

Original Research

# Genetic Analysis of Spring Wheat Germplasm against Water Deficit Conditions

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## Abstract

Spring wheat, also known as bread wheat, is greatly affected by drought, which is one of the most significant abiotic variables, and the lack of useful selection criteria restricts the breeding of resistant genotypes. This investigation aimed to evaluate 8 lines and 3 testers, along with their 24 hybrid crosses, for yield-related indices. The combining ability and gene action were also estimated using line tester analysis. Significant differences were found in the line  $\times$  tester analysis, demonstrating the highly significant variations of studied attributes among lines (L), testers (T), L  $\times$  T, and parents (P) vs. crosses (C) under both stressed and non-stressed conditions, except the tester revealed a non-significant difference for only plant height in the non-stressed condition. Out of 8 lines and 3 testers, L3 (Pasban-90) and T2 (Lasani-2008) proved to be good general combiners in non-stressed conditions, while in stressed water deficit conditions, L1 (Aas-11), L3 (Pasban-90), L4 (Chakwal-97), L6 (FSD-08), T2 (Lasani-2008), and T3 (Gomal-2008) exhibited good general combining ability. The hybrid crosses between L7  $\times$  T3 (Shahkar-13  $\times$  Gomal-2008) and L3  $\times$  T2 (Pasban-90  $\times$  Lasani-2008) demonstrated good specific combining ability (SCA) under both studied environments. The predominant non-additive type of gene action observed for all of the studied indices suggests the development of potential hybrids for drought tolerance.

**Keywords:** combining ability, drought tolerance, gene action, *Triticum aestivum* L.

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## Introduction

Due to its excellent nutritional value, wheat is a fundamental cereal being consumed by more than seven billion people worldwide. The majority of its uses are in manufacturing bread, biscuits, feed, and sweets. At the moment, wheat is the crop cultivated on the biggest scale in the world; it is grown on about 219 million hectares [1]. A little over 80 million farmers make their living on that crop. The global market for it exceeds the total market value of all other crops combined.

Wheat, which was formerly believed to be the staple diet of Western civilizations, is now consumed by people worldwide. Wheat is one of the main sources of carbohydrates and the source of about 13% of protein on a global level. Wheat contributes 20% of our daily protein and dietary calories, making it an essential food for human nutrition. It is considered a good source of dietary fiber and other micronutrients when consumed as a complete meal. Additionally, it has trace levels of vitamins, minerals, and fats. Following rice as the most important food crop in emerging nations worldwide, wheat ranks second [1]. In areas with limited water availability for cultivation, such as semi-arid and dry regions, abiotic stressors are the primary factor limiting productivity [2]. Due to climate change, drought stress is a major abiotic stressor that farmers experience globally [3]. An estimated 65% of the global population will face a total water deficit by 2025 and be forced to live in conditions that are stressful for water [4]. Inadequate irrigation is the main cause of the 14% difference in wheat output in emerging nations relative to advanced nations. Droughts often cause a 50 to 60% reduction in wheat production. In addition to reducing productivity, drought also accelerates soil erosion and environmental degradation [5].

Combining ability, known as productivity in crosses, refers to the capacity of varieties or parents to combine with one another during the hybridization process so that desirable alleles or traits are passed on to their progenies. Combining ability is a measure of estimating the significance of genotypes based on how well their offspring perform in a particular mating program [6]. According to [7], the effects of combining ability show the variations between genotypes as well as the sort of gene activity involved. The two basic objectives of most crop breeding management are finding the best-performing lines (for commercial distribution) and testers that may be used as parents in subsequent crossings [8].

By assessing combining ability, one may categorize parents according to their hybrid performance and gain a greater awareness of the nature of quantitative inherited attributes [9]. When selecting parents for hybrid cultivars, especially in cross-pollinated crops, plant breeders progressively consider knowledge of the general and specific combining abilities that impact yield, and their parts are more crucial. Line tester

analysis for specific traits has been used in many research works to investigate bread-wheat hybrid populations' combining capacities and gene activity [10]. A deeper comprehension of the genetic foundation of yield and the contributing indices, combining abilities (both general and specific), and the function of genes in the breeding material are critical components of an efficient breeding program. To choose the best parental materials for desired traits in the wheat improvement program, combining ability analysis is an effective method for differentiating between good and poor combiners [11].

The main objectives of this study were to determine which parents are most likely to generate these qualities in breeding programs, investigate the genetic behavior of associated traits and yield in wheat genotypes under drought stress, and conduct additional research on cross combinations for developing stress-tolerant cultivars. The information gathered from this research may be effectively applied to developing and implementing breeding plans aimed at producing new cultivars that embody these attributes.

## Materials and Methods

The research was carried out at the Department of Plant Breeding and Genetics (PBG) research facility at the Islamia University of Bahawalpur, Punjab/Pakistan (29.24°, 71.41°E) in November 2023. In this experiment, F1 seeds of 24 hybrids along with their parents (8 lines and 3 testers) were cultivated to test the physio-morphological attributes using randomized complete block design (RCBD) with three replications under water deficit and irrigated conditions. In this experiment, a line  $\times$  tester mating design was undertaken with 3 testers serving as males and 8 lines as females. Eight genotypes, L1 (Aas-11), L2 (Chakwal-86), L3 (Pasban-90), L4 (Chakwal-97), L5 (Kohistan-97), L6 (FSD-08), L7 (Shahkar-13), and L8 (AUR-09), were selected as drought-tolerant and also used as lines, and three accessions were used as testers, namely T1 (Mairaj-08), T2 (Lasani-2008), and T3 (Gomal-2008). At first, 10 seeds of each genotype were planted in rows spaced 6 inches apart among seedlings and with a 12 inch space between rows. Following germination, three plants from each genotype were selected. The normal trial included the application of prescribed irrigation at the stages: (1) tillering (35 days after sowing, or DAS); (2) booting (85 DAS); and (3) milking (112 DAS). In the stressed experiment, drought stress was applied at the tillering stage by skipping the irrigation. Throughout the growth season, all cultural practices were followed as recommended.

Data were gathered from eight guarded plants for each genotype under water stress and normal circumstances when the plants reached maturity. The following traits were studied in this experiment: plant height (cm), flag leaf area (cm<sup>2</sup>), spikelets per spike,

grains per spike, 1000-grain weight (g), tillers per plant, grain yield per plant, biological yield/plant, harvest index, relative water content, cell membrane thermostability, and total chlorophyll content.

For the above-mentioned traits, the data were subjected to analysis of variance (ANOVA) as given by [12]. The traits exhibiting significant differences were further analyzed using the line  $\times$  tester technique as delineated by [13]. The mean performance graph was made using MS Excel. The heat cluster maps were made using R Studio.

## Results

The analysis of variance for the present experiment was carried out for twelve attributes to test the

significance of differences among treatments. The analysis mentioned that variation due to all sources of influence was highly significant for all the studied traits under normal and water stress conditions, as depicted in Tables 1 and 2 (a and b). While in normal conditions, testers showed non-significant differences in plant height, as shown in Table 3.

### Mean Variability of Parents and Their Crosses (L $\times$ T) under Normal and Drought Conditions

For plant height, the best-performing line was L3 (101.50), and the best tester was T2 (92.50) under normal irrigated conditions, as presented in Table 3a), while under water scarcity conditions, the best performance was shown by line L3 (77.50) and tester T2 (63.50), as demonstrated in Table 4a). The high variability

Table 1a). Analysis of variances through L  $\times$  T for studied traits under normal conditions.

Traits	PH	FLA	NSS	NGS	TGW	NTP
Replication	25.95**	23.74**	1.23**	14.01**	11.57**	20.50**
Genotypes	40.30**	67.71**	19.93**	37.55**	28.41**	32.79**
Parents	33.47**	46.46**	32.91**	46.35**	13.74**	12.19**
Crosses	21.30**	11.61**	8.15**	17.73**	18.50**	6.13**
P. Vs. C	545.33**	1570.47**	161.13**	405.29**	403.09**	852.20**
Lines	104.00**	34.21**	30.75**	75.93**	116.75**	28.44**
Testers	5.37ns	36.83**	7.13**	22.62**	0.88**	9.13**
L x T	67.81**	31.26**	27.94**	52.69**	47.56**	16.44**
Error	3.11	1.62	0.21	0.12	0.09	0.25
Total	15.70	23.65	6.68	12.62	9.57	11.23

(PH) Plant height (cm), (FLA) Flag leaf area (cm<sup>2</sup>), (NSS) Spikelet per spike, (NGS) Grains per spike, (TGW) 1000-grain weight (g), (NTP) Tillers per plant.

Table 1b). Analysis of variances through L  $\times$  T for studied traits under normal conditions.

Traits	GYP	BYP	HI	RWC	CMT	TCC
Replication	42.84**	34.19**	31.82**	10.67**	8.99**	82.77**
Genotypes	27.26**	75.28**	18.88**	112.04**	59.95**	52.85**
Parents	5.51**	67.23**	36.34**	126.05**	31.57**	67.60**
Crosses	9.26**	7.69**	10.79**	7.04**	9.11**	10.61**
P. vs C	658.96**	1710.39**	30.08**	2386.99**	1513.01**	876.91**
Lines	34.44**	37.44**	15.39**	12.94**	28.75**	8.99**
Testers	4.63**	4.63**	19.32**	12.49**	5.38**	40.62**
L x T	33.69**	23.19**	44.72**	27.75**	35.31**	36.17**
Error	0.04	0.04	0.01	0.02	0.01	0.03
Total	9.76	25.29	6.79	36.85	19.78	18.89

(GYP) Grain yield per plant, (BYP) Biological yield/plant, (HI) Harvest index, (RWC) Relative water content, (CMT) Cell membrane thermostability, (TCC) Total chlorophyll content.

observed among hybrids  $L3 \times T2$  (108.50, 83.50) in both environments is depicted in Tables S1a and S2a. In the flag leaf area, among parents, the line L3 (38.85, 28.05) and tester T2 (29.45, 16.05), as shown in Tables 3a) and 4a), performed best under non-stressed conditions, while the cross  $L3 \times T2$  (44.05, 35.05) performed best under stressed conditions, as mentioned in Tables S1a and S2a. The spikelets per spike depicted that line L3 (27.27), T2 (18.81), and cross  $L3 \times T2$  (27.64) had the best results under normal environmental conditions as displayed in Tables 3a) and S1a, while L3 (20.64), T2 (12.64), and cross  $L3 \times T2$  (24.64) had excellent performance under water deficit conditions (Tables 4a) and S2a). In the number of grains/spike, among parents, the line L3 (69.69, 50.65) and tester T2 (59.65, 42.65), as shown in Tables 3a) and 4a), were best under non-

stressed conditions, while cross  $L3 \times T2$  (71.25, 55.65) performed best under stressed conditions, as mentioned in Tables S1a and S2a. The thousand-grain weight showed that among resultant crosses, the highest mean performance  $L3 \times T2$  value was 58.04 and 44.04 under both conditions, as given in Tables S1a and S2a. Among parents, the highest mean values for line L3 were 53.04 and 36.04, and for tester T2, 48.04 and 32.04, as mentioned in Tables 3a) and 4a) under the normal and non-irrigated situations.

The number of tillers/plant depicted that line L3 (20.57), T2 (15.57), and cross  $L3 \times T2$  (25.57) had the best results under normal environmental conditions as displayed in Tables 3a) and S1a, while L3 (16.64), T2 (12.47), and cross  $L3 \times T2$  (20.47) had excellent performance under water deficit conditions as shown

Table 2a). Analysis of variances through  $L \times T$  for studied traits under drought conditions.

Traits	PH	FLA	NSS	NGS	TGW	NTP
Replication	8.05**	12.76**	0.94**	12.31**	8.65**	19.01**
Genotypes	136.54**	105.26**	47.71**	52.84**	48.05**	15.02**
Parents	100.79**	68.73**	27.37**	32.24**	16.06**	14.17**
Crosses	7.37**	7.57**	5.32**	4.71**	12.96**	7**
P. Vs. C	3464.93**	2717.35**	1226.31**	1365.97**	1175.17**	274.73**
Lines	23.75**	21.00**	18.67**	10.29**	79.00**	19.61**
Testers	10.50**	4.50**	2.48**	5.22**	2.38**	0.38**
$L \times T$	25.25**	30.75**	19.99**	19.33**	33.81**	13.61**
Error	0.04	0.02	0.03	0.01	0.03	0.02
Total	44.82	34.67	15.64	17.52	15.90	5.29

(PH) Plant height (cm), (FLA) Flag leaf area ( $\text{cm}^2$ ), (NSS) Spikelets per spike, (NGS) Grains per spike, (TGW) 1000-grain weight (g), (NTP) Tillers per plant.

Table 2b). Analysis of variances through  $L \times T$  for studied traits under drought conditions.

Traits	GYP	BYP	HI	RWC	CMT	TCC
Replication	42.67**	35.99**	62.62**	11.20**	9.99**	85.83**
Genotypes	13.69**	62.66**	35.21**	77.83**	34.82**	59.21**
Parents	13.96**	51.04**	60.95**	131.78**	44.52**	78.49**
Crosses	4.43**	3.26**	14.22**	3.62**	3.46**	3.79**
P. vs C	223.97**	1545.18**	260.54**	1245.14**	659.08**	1140.88**
Lines	16.94**	24.44**	33.43**	6.02**	10.28**	6.32**
Testers	1.63**	3.50**	18.52**	1.91**	5.41**	19.27**
$L \times T$	16.19**	4.75**	55.79**	16.84**	12.77**	9.04**
Error	0.07	0.05	0.02	0.08	0.02	0.14
Total	5.34	21.21	12.72	25.71	11.59	21.10

(GYP) Grain yield per plant, (BYP) Biological yield/plant, (HI) Harvest index, (RWC) Relative water content, (CMT) Cell membrane thermostability, (TCC) Total chlorophyll content.

in Tables 4a) and S2a. For grain yield/plant, the best-performing line was L3 (35.00), and the best tester was T2 (34.00) under normal irrigated conditions, as presented in Table 3b), while under water scarcity conditions, the best performance was shown by line L3 (29.81) and tester T2 (25.00), as demonstrated in Table 4b). A high variability was observed among hybrid L3  $\times$  T2 (42.00, 31.00) in both environments, as depicted in Tables S1b and S2b. In biological yield per plant, among parents, the line L3 (73.06, 48.49) and tester T2 (60.49, 38.49), as shown in Tables 3b) and 4b), performed best under non-stressed conditions, while cross L3  $\times$  T2 (76.49, 52.49) performed best under stressed conditions, as mentioned in Tables S1b and S2b.

Concerning the harvest index among resultant crosses, the value of the highest mean performance L3  $\times$  T2 was 55.02 and 57.17 under both conditions, as

given in Tables S1b and S2b. Among parents, the highest mean values for line L3 were 52.07 and 65.80, and for tester T2, 54.71 and 62.47, as mentioned in Tables 3b) and 4b) under the normal and non-irrigated situations. The higher mean value for relative water content was found by line L3 (77.96, 66.40), tester T2 (62.48, 51.48), and cross L3  $\times$  T2 (82.48, 67.48) in both environments, as depicted in Tables 3b, 4b, S1b, and S2b. For total chlorophyll content, the best-performing line was L3 (59.31), and the best tester was T2 (47.84) under normal irrigated conditions, as presented in Table 3b), while under water scarcity conditions, the best performance was shown by line L3 (49.05) and tester T2 (38.18), as demonstrated in Table 4b). High variability was observed among hybrids L3  $\times$  T2 (63.84, 53.66) in both environments, as depicted in Tables S1a and S2a.

Table 3a). Mean variability of parents (L and T) under normal conditions.

		PH	FLA	NSS	NGS	TGW	NTP
Lines	L1	97.50	28.85	23.81	63.65	49.98	16.57
	L2	95.17	36.51	21.81	62.65	51.04	17.57
	L3	101.50	38.85	27.27	69.69	53.04	20.57
	L4	97.17	31.85	18.87	62.65	49.04	17.57
	L5	96.83	29.85	21.81	61.65	48.04	18.27
	L6	95.83	28.85	19.81	63.65	47.04	15.57
	L7	99.50	36.85	25.81	64.65	51.04	18.57
	L8	97.17	30.85	20.81	60.10	47.58	15.57
Testers	T1	91.50	28.45	17.81	57.65	47.04	14.57
	T2	92.50	29.45	18.81	59.65	48.04	15.57
	T3	90.50	27.45	16.81	54.65	46.04	13.57

Table 3b). Mean variability of parents (L and T) under normal conditions.

		GYP	BYP	HI	RWC	CMT	TCC
Lines	L1	30.81	68.49	43.92	76.48	56.74	56.84
	L2	34.63	63.49	46.85	74.48	57.74	55.84
	L3	35.00	73.06	52.07	77.96	59.74	59.31
	L4	34.00	66.49	49.65	70.48	54.74	54.55
	L5	33.00	65.49	48.91	71.48	55.74	55.84
	L6	32.00	62.49	49.68	66.48	54.74	56.84
	L7	35.00	70.49	53.52	76.48	58.74	57.84
	L8	33.00	61.08	46.91	65.32	53.38	55.84
Testers	T1	33.00	59.49	54.53	61.48	51.74	46.84
	T2	34.00	60.49	54.71	62.48	52.74	47.84
	T3	32.00	58.49	52.16	60.48	48.74	45.84

Table 4a). Mean performance of parents (L and T) under drought conditions.

		PH	FLA	NSS	NGS	TGW	NTP
Lines	L1	74.50	25.05	19.64	49.65	34.04	15.66
	L2	71.50	26.05	17.64	48.65	33.04	14.64
	L3	77.50	28.05	20.64	50.65	36.04	16.64
	L4	68.50	23.05	15.64	45.65	33.04	14.57
	L5	67.50	22.05	15.64	45.65	33.04	15.57
	L6	65.50	21.05	13.64	43.09	27.93	11.46
	L7	76.17	16.00	13.64	44.65	35.04	15.57
	L8	64.46	20.05	12.96	44.15	34.04	13.57
Testers	T1	62.50	15.05	12.64	41.65	31.04	10.57
	T2	63.50	16.05	12.64	42.65	32.04	12.47
	T3	60.50	14.05	11.64	40.65	30.04	10.57

Table 4b). Mean performance of parents (L and T) under drought conditions.

		GYP	BYP	HI	RWC	CMT	TCC
Lines	L1	25.00	44.49	54.21	60.48	55.65	47.95
	L2	27.00	45.49	57.30	62.48	54.65	47.30
	L3	29.81	48.49	65.80	66.40	56.65	49.05
	L4	26.00	42.03	58.99	61.48	52.65	46.51
	L5	23.55	46.49	49.83	61.48	51.65	45.58
	L6	26.00	40.49	61.83	54.02	45.70	42.93
	L7	25.00	43.49	55.43	55.48	54.65	45.66
	L8	27.00	38.84	57.81	64.48	53.65	47.63
Testers	T1	23.00	37.49	62.19	50.48	47.74	35.84
	T2	25.00	38.49	62.47	51.48	48.06	38.18
	T3	22.00	35.49	56.39	45.48	47.08	34.84

### General Combining Ability (GCA) and Specific Combining Ability (SCA)

Plant height in line L3 (3.00) showed a significant GCA effect in the desired direction under normal conditions, while line L1 (0.42) under water stress conditions indicated positive and significant GCA effects. Among testers, T2 (0.54, 0.5) was observed to be a good general combiner under well-irrigated and water-stressed conditions, as shown in Tables 5a) and 6a), respectively. For plant height, the SCA effect was observed for L3  $\times$  T2 (4.13, 2.17) under normal and water stress conditions, as demonstrated in Table S3a and Table S4a, respectively. For the flag leaf area, line L3 (0.77) showed a significant GCA effect in the desired direction under normal conditions, while line L4 (0.5) under water stress conditions reflected positive and significant GCA effects. Among testers, T2 (1.42, 0.25) was observed to

be a good general combiner under both well-irrigated conditions and water-stressed conditions, as shown in Tables 5a) and 6a). Under normal conditions, the best cross for flag leaf area was L4  $\times$  T3 (2.49), while under water deficit conditions, the best-performing cross, L3  $\times$  T2 (2.42), was found, as mentioned in Table S3a and Table S4a.

The parameter number of spikelets per spike demonstrated that L6 (1.08) under both normal conditions was positive and significant and proved to be a good general combiner, whereas, under water-stressed conditions, line L3 (0.15) was found to give significantly superior general combiners. Among testers under normal and water-stressed conditions, T2 (0.38, 0.19) was found to be a promising general combiner, as shown in Tables 5a) and 6a). Based on SCA effects, the best cross for this character was L3  $\times$  T2 (2.63) under water-irrigated conditions, whereas under drought stress



Table 5a). General combining ability of lines and testers under normal conditions.

		PH	FLA	NSS	NGS	TGW	NTP
Lines	L1	0.67*	-0.83*	0.42*	-0.13*	2.58*	-1.29*
	L2	0.33ns	1.57ns	0.75ns	-1.79ns	-0.08ns	-0.63ns
	L3	3*	0.77*	0.75*	2.21*	2.58*	1.38*
	L4	-1*	0.43*	-1.58*	0.21*	-1.75*	1.04*
	L5	2ns	0.77ns	-0.92ns	1.54ns	-3.08ns	0.71ns
	L6	-2*	-0.23*	1.08*	0.54*	-0.08*	-0.63*
	L7	-1.33ns	-1.23ns	-0.92ns	-0.13ns	-0.08ns	-0.63ns
	L8	-1.67ns	-1.23ns	0.42ns	-2.46ns	-0.08ns	0.04ns
Testers	T1	-0.33ns	-0.6ns	0.25ns	0.29ns	-0.21ns	-0.29ns
	T2	0.54*	1.42*	0.38*	0.79*	0.04*	-0.42*
	T3	-0.21*	-0.83*	-0.63*	-1.08*	0.17*	0.71*

Table 5b). General combining ability of lines and testers under normal conditions.

		GYP	BYP	HI	RWC	CMT	TCC
Lines	L1	-1.38*	-1.04*	-1.13*	0.13*	0.25*	0.67*
	L2	-1.04ns	-1.38ns	-0.42ns	-0.54ns	-1.42ns	0ns
	L3	1.63*	1.29*	1.25*	0.79*	1.25*	0.67*
	L4	-0.71*	-1.04*	-0.22*	0.79*	-0.08*	-0.67*
	L5	0.96ns	1.29ns	0.37ns	-0.21ns	-0.75ns	-0.67ns
	L6	0.29*	0.63*	-0.03*	-0.87*	0.25*	0.33*
	L7	0.63ns	0.63ns	0.41ns	-0.54ns	-0.75ns	-0.33ns
	L8	-0.38ns	-0.38ns	-0.22ns	0.46ns	1.25ns	0ns
Testers	T1	0.42ns	-0.46ns	0.89ns	-0.83ns	-0.54ns	-1.46ns
	T2	-0.46*	0.42*	-0.91*	0.42*	0.21*	1.04*
	T3	0.04*	0.04*	0.02*	0.42*	0.33*	0.42*

conditions, the best cross was L2 × T3 (0.94), as shown in Tables S3a and S4a. As for the number of grains per spike observed, line L3 (2.21) under normal conditions was found to be positive and significant and proved to be desirable and good for the general combining ability under the well-irrigated conditions, and line L4 (0.27) was found to be a significantly superior general combiner under drought. Among testers under normal and water-stressed conditions, T2 (0.79, 0.1) was found promising for general combiners, as represented in Tables 5a) and 6a). Nine cross-combinations in normal and ten cross-combinations in water-stressed conditions showed positive and significant SCA effects for this property, as depicted in Tables S3a and S4a. In thousand-grain weight, testers T2 (0.04) and T3 (0.17) demonstrated significant positive GCA effects under well-irrigated conditions, whereas tester T2 (0.29) had significantly positive GCA effects under water deficit conditions.

However, among the lines, the L3 (2.58, 2.5) under both conditions showed the maximum desirable GCA effect and thus was found to be a good combiner for this trait, as shown in Tables 5a) and 6a). Nine crosses, under normal water conditions, had significantly positive SCA effects, while twelve crosses had significantly positive SCA effects under water stress conditions, as shown in Tables S3a and S4a. The parameter number of tillers per plant demonstrated positive and significant GCA effects for line L3 (1.38, 1.37) under water-irrigated and water scarcity conditions. Tester T3 (0.71) proved to be a good general combiner in order of merit under non-stress conditions, and tester T2 (0.14) under water stress conditions, as displayed in Tables 5a) and 6a). Three crosses under normal and nine crosses under water deficit conditions showed positive and significant SCA effects for this property, as shown in Tables S3a and S4a.

Concerning the grain yield per plant, the L3 (1.63, 1.46) among the lines under well-irrigated conditions and water stress conditions demonstrated to be a good combiner, following the testers T3 (0.04, 0.21), which showed desirable GCA effects under non-stress and stress conditions, as shown in Tables 5b) and 6b). Nine cross combinations in normal and seven crosses in water stress conditions showed positive and significant SCA effects for this parameter, as mentioned in Tables S3b and S4b. Biological yield per plant exhibited that the tester T2 (0.42) and T3 (0.04) performed well under irrigated conditions, whereas under water deficit conditions, the tester T2 (0.42) demonstrated significantly desirable GCA effects. Among the lines, L3 (1.29, 0.96) under both normal and drought conditions was observed as a good general combiner for biological yield per plant, as shown in Tables 5b) and 6b). Nine crosses in this experiment demonstrated the desirable and significant crosses under

water-sufficient conditions, seven crosses under the condition of stress, and seven crosses under drought conditions, as depicted in Tables S3b and S4b. For the harvest index, under both conditions, line L3 (1.25, 1.74) showed significant GCA effects, whereas the tester T3 (0.02, 0.49) was observed as a good general combiner, as displayed in Tables 5b) and 6b). Eleven cross combinations were found under normal conditions, and ten crosses were found under water stress conditions, which had significantly positive SCA effects, as shown in Tables S3b and S4b, respectively. Relative water content revealed that the L3 (0.79) showed significant GCA effects, and under stressed conditions, L4 (0.12) showed significant GCA effects, whereas the tester T2 (0.42) under normal and tester T2 (0.33) under drought conditions was observed as a good general combiner, as depicted in Tables 5b) and 6b). Some crosses indicated positive significant SCA following the 11 crosses

Table 6a). General combining ability of lines and testers under water stress conditions.

		PH	FLA	NSS	NGS	TGW	NTP
Lines	L1	0.42*	-1.17*	0.55*	0.02*	-0.17*	-0.91*
	L2	0.42ns	0.17ns	1.19ns	0.69ns	-2.5ns	-0.32ns
	L3	0.08*	0.83*	0.15*	0.76*	2.5*	1.37*
	L4	-0.58*	0.5*	-0.18*	0.27*	0.17*	0.1*
	L5	-0.92ns	-0.5ns	-1.18ns	-0.81ns	1.17ns	-0.4ns
	L6	0.42*	-0.83*	-0.85*	-0.64*	-1.83*	-0.67*
	L7	-1.25ns	1.17ns	-0.18ns	-0.31ns	0.5ns	0.97ns
	L8	1.42ns	-0.17ns	0.49ns	0.02ns	0.17ns	-0.13ns
Testers	T1	0.25ns	0.25ns	0.18ns	0.51ns	0.04ns	-0.09ns
	T2	0.5*	0.25*	0.19*	-0.1*	0.29*	0.14*
	T3	-0.75*	-0.5*	-0.37*	-0.41*	-0.33*	-0.06*

Table 6b). General combining ability of lines and testers under water stress conditions.

		GYP	BYP	HI	RWC	CMT	TCC
Lines	L1	-0.54*	-0.04*	-0.97*	0.12*	-0.32*	-0.46*
	L2	0.12ns	0.96ns	-0.8ns	-1.02ns	0.02ns	0.35ns
	L3	1.46*	0.96*	1.74*	-0.05*	0.82*	0.31*
	L4	-0.88*	-1.71*	0.15*	0.12*	0.96*	0.73*
	L5	-0.54ns	-0.38ns	-0.65ns	0.38ns	-0.36ns	0ns
	L6	0.46*	-0.04*	0.93*	-0.05*	-0.33*	-0.5*
	L7	0.12ns	-0.38ns	0.63ns	0.25ns	-0.25ns	0.03ns
	L8	-0.21ns	0.63ns	-1.05ns	0.25ns	-0.55ns	-0.44ns
Testers	T1	0.08ns	-0.33ns	0.52ns	-0.18ns	-0.49ns	-0.6ns
	T2	-0.29*	0.42*	-1.01*	0.33*	0.46*	1.03*
	T3	0.21*	-0.08*	0.49*	-0.15*	0.02*	-0.43*



in normal and 10 under the drought condition, as shown in Tables S3b and S4b.

Cell membrane thermostability showed that the line L3 (1.25) demonstrated significant GCA effects under well-irrigated conditions, while under stressed conditions, the line L4 (0.96) performed as a good combiner, whereas the testers T3 (0.33) in non-stress conditions and T2 (0.46) under water stress conditions were observed as good general combiners, as depicted in Tables 5b) and 6b). The experiment indicated that nine crosses gave significantly positive SCA following seven crosses under water stress conditions, as shown in Tables S3b and S4b. Total chlorophyll content revealed line L3 (0.67) to be significant and positive under the water-irrigated conditions and proved to be a good general combiner, whereas, under water-stress conditions, line L4 (0.73) was found to be significantly superior to the general combiners. The tester T3 (0.42) was found to be promising for general combiners under water-irrigated conditions and T2 (1.03) under water-stressed conditions, as represented in Tables 5b) and 6b). The SCA effects were found to be significant in nine crosses under well-irrigated conditions and five crosses under drought stress conditions, as shown in Tables S3b and S4b.

#### Gene Action and Proportional Contribution of Parents and their Crosses under Normal and Drought Conditions

Plant height exhibited a non-additive type of gene action. The contribution of parents and their crosses is also shown in Table 7. The table shows that, at plant height, the crosses contribute more than lines and testers in both normal and water deficit conditions. The results of the flag leaf area demonstrate that the crosses  $L \times T$  performed better than the parents in water deficit conditions, while lines contribute equally in both conditions and testers contribute more in a normal environment. The flag leaf area presented a dominant type of gene action, as shown in Table 7. Spikelets per spike had a non-additive type of gene action. The contribution of lines and F1 hybrids was more in the trait spikelet per spike, while the tester's contribution was less. For grains per spike, the contribution of lines was more predominant in the normal conditions, while crosses showed their stronger contribution in water deficit conditions. Grains per spike also demonstrated dominant gene action (Table 7). The parameter thousand-grain weight had more lines of contribution in the non-stressed environment, and crosses displayed equal contribution. In contrast, the testers had no contribution in the normal condition.

The attribute of tillers per plant also exhibited the same result as 1000-grain weight. In this trait, the lines also had a predominant contribution in both situations, and crosses contributed more under water deficit conditions, as mentioned in Table 7. Grain yield per plant showed dominant gene action under

both normal and stressed conditions, while it had negative additive gene action under both environments. The line and interaction of line  $\times$  tester contributed equally under both normal and water deficit conditions. The biological yield per plant attribute also exhibited a dominant type of gene action, as displayed in Table 7. The harvest index had a non-additive type of gene action. In this index, the parents' contribution presented the same results as biological yield per plant, as it had a dominant line contribution in water deficit conditions, while line  $\times$  tester had more contribution under normal conditions. The trait relative water content showed positive dominance gene action under both conditions. In this trait, the lines and testers had equal contributions under the normal environment, while  $L \times T$  contributed predominantly under water-stressed conditions (Table 7). The dominant type of gene action was shown by cell membrane thermostability. The line and line  $\times$  tester both contributed equally under the conditions, i.e., non-stressed and stressed environments. Total chlorophyll content also had a non-additive type of gene action. In total chlorophyll content, the contribution of lines and testers showed their performance under stressed conditions, while hybrid crosses had more contribution under non-stressed conditions, as shown in Table 7.

#### Discussion

Significant results indicated the variations desired by plant breeders for further genetic study, and non-significant results showed no variation, which is not desirable. Significant variation present among yield-related traits has also been reported by [5, 14]. Researchers [15] and [16] mentioned in their studies that variations in plant height among different genotypes can express differences in their ability to maintain development and growth under drought conditions in wheat. Under water-limited conditions, flag leaf area plays an important role in grain production in wheat because it has a strong association with the process of photosynthesis, buildup, synthesis, and partitioning of carbohydrates. Previous research revealed that many indices, including the number of tillers per plant and the 1000-grain weight, were the main factors influencing and managing grain output. Grain yield could be indirectly increased by enhancing these yield-attributing traits in wheat [17-19]. The trait number of tillers per plant was observed to have a negative impact due to water drought conditions in various rice, wheat, and barley crops, resulting in a decline in crop growth and the photosynthesis process [20]. Similar findings were witnessed in the current research. The  $L \times T$  interaction populations have demonstrated the highest share (compared to lines and testers) in the corresponding proportion of populations to total variation for the majority of the characteristics, such as grain yield and biological yield per plant [21]. When the biomass decreases, the assimilated portion to the spike enhances

Table 7. Estimation of components of variance and proportional contribution of parents and crosses under both conditions.

Plant traits	Gene action				Contribution of parents and their crosses					
	Additive		Dominance		Line		Tester		L×T	
	N	WD	N	WD	N	WD	N	WD	N	WD
Plant height	0.21	-0.07	21.57	8.40	42	28	2	12	55	60
Flag leaf area	0.05	-0.20	9.88	10.24	26	24	27	5	47	71
Spikelets per spike	-0.04	-0.07	9.24	6.65	33	31	8	4	60	65
Grains per spike	0.17	-0.05	17.52	6.44	37	19	11	10	52	71
1000-grain weight	0.65	0.42	15.82	11.26	55	53	0	2	45	45
Tillers per plant	0.12	0.03	5.40	4.53	40	42	13	1	47	58
Grain yield per plant	-0.09	-0.04	11.22	5.37	32	33	4	3	63	64
Biological yield/plant	0.10	0.23	7.72	6.26	42	65	5	9	52	25
Harvest index	-0.43	-0.39	14.90	18.59	12	20	16	11	72	68
Relative water content	-0.22	-0.18	9.24	5.59	16	14	15	5	69	81
Cell membrane thermostability	-0.18	-0.04	11.77	4.01	27	26	5	14	67	61
Total chlorophyll content	-0.30	0.00	12.05	2.97	7	14	33	44	59	41

the growth of the harvest index and resistance to lodging under drought conditions [22]. It has been demonstrated that wheat genotypes with higher RWC have increased resilience to drought stress. Furthermore, the relative water content and cell membrane thermostability were regarded as essential selection variables for wheat resistant to drought stress [23]. CMT is a useful metric for quickly assessing wheat breeding response to drought. Under drought conditions, this is the sole positive and adaptive reaction that benefits the plant [24]. Water stress conditions reduce the amount of water that leaves absorbed from their root system. As a result, it reduces the ability to store water and stomatal movement, limiting chlorophyll production, the uptake of CO<sub>2</sub> by leaves, and photosynthesis [25-27].

Negative GCA effects were highlighted, particularly for plant height, suggesting a focus on selecting short-stature plants for their responsiveness to fertilizers and lodging tolerance under normal conditions [28]. The outcomes were comparable to those of [21]. A longer spike has the potential to generate more grains and spikelets per spike, which improves the output of wheat grains overall. Scientists have also reported significant and positive effects of GCA on a variety of plant parameters, including spikelets per spike, grain weight per spike, and grain yield per plant [29]. The genotypes possessing positive and significant GCA and SCA for thousand-grain weight were selected in this experiment. Additionally, the results showed that grain weight had beneficial, significant, and specific combining ability impacts [30]. A parent demonstrated the high GCA for the number of tillers per plant contributed to the increased number of tillers produced per plant, while the specific combining ability with enhanced value showed

that the specific parental lines lead to better or worse performance based on the GCA for the number of tillers per plant. The research by [31] showed that most crosses demonstrated a good general combiner ability for the number of tillers per plant. Wheat breeders determined the significance of SCA impacts on biological yield in wheat. However, many other researchers have mentioned both GCA and SCA effects on biological yield [32]. Previously, scientists [33] reported the positive and significant GCA for the harvest index, while SCA is an accurate indicator of both conditions for RWC. This implies a duration of water scarcity; these features might be used as crucial indirect selection factors to increase grain output [6, 34, 35]. Past studies have shown that some populations have been demonstrated to be the best specific combiners for cell membrane thermostability in wheat and that lines and testers had strong positive GCA impacts [36-38]. Similar outcomes were reported by [39], who observed that under stressful conditions at the anthesis stage, there were positive GCA and SCA effects on the chlorophyll content in the parents and F1 hybrids of wheat genotypes.

According to [40], the hexaploid cultivars exhibited the dominating impact of traits such as flag leaf area. While the GCA variances for tillers per plant, number of grains per spike, and 1000-grain weight were lower than SCA, indicating the non-additive type of gene action, the GCA variances for flag leaf area, number of spikelets per spike, and grain yield per plant were higher than SCA. Positive and significant heterosis for grain-related traits and tillers per plant was reported by [41]. The higher non-additive genetic effects on the harvest index suggested that for the improvement of the harvest index, selection must be made late [42-44].

Under favorable and stressful conditions, non-additive gene action for RWC and TCC is further supported by larger dominance variance than additive. Multiple research investigations that reported non-additive gene activity for grain production in wheat under both normal and stressful situations also reported similar findings [45].

## Conclusions

The purpose of this investigation was to determine the combining ability of 8 lines and 3 testers, as well as 24 hybrid crosses (making a total of 35 genotypes). The combining ability and gene action were estimated using line tester analysis. Significant differences were found in the line  $\times$  tester analysis, demonstrating the highly significant variations of studied attributes among lines, testers,  $L \times T$ , and parents (P) vs. crosses under both water deficit and normal conditions, except for testers showing non-significant differences for only plant height in non-stressed conditions. Out of 8 lines and 3 testers, L3 (Pasban-90) and T2 (Lasani-2008) proved to be good general combiners under non-stressed conditions, while under stressed water deficit conditions, L1 (Aas-11), L3 (Pasban-90), L4 (Chakwal-97), L6 (FSD-08), T2 (Lasani-2008), and T3 (Gomal-2008) exhibited good general combining ability, suggesting that crosses good performance for drought tolerance. The hybrid crosses between  $L7 \times T3$  (Shahkar-13  $\times$  Gomal-2008) and  $L3 \times T2$  (Pasban-90  $\times$  Lasani-2008) demonstrated good specific combiners under both studied environments. The predominant non-additive type of gene action was observed for all the studied indices. The lines and crosses showed more contribution among parents. The information gathered from this research may be effectively applied to developing and implementing breeding plans aimed at producing new cultivars embodying these attributes.

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## Conflict of Interest

Authors declare no conflict of interest.

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## SUPPLEMENTARY MATERIALS

Link to supplementary materials: <https://www.pjoes.com/SuppFile/203101/1/>