228 (4.15), and parent E121 (5.32) demonstrated more tillers per plant (Figure 3). In contrast, parents C271 (3.9), Fsd-08 (3.63), E145 (3.1), Akbar 2019 (2.82), E133 (2.54), E116 (2.33), C228 (2.09), E109 (1.48), and Punjab 76 (1.36) gave a lower number of tillers. It is evident from Figure 4 that breeding more tillers per plant is likely to increase the grain yield. The correlation coefficients revealed tillers per plant showed links with seed production by the vital correlation with the number of seeds per spike (0.92) and cell membrane thermostability (0.87). Subsequently, it showed an increased number of tillers results in each plant producing more seeds to boost the grain yield per plant. This result gains support from other studies revealing the important influence of tiller numbers on increasing yields (Ding *et al.*, 2021).

The number of seeds per spike appeared the highest in F₃ populations Punjab 76 × E109 and Akbar 2019 × E145 and in parent E121, i.e., 75.83, 75.36, and 74.16, respectively. In comparison, seeds per spike in parents C228 (54.2), Akbar 2019 (54.13), E109 (52.51), and Punjab 76 (49.53) were notable. It was apparent in Figure 4 that more seeds in a spike showed a strong relation to a greater yield per plant in the studied wheat genotypes. Therefore, a strong association (0.93) between the number of seeds per plant and grain yield per plant suggested that seed number did benefit grain yield in wheat (Philipp *et al.*, 2018).

The grain yield per plant showed significant variations across the genotypes and reached the topmost yields, as shown in Figure 3, by F₃ populations Punjab 76 × E109 (17.20), Akbar 2019 × E145 (12.07), E116 × C228 (11.53), Fsd-08 × E133 (10.83), and E121 × C271 (9.26). Among parents, wheat genotype E121 (11.90) gave the maximum grain yield, while the remaining genotypes, E145 (8.54), C271 (6.34), E133 (5.7), Akbar 2019 (5.68), Fsd-08 (5.28), E109 (3.41), E116

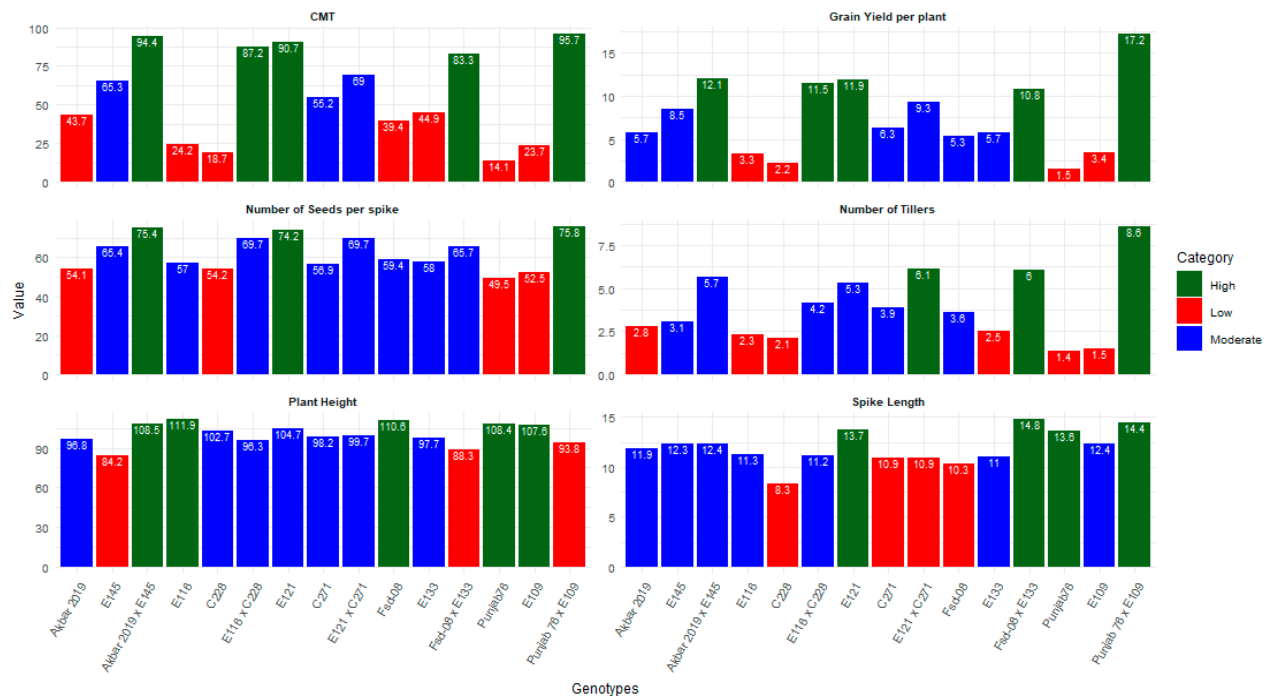


Figure 3. Mean comparison of studied wheat parents and their F_3 populations for six plant traits.

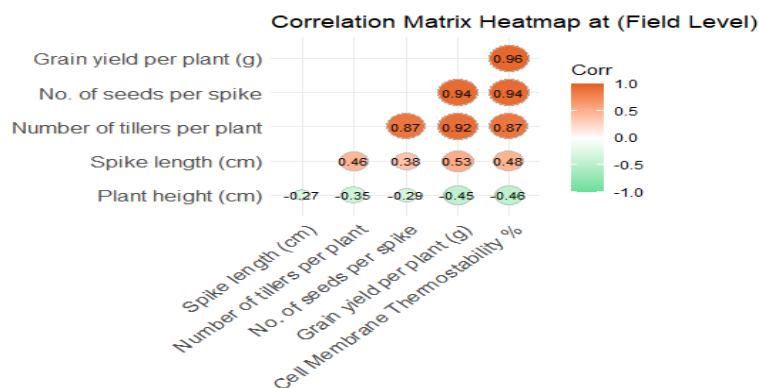


Figure 4. Correlation matrix of six plant traits for five F_3 populations.

(3.28), C228 (2.17), and Punjab 76 (1.52), expressed low yields. Correlation coefficients of grain yield per plant displayed a strongly positive association with the number of seeds per spike (0.93) and the number of tillers per plant (0.92). It indicates that improving these traits would lead to higher grain yields (Figure 4). The negative correlation with plant height (-0.46) suggested a selection of semi-dwarf plants in F_3 populations would be helpful to

improve yield in the next filial generations. Yang *et al.*, (2019) reported optimizing multiple traits, including seed number and tiller production, could remarkably enhance grain yield.

For cell membrane thermostability, F_3 populations Punjab 76 x E109 (95.65), Akbar 2019 x E145 (94.38), E116 x C228 (87.20), Fsd-08 x E133 (83.31), and E121 x C271 (68.95) showed better CMT than other

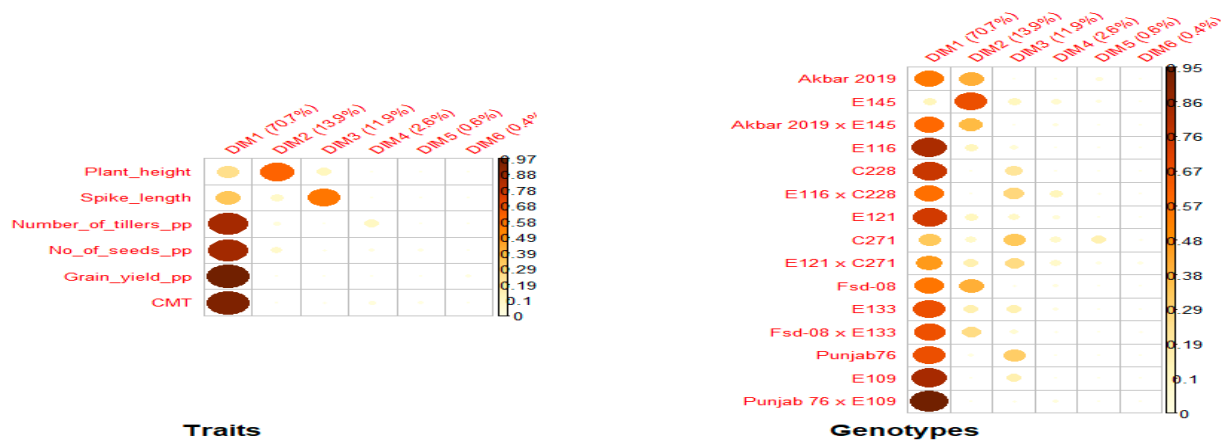


Figure 5. Contribution of genotypes (parents and F_3 populations) and traits in PC_1 to PC_6 .

populations. Similarly, parental lines E121 (90.73), E145 (65.26), and C271 (55.17) displayed good CMT to withstand heat stress, as shown in Figure 3. A positive correlation between cell membrane thermostability and grain yield per plant (0.96) implied a strong association. This suggested selection of heat stress-tolerant wheat plants in studied genotypes would be favorable to enrich grain yield of these populations because CMT had a direct effect on yield and the reproduction of plants (Ullah *et al.*, 2022). However, the negative correlation with plant height (-0.46) indicated that semi-dwarf plants may be more resistant to heat stress, aligning with other studies (Riaz *et al.*, 2021).

Principal component analysis

The biplot plot for parents and F_3 generations illustrated the first principal component (PC_1) explained 70.69% of the total variance, which was consistent with previous studies, as the first principal component often captures the most significant variation in complex datasets (Hussain *et al.*, 2024). The second principal component (PC_2) shared 13.92% of the variability of cumulative variance (Figure 5). The remaining PCs contributed progressively less, as PC_3 explained 11.86%. This large drop-off in explained variance after PC_1 highlights the importance of the first two components in capturing most of the data's

structure. PCA evaluated the connections between grain yield and morphological traits in wheat genotypes and crosses through a graphical representation (Figure 6). The factor number of tillers per plant showed a positive relationship with grain yield, as described by Sokoto *et al.* (2012). The PCA biplot analysis detailed that F_3 populations Punjab 76 \times E109, Fsd-08 \times E133, and C250 \times E117 displayed both yield stability and adaptability to different environmental settings, as already reported by Ali *et al.*, (2015). Plant height characteristics revealed an association with the genotypes located in Quadrant I. Five parental genotypes, including C228, Faisalabad 2008, and E116, displayed this grouping pattern (Figure 6). The wheat genotypes demonstrating a survival strategy proved their plants to grow taller, according to Kumar *et al.*, (2017). The wheat populations comprising E121 \times C271, Akbar 2019 \times E145, and E116 \times C228, and parent E121 belonged to Quadrant II. This refers to their association with the number of tillers per plant, cell membrane thermostability, the number of seeds per spike, and grain yield per plant (Figure 6). The selected populations and their parents expressed high-yield levels, which increased their effectiveness in stressful environments (Reynolds *et al.*, 2012). The wheat genotypes of Quadrant III displayed a trait of spike length. The quadrant contained genotypes, such as populations Punjab 76 \times E109 and Fsd-08 \times E133 and parent E145,

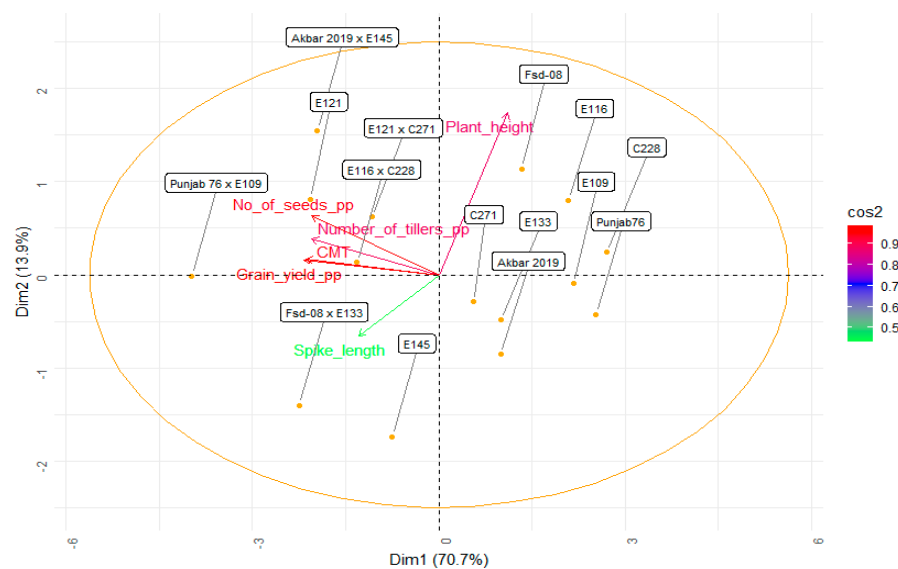


Figure 6. Biplot for parents and F₃ generations of wheat using principal component analysis.

based on a study of Figure 6. The evaluated genotypes focused on spike length having a positive relation with grain yield (Foulkes *et al.*, 2011). The parent E116, along with E133, Punjab 76, and parent E109, were also in quadrant IV, which presented cell-membrane injury characteristics.

CONCLUSIONS

Climate change is adversely affecting wheat (*T. aestivum* L.) yield in Pakistan due to abrupt changes in temperature at different growth stages, especially at the reproductive phase. It is highly critical to develop wheat cultivars that survive better in harsh environments. The results of the current study revealed that four F₃ populations, i.e., Punjab 76 × E109, Akbar 2019 × E145, E116 × C228, and Fsd-08 × E133, showed good performances for grain yield per plant under the agroclimatic conditions of Sargodha, Pakistan. Therefore, the selected plants from F₃ populations based on high grain yield and heat-stress tolerance would be valuable to develop climate-resilient wheat cultivars to ensure food security in Pakistan.

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