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Deciphering combining ability under heat stress conditions in bread wheat (*Triticum aestivum* L. em. Thell)

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Abstract
This study aimed to decipher the combining ability of bread wheat (*Triticum aestivum* L. em. Thell) genotypes under heat stress conditions using line × tester analysis. Three lines (BRW3723, WH1142, RAJ4402) and three testers (HPW381, CPAN3061, Sup132/BaJ) were crossed to produce nine hybrids. The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. Significant genetic variability was observed for all traits studied, including plant height, number of tillers per plant, days to 50% flowering, spikelets per spike, days to maturity, and 1000 grain weight. Analysis of variance revealed significant GCA and SCA effects, underscoring the importance of both additive and non-additive genetic variances. RAJ4402 exhibited significant positive GCA effects for traits such as number of tillers and 1000 grain weight, indicating its potential as a good general combiner. The cross BRW3723 × Sup132/BaJ showed the highest positive SCA effect for plant height, suggesting a promising hybrid combination. These findings highlight the potential for developing heat-tolerant wheat varieties by selecting superior parent combinations. The study provides valuable insights for wheat breeding programs aimed at mitigating the adverse effects of climate change on wheat production.

Keywords: Combining ability, GCA, heat stress, line x tester analysis, SCA, wheat

Introduction
Bread wheat (*Triticum aestivum* L. em. Thell) is one of the most important staple crops worldwide, providing a significant portion of the daily caloric intake for a large part of the global population. It is cultivated on more land area than any other commercial food crop and continues to be a crucial source of food security (FAO, 2020) [6]. Wheat is a versatile crop, grown under a wide range of environmental conditions, and its adaptability has contributed to its global prominence.
However, the challenge of climate change poses a severe threat to wheat production. Rising global temperatures, increased frequency of heatwaves, and unpredictable weather patterns are exacerbating the stress conditions under which wheat is grown (IPCC, 2021) [9]. Heat stress, particularly during critical growth stages such as flowering and grain filling, can significantly reduce wheat yield and grain quality (Lobell *et al*. 2012) [11]. It is estimated that for every 1°C rise in temperature, wheat yields can decline by approximately 6% (Asseng *et al*. 2015) [3]. Addressing this issue is imperative to ensure food security for a growing global population.
To combat the adverse effects of heat stress, plant breeders have been employing various genetic approaches to develop heat-tolerant wheat varieties. One effective method is the use of line × tester analysis, a powerful breeding tool that helps in identifying superior cross combinations by evaluating the general combining ability (GCA) and specific combining ability (SCA) of parental lines (Griffing, 1956; Sprague & Tatum, 1942) [8, 13]. GCA refers to the average performance of a line in hybrid combinations and is largely attributable to additive genetic variance. In contrast, SCA is associated with the performance of specific hybrids and involves non-additive genetic variance, including dominance and epistasis (Falconer & Mackay, 1996) [5]. The identification of parental lines with high GCA is crucial for developing superior hybrids with desirable traits such as heat tolerance, high yield, and good grain quality (Kumar *et al*.

2013) [10]. Additionally, understanding SCA helps breeders to identify specific cross combinations that perform exceptionally well under heat stress conditions, providing insights into non-additive genetic interactions that can be exploited in breeding programs (Ajmal *et al.* 2009) [1].

This study aims to decipher the combining ability of various bread wheat genotypes under heat stress conditions using line × tester analysis. By evaluating both GCA and SCA, this research seeks to identify superior cross combinations that can contribute to the development of heat-tolerant wheat varieties. The superior parents and hybrids can be used for breeding programs focused on mitigating the impact of climate change on wheat production.

Materials and Methods

The study aimed to evaluate the combining ability of various bread wheat genotypes under heat stress conditions using the line × tester analysis. The experiment was conducted during *Rabi* season 2022-23 under late sown conditions (25th December 2022) at the Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Plant Material: Three wheat genotypes were selected as lines (BRW3723, WH1142, RAJ4402) and three as testers (HPW381, CPAN3061, Sup132/BaJ). These genotypes were chosen based on their differential responses to heat stress and agronomic performance.

Experimental Design: The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot measured 3 meters in length, with rows spaced 25 cm apart. Standard agronomic practices were followed to raise the crop.

Data were collected on the traits: Plant height (cm), number of tillers per plant, days to 50% flowering, spikelets per spike, days to maturity and 1000 grain weight (gm)

Statistical Analysis: The data collected were subjected to analysis of variance (ANOVA) using the line × tester method as described by Griffing (1956) [8]. The general combining ability (GCA) and specific combining ability (SCA) effects were estimated for all traits. Significant differences were determined at the 5% and 1% probability levels.

Results

Table 1 explains the ANOVA for different wheat traits under heat stress conditions and highlights significant genetic variability among the genotypes for all traits studied, including plant height, number of tillers per plant, days to 50% flowering, spikelets per spike, days to maturity, and 1000 grain weight. The significant genotype effects suggest substantial genetic diversity among the wheat genotypes. The significant variance due to parents, lines, and testers for multiple traits indicates that both parental lines and testers contribute to the observed variation. Moreover, the interaction effects (Line × Tester) are significant for several traits, demonstrating that specific combinations of lines and testers significantly impact these traits, underscoring the importance of non-additive genetic effects. The significant "Parents vs. Crosses" effects suggest the presence of heterosis indicating that the crosses differ significantly from their parent lines.

Table 1: ANOVA for different traits under heat stress conditions in wheat

Source of variation	df	Mean Sum of Square					
		Plant height (cm)	No. Tillers per plant	Days to 50 percent Flowering	Spikelets per spike	Days to maturity	1000 grain weight (g)
Replication	2	2.24	0.39	5.31	2.39	2.16**	1.33
Genotype	13	198.83**	38.59**	48.30**	16.35**	89.69**	52.67**
Parents	5	158.21**	3.00	18.96	18.47**	9.32**	49.44**
Line	2	62.34*	2.21	19.42	6.46*	3.15	60.43**
Testers	2	303.17**	1.41	15.57	62.51**	11.44*	10.41
Line vs Tester	1	388.21**	18.49	17.40	6.03	24.52**	89.43**
Parents vs Crosses	1	2100.62**	443.53**	376.53**	148.52**	174.29**	202.41**
Crosses	8	103.66**	25.35**	36.43**	6.62*	129.52**	46.38**
Line Effect	2	17.45	22.43	25.32	1.32	202.37**	8.95
Tester Effect	2	515.38*	103.52*	0.84	4.68	578.43**	32.57
Line * Tester	12	79.35**	18.48**	47.37**	8.72**	6.63*	72.21**
Error	60	21.63	5.49	9.63	2.38	2.26	3.17
Total	92	77.28	14.73	21.83	6.96	32.73	18.42

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

The general combining ability (GCA) estimates for six wheat parents (three lines: BRW3723, WH1142, RAJ4402, and three testers: HPW381, CPAN3061, Sup132/BaJ) for various traits are presented in the Table 2.

Plant Height (cm): BRW3723 shows a positive GCA (0.905), indicating a favorable additive effect for taller plants and similarly for WH1142 and HPW381 exhibit positive GCA values (0.429 and 2.206, respectively), suggesting taller plant height, which might be beneficial for certain breeding objectives. RAJ4402 has a positive GCA (1.016).

Number of Tillers per Plant: RAJ4402 has a significant positive GCA (3.429**), indicating its strong potential to increase tiller number, a desirable trait for higher yield. WH1142 shows a significant positive GCA (1.905*), suggesting an increase in tiller number. BRW3723 and CPAN3061 exhibit slightly negative GCA values (-0.571 and -0.349, respectively), indicating lesser contributions to tiller number.

Days to 50% Flowering: Sup132/BaJ shows a positive GCA (1.984), indicating a delay in flowering time. WH1142 has a significant negative GCA (-2.683*), indicating earlier flowering, which can be advantageous for heat escape. BRW3723 has a positive GCA (1.429), suggesting a tendency towards later flowering.

Spikelets per Spike: BRW3723 and CPAN3061 both exhibit positive GCA values (0.349), indicating their potential to increase the number of spikelets per spike. Sup132/BaJ shows a negative GCA (-0.429), suggesting a reduction in spikelet number. The GCA values for the other parents are relatively low, indicating a minor contribution to this trait.

Days to Maturity: Sup132/BaJ has a significant positive GCA (2.603**), indicating a longer maturity period, which might help in avoiding terminal heat stress. BRW3723 shows a significant negative GCA (-5.841**), indicating earlier maturity, which can be beneficial for avoiding heat stress during grain filling. CPAN3061 also has a significant negative GCA (-2.619**), suggesting a shorter maturity period.

1000 grain Weight (gm): RAJ4402 exhibits a positive GCA (0.717), indicating its potential to increase grain weight. HPW381 shows a significant negative GCA (-2.205**), suggesting a reduction in grain weight. WH1142 has a positive GCA (0.362), contributing moderately to grain weight. Studies by Ali *et al*. (2008) [2] and Ajmal *et al*. (2009) [1] have shown significant genetic variability and high heritability for traits such as plant height, number of tillers, and grain yield in wheat. The significant GCA values observed in this study for these traits are consistent with their findings, indicating that these traits are controlled by additive genetic effects and can be improved through selection.

Kumar *et al*. (2013) [10] highlighted the importance of selecting parents with high GCA for traits such as days to maturity and grain yield. The positive GCA values for Sup132/BaJ for days to maturity and RAJ4402 for grain yield align with these findings, suggesting their suitability for breeding programs aimed at improving heat stress tolerance.

Table 2: General Combining Ability of lines and testers for different traits under heat stress

Parents	Plant height (cm)	No. of tillers per plant	Days to 50 percent flowering	Spikelets per spike	Days to maturity	1000 grain weight (g)
BRW3723	0.905	-0.571	1.429	0.349	-5.841 **	-0.083
WH1142	0.429	1.905 *	-2.683 *	-0.317	1.270 *	0.362
RAJ4402	1.016	3.429 **	-1.571	-0.095	-3.063 **	0.717
HPW381	2.206	0.429	-1.127	0.127	-1.175 *	-2.205 **
CPAN3061	-0.873	-0.349	1.54	0.349	-2.619 **	0.129
Sup132/BaJ	2.127	-0.683	1.984	-0.429	2.603 **	0.029
CD 95% GCA (Line)	3.122	1.586	2.175	1.146	1.145	1.311
CD 95% GCA (Tester)	2.044	1.038	1.424	0.75	0.749	0.859

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

The SCA analysis provides a comprehensive understanding of the non-additive genetic effects for various traits under heat stress conditions. The significant SCA values were observed for traits such as plant height, number of tillers, days to 50% flowering, spikelets per spike, days to maturity, and 1000 grain weight (Table 3).

Plant Height (cm): The highest positive SCA effect was observed in the cross BRW3723 x Sup132/BaJ (0.79**), indicating a significant interaction for increased plant height. Conversely, BRW3723 x CPAN3061 showed a negative SCA effect (-0.58), suggesting shorter plants. Ali *et al*. (2008) [2] found similar variability in plant height.

Number of Tillers per Plant: The cross BRW3723 x HPW381 exhibited a significant positive SCA (1.25**), indicating an increase in the number of tillers. On the other hand, BRW3723 x Sup132/BaJ had a significant negative SCA (-1.05*). Ajmal *et al*. (2009) [1] reported that tiller number is a vital trait for yield enhancement, with positive SCA effects being essential for selecting superior genotypes.

Days to 50% Flowering: RAJ4402 x HPW381 showed a significant positive SCA (1.06**), indicating delayed flowering. In contrast, BRW3723 x Sup132/BaJ had a significant negative SCA (-1.18**) suggesting earlier flowering.

Spikelets per spike: RAJ4402 x Sup132/BaJ had the highest positive SCA (0.89**), indicating an increase in the number of spikelets. Negative SCA was noted in WH1142 x Sup132/BaJ (-0.67*). Gao *et al*. (2016) [7] emphasized the role of spikelet number in determining yield potential, with positive SCA effects being crucial for high-yielding hybrids. Ajmal *et al*. (2009) [1] found that spikelet number is an important trait for yield improvement, aligning with the significant positive SCA effects observed in this study.

Days to Maturity: WH1142 x HPW381 showed a significant positive SCA (1.52**), suggesting a longer maturity period. Conversely, BRW3723 x HPW381 had a significant negative SCA (-2.15**), indicating earlier maturity.

1000 grain Weight (gm): WH1142 x CPAN3061 exhibited a significant positive SCA (0.45**), indicating an increase in grain weight. Negative SCA was observed in BRW3723 x Sup132/BaJ (-0.39*). Ali *et al*. (2008) [2] and Ajmal *et al*. (2009) [1] found that grain weight is a critical component of yield, with positive SCA effects being essential for developing high-yielding hybrids. Gao *et al*. (2016) [7] also highlighted the significance of grain weight in determining overall yield, supporting the need for positive SCA effects in breeding programs.

Table 3: Specific Combining Ability for different traits under heat stress in wheat

Cross	Plant Height (cm)	No. of Tillers per Plant	Days to 50% Flowering	Spikelets per Spike	Days to Maturity	1000 Grain Weight (g)
BRW3723 x HPW381	0.34	1.25**	-0.92	0.67*	-2.15**	0.24
BRW3723 x CPAN3061	-0.58	0.95*	1.12**	-0.32	1.87**	-0.47
BRW3723 x Sup132/BaJ	0.79**	-1.05*	-1.18**	0.21	0.72*	-0.39*
WH1142 x HPW381	-0.42	-0.89*	0.48	-0.76**	1.52**	-0.31
WH1142 x CPAN3061	0.56*	1.17**	-0.74*	0.53*	-1.26**	0.45**
WH1142 x Sup132/BaJ	-0.14	-0.28	0.62*	-0.67*	-0.52*	0.38*
RAJ4402 x HPW381	0.45	-0.47	1.06**	0.78**	-1.18*	-0.21
RAJ4402 x CPAN3061	-0.37	-0.93*	0.85**	-0.56*	0.62*	-0.24*
RAJ4402 x Sup132/BaJ	-0.08	1.24**	-0.74*	0.89**	1.03**	-0.28*

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

Discussion

The ANOVA revealed significant genetic variability among the wheat genotypes for all traits studied under heat stress conditions. The significant genotype effects for plant height, number of tillers per plant, days to 50% flowering, spikelets per spike, days to maturity, and 1000 grain weight suggest substantial genetic diversity, which is crucial for breeding heat-tolerant wheat varieties. The GCA effects indicated that the lines and testers contributed differently to the traits studied. For plant height, BRW3723 and RAJ4402 showed positive GCA effects, suggesting their potential to contribute to taller plants while for the number of tillers per plant, RAJ4402 exhibited a significant positive GCA effect.

The SCA effects were significant for several traits, indicating the importance of non-additive genetic variance. The cross BRW3723 x Sup132/BaJ showed the highest positive SCA effect for plant height, suggesting a significant interaction for increased plant height. For the number of tillers per plant, the cross BRW3723 x HPW381 exhibited a significant positive SCA effect, indicating an increase in tiller number. The significant positive SCA effect for RAJ4402 x HPW381 for days to 50% flowering indicates delayed flowering, which can help in avoiding terminal heat stress (Gao *et al*. 2016) [7].

The significant GCA and SCA effects observed in this study highlight the importance of selecting appropriate parent combinations for breeding programs aimed at improving heat stress tolerance in wheat. The positive GCA effects for traits such as days to maturity and 1000 grain weight suggest that these traits are controlled by additive genetic effects and can be improved through selection. The significant SCA effects indicate that specific cross combinations can produce superior hybrids with enhanced performance under heat stress conditions. The cross BRW3723 x Sup132/BaJ, with significant positive SCA effects for plant height and delayed flowering, can be a valuable genetic resource for developing heat-tolerant wheat varieties.

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