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# Genetic Analysis of Heat Stress Tolerance in Wheat (*Triticum aestivum* L.) Using Line x Tester Mating Design

**Tushadri Singh [a*], J. P. Jaiswal [a] and Ashish Sheera [b]**

[a] *Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, 263145, India.*
[b] *College of Agriculture (SKN Agriculture University), Baseri, Dholpur, 328027, Rajasthan, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Bread wheat (*Triticum aestivum* L.) is a critical cereal crop, providing sustenance for over 35% of the global population. Bread wheat possesses remarkable adaptability to diverse climates and soil types. However, heat stress, exacerbated by global climate change, poses a significant threat to wheat production. Developing heat-tolerant wheat varieties is essential to ensuring food security. This study identified to identify genetic variance in heat tolerance through the Line × Tester analysis, a breeding tool that evaluates the combining ability of parental lines. The experimental material comprised 16 crosses derived from four high-yielding lines and four heat-tolerant testers. These were cultivated in Pantnagar, India, under late-sown conditions to replicate heat stress. Agronomic traits such as plant height, tiller number, grains per spike, days to maturity, and grain

**Corresponding author: E-mail: tushadri@rediffmail.com;*

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yield were evaluated. Analysis of variance (ANOVA) was used to estimate general combining ability (GCA) and specific combining ability (SCA), providing insights into additive and non-additive genetic variances. Results indicated significant genetic variability among genotypes, with substantial non-additive genetic components influencing most traits. Plant height, for instance, demonstrated significant GCA and SCA variances, with SCA effects being more pronounced. Similarly, traits like the number of tillers per plant and grains per spike were predominantly controlled by non-additive genetic factors. The study revealed that hybrid combinations significantly influenced growth and yield traits, underscoring the importance of both GCA and SCA in breeding programs. The significant Line × Tester interactions suggest that specific combinations of parental lines and testers are crucial for achieving superior phenotypes. This study supports the notion that both additive and non-additive genetic effects are vital for crop improvement under heat stress, providing a robust foundation for future breeding programs aimed at enhancing wheat resilience to increasing temperatures.

*Keywords: Additive genetic variance; heat stress; hybridization; line × tester analysis; non-additive genetic variance; wheat breeding.*

## 1. INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most crucial cereal crops globally, serving as a primary food source for over 35% of the world's population [1]. This hexaploid species originated approximately 8,000-10,000 years ago in the Fertile Crescent region, through natural hybridization and subsequent polyploidization events involving three diploid progenitors: *T. urartu* (A genome), *Aegilops speltoides* (B genome), and *Aegilops tauschii* (D genome) [2]. This unique genetic composition has endowed bread wheat with remarkable adaptability to diverse climatic conditions and a wide range of soil types, making it a staple in both temperate and subtropical regions [3].

Heat stress is a significant abiotic factor that adversely affects wheat production, particularly in the context of global climate change, which is anticipated to increase the frequency and intensity of heatwaves [4]. Developing heat stress-tolerant wheat lines is thus imperative for ensuring food security. Heat tolerance in wheat is a complex trait governed by multiple genes, influencing various physiological and biochemical pathways [5]. The importance of genetic diversity in developing climate-resilient crops is well realized [6,7].

Breeding for heat tolerance involves the identification and incorporation of desirable traits from heat-tolerant lines into high-yielding cultivars. This can be effectively achieved through the Line × Tester analysis, a powerful breeding tool used to evaluate the combining ability of different parental lines [8]. The approach helps in assessing the general combining ability (GCA) of parents and the specific combining ability (SCA) of crosses, providing insights into the additive and non-additive genetic variances influencing heat tolerance and yield traits [9].

The percent contribution of each trait to the overall performance under heat stress is calculated to prioritize traits for selection [10-13]. Traits such as grain filling duration, canopy temperature depression, and chlorophyll content have been identified as critical determinants of heat tolerance in wheat [14]. By leveraging the genetic diversity within wheat and employing advanced breeding techniques, it is possible to develop heat-tolerant, high-yielding wheat varieties that can sustain productivity under increasing temperature regimes.

This study was carried out by crossing heat stress tolerant genotypes with high yielding genotypes in line X tester mating design to identify the variability among different traits, general combining ability and specific combining ability variance and percent contribution of Lines and testers to traits under study.

## 2. MATERIALS AND METHODS

Experimental Material: Experimental material consisted of 16 crosses derived from 4 lines and 4 testers crossed in Line x Tester fashion where each tester was crossed to each line. The lines and testers used in the study are: lines (female): CPAN3061, Sup132/BaJ, HD3098, PBW791 and testers (male): BRW3723, ATTILA/3*BCN/1, ATTILA*2/PBW65, HEILO//MILAN/.

The experiment was sown at Norman E. Borlaug Crop Research Centre, GBPUAT, Pantnagar during *rabi* season 2021-22 to develop $F_1$ seeds which were sown along with parents in Randomized Block Design under late sown conditions in *rabi* season 2022-23 (15th December 2022) to replicate heat stress conditions. Pantnagar is situated at 29º N latitude and 79.30º E longitude, with an elevation of 243.84 meters, experiencing a humid subtropical climate. The region has hot, dry summers and cool winters, receiving an average annual rainfall of 1433.3 mm, mostly during the rainy season. The highest temperatures occur in May and June, while the lowest are in December and January. Relative humidity is high, between 80-90%, from mid-June to February, with occasional winter showers and frost usually in late December and sometimes January.

The plants were systematically planted in a designated plot, covering 2.70 square meters (3 meters × 0.9 meters) with four rows. The planting arrangement included a row-to-row distance of 30 cm and a plant-to-plant distance of 10 cm, achieved through thinning at 30 days post-sowing. All the standard agronomic practices were followed with strict weed control.

In Line × Tester analysis, selected heat-tolerant lines (testers) were crossed with high-yielding lines (lines) to generate $F_1$ progenies. These progenies were then evaluated for various agronomic traits under heat stress conditions. Analysis of variance (ANOVA) was conducted to partition the total phenotypic variance into its components, allowing for the estimation of GCA and SCA effects. This statistical approach helped in identifying the proportion of variance attributable to each trait [15].

## 3. RESULTS AND DISCUSSION

The Analysis of Variance (ANOVA) table for the wheat crop includes data on various growth and yield parameters. The source of variation, degrees of freedom (df), and mean sum of squares for plant height, number of tillers per plant, number of grains per spike, days to maturity, and grain yield per plant are analyzed under heat stress conditions (Table 1).

**Plant Height (cm):** Different genotypes showed highly significant variation (127.15**), indicating substantial genetic diversity among the genotypes studied. Significant variation among parents or plant height (49.45*), suggesting differences among the parental lines, similarly for lines, significant variation (147.56**) existed, highlighting the impact of different lines on plant height and also for testers (86.52**) was observed. Significant interaction for Line vs Tester (95.11**), Parents vs Crosses (140.80**) and crosses (152.70**) also existed.

**Number of Tillers per Plant:** Genotypes were highly significant (99.15**), indicating genetic differences in tiller production. Similarly, parents (12.07*) were also significant depicting variability among parent lines, along with lines (284.86**), Line vs Tester (14.13*), Parents vs Crosses (450.37**) and crosses (122.51**) were significant. However, testers were not significant for number of tillers per plant (10.88)

**Number of Grains per Spike:** Genotypes were highly significant (15.28**) for number of grains per spike, indicating genetic differences. Parents (6.79*), lines (47.87**), Line vs Tester (6.78*), Parents vs Crosses (96.97*) and crosses (16.02**) also showed significant variation for number of grains per spike.

**Days to Maturity:** Genotypes were highly significant (97.98**), showing genetic differences for days to maturity. Similarly, parents (10.84*), lines (398.90**), testers (22.44**), line vs tester (23.10**), parents vs crosses (770.77**) and crosses (109.77**) were significant for days to maturity, indicating presence of variation.

**Grain Yield per Plant (g):** Genotypes were highly significant (235.62**), showing substantial genetic differences for grain yield per plant. Similarly, parents (7.31**), lines (277.61**), testers (47.49**), line vs tester (103.13**), parents vs crosses (667.54**) and crosses (300.65**) were significant for the trait under study, highlighting presence of sufficient genetic variability.

The results indicate that genotype, lines, and their interactions have a highly significant impact on most growth and yield parameters of wheat. These findings are consistent with previous research indicating the substantial role of genetic diversity and hybridization in crop improvement. Studies by Fischer et al. [16] and USDA [17] support the significant impact of genetic variability on plant height and grain yield. They found that hybridization often results in superior phenotypic traits, which is reflected in the highly significant variation observed in the Parents vs Crosses and Crosses categories.

**Table 1. ANOVA for different traits under study in heat stress conditions in bread wheat**

| Source of Variation | df | Mean Sum of Squares | | | | |
|---|---|---|---|---|---|---|
| | | Plant Height (cm) (1) | No. of Tillers per Plant (2) | No. of Grains per Spike (3) | Days to Maturity (4) | Grain Yield per Plant (g) (5) |
| Replication | 2 | 8.76 | 7.30 | 9.63 | 5.98 | 12.51 |
| Genotype | 24 | 127.15** | 99.15** | 15.28** | 97.98** | 235.62** |
| Parents | 9 | 49.45* | 12.07* | 6.79** | 10.84* | 7.31** |
| Line | 4 | 147.56* | 284.86* | 47.87* | 398.90* | 277.61** |
| Testers | 4 | 86.52** | 10.88** | 3.58** | 22.44** | 47.49** |
| Line vsTester | 9 | 95.11** | 14.13* | 6.78* | 23.10** | 103.13** |
| Parents vs Crosses | 1 | 140.80* | 450.37** | 96.97* | 770.77* | 667.54** |
| Crosses | 15 | 152.70** | 122.51** | 16.02* | 109.77* | 300.65** |
| Error | 30 | 17.84 | 2.00 | 1.99 | 3.29 | 9.96 |

*,** significant at 5% and 1%, respectively*

**Table 2. Components of variance for different agronomic traits in wheat under heat stress conditions**

| Components | Plant Height (cm)(1) | No. of Tillers per Plant (2) | No. of Grains per Spike (3) | Days to Maturity (4) | Grain Yield per Plant (g)(5) |
|---|---|---|---|---|---|
| $\sigma^2$ GCA | 0.950 | 1.81 | 0.149 | 1.436 | 3.19 |
| $\sigma^2$ SCA | 49.29 | 48.39 | 5.66 | 42.92 | 100.23 |
| $\sigma^2$ SCA/$\sigma^2$ GCA | 51.88 | 26.73 | 37.98 | 29.88 | 31.42 |
| $\sigma^2$GCA/$\sigma^2$ SCA | 0.019 | 0.037 | 0.026 | 0.033 | 0.031 |
| $\sigma^2$ Line | 5.20 | 9.72 | 0.78 | 7.77 | 18.18 |
| $\sigma^2$ Tester | 0.10 | 0.05 | 0.06 | 0.05 | 0.53 |
| $\sigma^2$a (F = 0) | 3.15 | 7.39 | 22.59 | 5.28 | 11.32 |
| $\sigma^2$d (F = 0) | 190.24 | 191.48 | 19.80 | 165.36 | 419.24 |
| $\sigma^2$ a / Var.D | 0.01 | 0.03 | 1.05 | 0.033 | 0.029 |

**Table 3. Per cent contribution of lines, testers and their interactions to total variance for various traits in $F_1$ generation in wheat under heat stress conditions**

| Traits | Lines | Testers | L x T |
|---|---|---|---|
| Plant height (cm) | 52.5180 | 4.3587 | 43.1233 |
| No. tillers per plant | 91.3286 | 0.6837 | 7.9878 |
| No. of grains/spike | 68.9463 | 1.7214 | 29.3324 |
| Days to maturity | 83.8584 | 1.5728 | 14.5688 |
| Grain yield per plant (g) | 75.0368 | 1.2152 | 23.7480 |

Moreover, the significant effect of lines and testers on plant height and tiller number aligns with findings by CIMMYT [18], which emphasized the importance of selecting appropriate parental lines for breeding programs. The significant interaction between lines and testers (Line vs Tester) suggests that the specific combination of genetic material plays a crucial role in determining the final phenotype, as also observed by Fischer et al. [16].

The non-significant replication effects across all parameters suggest that the experimental setup and environmental conditions were uniformly controlled, thereby ensuring that the observed variations are primarily due to genetic factors.

Table 2 presents various components of variance for different agronomic traits in wheat under heat stress conditions. These components are crucial for understanding the genetic control of traits and the potential for breeding heat-tolerant wheat varieties.

**Plant Height:** GCA variance for plant height (0.950) indicates the contribution of additive genetic variance, suggesting that selection for plant height can be effective through parental lines with high GCA. The SCA variance is significantly higher at 49.29, indicating a substantial non-additive genetic component. This suggests that specific hybrid combinations can produce superior plant height due to dominance and epistatic effects. The ratio of GCA to SCA variance is 51.88, highlighting the predominance of SCA effects over GCA in determining plant height. The results align with the findings of Fahad et al. [19], who reported significant GCA and SCA effects for plant height in wheat under heat stress conditions. This suggests that both additive and non-additive genetic variances are essential for breeding programs targeting plant height improvement under stress conditions.

**Number of Tillers per Plant:** The GCA variance (1.81) indicates the influence of additive genetic factors. The SCA variance is much higher at 48.39, demonstrating the dominance of non-additive genetic effects. The GCA/SCA ratio is 26.73, indicating that SCA effects are more influential than GCA effects. These results are in line with those reported by Ali et al. [20], who found that both GCA and SCA significantly impact the number of tillers per plant under heat stress, with non-additive variance playing a more crucial role.

**Number of Grains per Spike:** GCA variance (0.149) is relatively low while the SCA variance (5.66) indicates a substantial contribution from non-additive genetic effects. The GCA/SCA ratio is 37.98, highlighting the dominance of SCA effects. These findings are consistent with those of Liu et al. [21], who reported that the number of grains per spike in wheat is heavily influenced by non-additive genetic factors, especially under heat stress conditions.

**Days to Maturity:** The GCA variance (1.436) suggests moderate additive genetic effects while the SCA variance (42.92) indicates a significant non-additive genetic component. The GCA/SCA ratio is 29.88, showing the predominance of SCA effects. This is supported by the work of Farooq et al. [22], who highlighted the importance of both GCA and SCA in determining days to maturity in wheat under stress conditions, with a stronger emphasis on non-additive effects.

**Grain Yield per Plant:** The GCA variance is 3.19, indicating some additive genetic effects while the SCA variance is 100.23, demonstrating a significant non-additive genetic contribution. The GCA/SCA ratio is 31.42, emphasizing the importance of SCA effects. These results are similar to those found by Tadesse et al. [23], who showed that grain yield per plant in wheat under heat stress is significantly influenced by non-additive genetic factors, with SCA playing a crucial role.

Table 3 provides the percent contribution of lines, testers, and their interactions (L × T) to the total variance for various traits in the $F_1$ generation of wheat under heat stress conditions.

**Plant Height (cm):** Lines contribute 52.518% to the total variance while testers contribute 4.358% and L × T interaction contributes 43.123% to the total variance. The significant contribution of lines indicates that additive genetic effects are predominant in determining plant height. The relatively lower contribution of testers suggests a minor role for specific parental testers. However, the substantial L × T interaction implies that specific combinations of lines and testers significantly influence plant height. These findings are consistent with Ahmed et al. [24], who found significant additive and non-additive genetic variances for plant height under heat stress conditions. Baloch et al. [25] also highlighted the importance of specific line-tester combinations in achieving optimal plant height under stress.

**Number of Tillers per Plant:** Lines contribute 91.328% to the total variance while testers contribute 0.683% and L × T interaction contributes 7.987% to the total variance. The overwhelming contribution of lines suggests that the number of tillers per plant is predominantly controlled by additive genetic effects. The minimal contribution of testers and the low L × T interaction indicate that specific testers and their combinations with lines have little effect. This is supported by Sharma et al. [26], who reported that tillering in wheat is largely influenced by additive genetic factors under heat stress. Similarly, Islam et al. [27] found that the number of tillers is highly heritable and influenced by additive effects.

**Number of Grains per Spike:** Lines contribute 68.946% to the total variance while testers contribute 1.721% and L × T Interaction contributes 29.324% to the total variance. The significant contribution of lines and the considerable L × T interaction indicate that both additive and non-additive genetic effects are important for the number of grains per spike. The low contribution of testers suggests limited influence from specific parental testers. These results align with Singh et al. [28], who highlighted the importance of both genetic effects in controlling grains per spike in wheat under heat stress conditions. Liu et al. [29] have shown similar patterns of additive and non-additive genetic influences on this trait.

**Days to Maturity:** Lines contribute 83.858% to the total variance while testers contribute 1.572% and L × T interaction contributes 14.568% to the total variance. The high contribution of lines suggests that days to maturity are largely influenced by additive genetic effects. The low contribution of testers and the moderate L × T interaction indicate that while additive effects are predominant, specific combinations of lines and testers can also impact maturity. These findings are supported by Kumar et al. [30], who reported that days to maturity in wheat are primarily controlled by additive genetic effects, with some influence from specific line-tester combinations. Recent research by Qin et al. [31] also supports these findings, emphasizing the role of additive genetic effects in determining maturity under heat stress.

**Grain Yield per Plant (g):** Lines contribute 75.036% to the total variance while testers contribute 1.215% and L × T interaction contributes 23.748% to the total variance. The substantial contribution of lines indicates that additive genetic effects are critical for grain yield per plant. The low contribution of testers and the significant L × T interaction suggest that while specific parental testers have limited impact, certain combinations of lines and testers can significantly enhance grain yield. This aligns with findings by Wang et al. [32], who emphasized the importance of both additive and non-additive genetic effects in improving grain yield under heat stress. Furthermore, studies by Langridge and Reynolds [33] highlight the critical role of genetic interactions in achieving high grain yield under stress conditions.

## 4. CONCLUSION

The analysis reveals that non-additive genetic variance (SCA) is predominant for most traits studied under heat stress conditions. This suggests that hybrid breeding strategies, which exploit SCA, can be highly effective for improving these traits. The relatively lower GCA variance indicates that while additive effects are present, they are less influential than non-additive effects for these traits under heat stress. This study corroborates findings from other researchers, emphasizing the importance of both additive and non-additive genetic effects in wheat breeding under heat stress. Effective breeding strategies should consider both GCA and SCA to develop heat-tolerant wheat varieties.

Moreover, the significant L × T interactions for traits like plant height, number of grains per spike, and grain yield per plant suggest that non-additive genetic effects also play a crucial role. This highlights the importance of hybrid breeding strategies that exploit both additive and non-additive genetic variances for developing heat-tolerant wheat varieties. The minimal contribution of testers across most traits indicates that specific parental testers have limited influence on these traits, likely due to the specific genetic backgrounds of the testers used in this study. Therefore, selecting superior lines and optimizing their combinations can be an effective strategy for breeding heat-tolerant wheat.

Overall, the analysis underscores the importance of genetic diversity, parental selection, and hybridization in wheat breeding programs to enhance growth and yield traits. These results corroborate the findings of other researchers and provide a robust foundation for further genetic improvement of wheat.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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**Name:** ChatGPT

**Version:** GPT-4

**Model:** GPT-4 architecture

**Source:** Developed and maintained by OpenAI

2. Input prompts: Check all the references if covered both in text and in literature cited.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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