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Assessment of Genetic Variability and Combining Ability for Agronomic and Physiological Traits in Maize (*Zea mays* L.) Using a Line × Tester Design

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Abstract

In maize (*Zea mays* L.) breeding, the aim is to improve genetically by making use of the variability between different lines and by being able to recognize parent lines that, when crossed, give high-yielding hybrids. Hence, the objective of this research was to evaluate five lines and five testers along with their 25 F1 hybrids for 12 morphological and physiological traits. The experiment was laid out in a randomized complete block design with three replications at the University of Agriculture, Faisalabad. It was found through analysis of variance that there were highly significant differences among the genotypes for various traits under study. Parents such as SG-1 were good sources of variability and also recorded higher means for traits like cob length, seeds per row, and 100-grain weight than other parental lines. The general combining ability (GCA) effect further portrayed that SG-1 contributed positively towards increased plant height, ear height, cob length, stem diameter, seeds per row, internodal length & cob diameter. On the other hand, results obtained from hybrids revealed that one cannot rely on the superior performance of a parent to predict how well the cross will do. While the SG-1 & WD-7 combination gave positive specific combining ability effects (SCA) for most of the traits, including the highest cob diameter, SG-5 & WD-8 were ranked among the top crosses with respect to 100 grain weight. Traits like plant height, ear height, and internodal length showed positive phenotypic correlations, whereas leaf temperature had an inverse relationship with chlorophyll content. The study recommends some parental and cross combinations that can be used further in breeding.

Keywords:
Maize; genetic variability; line × tester; general combining ability; specific combining ability; hybrid breeding; agronomic traits; physiological traits

Introduction:

Maize (Zea mays L.) is cultivated throughout the world for food, animal feed, and industrial use. Maize grain starch is extracted for further processing into oil, protein products, and other high-value products for the industrial sector. As a result of the various production systems available to the grower, and the widespread geographical area in which maize is grown across South Asia for both spring and autumn harvests, maize represents a significant proportion of the cereal-based production systems. Breeding programs continue to use a variety of germplasm resources and methods to help identify the best parental combinations (of hybrids) for maize.

The efficiency of selection is built on when there's an understanding of the genetic basis for desirable traits. Modern maize breeding practices have become increasingly reliant on genetic markers, advanced phenotyping, multi-environmental evaluations, and other methods to take advantage of this knowledge to reduce both the length of the breeding cycle and improve the quality of selection decisions (Chňapek et al., 2024; Adak et al., 2024). Additionally, there is increasing pressure to find high-performing parents and hybrids across differing growing conditions (Shrestha et al., 2026; Zhao et al., 2025) as a result of varying climatic conditions, limited water resources, plant heat stress, and fluctuating yield potential. Therefore, a major challenge is determining whether each parent has the ability to produce good hybrids over a wide range of environments or whether they will only produce good hybrids under specific conditions.

One should keep in mind that GCA and SCA can assist in finding out why some crosses between parents succeed.

The performance of the specific parent when crossed with several others is what the GCA stands for. Therefore, this GCA depends on the additive genes. If a parent possesses a high GCA, then it can be trusted to transmit the good characters to many of its offspring. On the other hand, SCA represents an additional advantage (or disadvantage) of a given combination of parents over and above what is expected on average from the parents involved. Hence, this SCA is greater than the parental average. The SCA is a result of certain genetic factors like dominance and gene interaction. At times, a non-exceptional parent may give rise to an exceptional hybrid when crossed with another parent. GCA and SCA have been shown to be important in analyzing yield and other related characteristics in maize (Habiba et al., 2022; Dharani et al., 2024; Shrestha et al., 2026).

The line × tester design is one effective approach for isolating the effect of one parent in a cross. This involves crossing every member of one group with every member of another group, so that after this, we can compare how the parents performed, estimate GCA, take note of those crosses that have high SCA, etc. It is a very easy method. Nowadays, researchers also apply this approach in their studies on maize under various conditions, including normal, drought, and high temperatures as well as nutrient deficiencies (Amzeri et al., 2025; Kamara et al., 2025; Manigben et al., 2024; Mebratu et al., 2024). The design also proves useful in situations where the parents have been selected for the purpose of producing hybrids because then one can relate the values of combining ability with other indicators of genetic variability such as molecular or phenotypic ones (Chňapek et al., 2024; Kamara et al., 2024).

The primary objective of breeding maize is grain yield, which is under polygenic control and greatly affected by the environment. Therefore, it follows that traits related to yield may give some information during the early evaluation, but again they must not substitute for actual yield data. Plant and ear structure can be defined by the following characteristics: plant height, ear height, stem diameter, internodal length, cob dimensions, number of rows, and seeds per row. Studying how these features relate to each other may also be of assistance to breeders in determining certain combinations that would promote a well-balanced plant architecture and reproductive efficiency. To illustrate this further, too much plant height with high ear placement, on the other hand, may lead to increased risk of lodging, but a well-developed stem would enhance the stability of the plant.

Conventional morphological assessment can be enhanced with physiological characteristics. The amount of chlorophyll indicates how much photosynthetic activity there is, whereas leaf temperature is useful in determining the canopy water status and transpiration, provided that it is properly measured. Both nature and nurture play a role in determining these traits, because they are influenced by genotype and environmental factors like radiation, air temperature, humidity, and soil moisture. As such, one should be very cautious while employing them in selection. It is known from recent studies that during certain critical growth periods, the physiology of maize responds significantly to high temperature and/or water stress (Fan et al., 2024; Liu et al., 2023; Mallhi et al., 2025).

A single test in a particular location cannot prove that a hybrid which looks promising is really of any use. The interaction between genotype and environment may result in different rankings of parents as well as hybrids over space and time. A few recent studies employing multi-environment testing protocols have called for consideration of combining ability, environmental response as well as performance for selecting maize hybrids (Hossain et al., 2026; Zhao et al., 2025). Therefore, this study aimed to assess some maize lines, certain testers, and their F1 combinations in relation to specific agronomic as well as physiological characteristics.

Five maize lines, five testers, and twenty-five F1 combinations were evaluated for agronomic and physiological traits in the current investigation. Through this, the researchers aimed to discover parental lines that exhibit positive general combining ability as well as identify those cross combinations that manifest good specific combining ability for future breeding program considerations.

Materials and Methods

Experimental Site and Genetic Material

This field experiment took place at the University of Agriculture Faisalabad, within the Research farm of the Department of Plant Breeding and Genetics. Ten maize parental genotypes obtained from the university germplasm collection were employed. These included five lines named SG-1 through SG-5 and five male testers named WD-6 through WD-10.

In every case, a line was combined with a tester, resulting in 25 F1 crosses. Therefore, there were 35 entries altogether, comprising 10 parents and 25 hybrids, which were tested and analyzed using a line x tester mating design to separate general parental effects from line x tester interactions while assessing the performance of parents. The study followed maize genetic evaluation practices that involve using diverse germplasm and complementary phenotypic information (Dossa et al., 2024; Guo et al., 2025).

Experimental Design and Crop Management

In this experiment, there was a use of a randomized complete block design (RCBD) which had replications of three. The design lessened the impact of any differences that may arise in the field while also giving many copies of data on how certain traits performed.

Plant spacing: Approximately 9 inches between plants within rows.

Row spacing: Approximately 30 inches between adjacent rows.

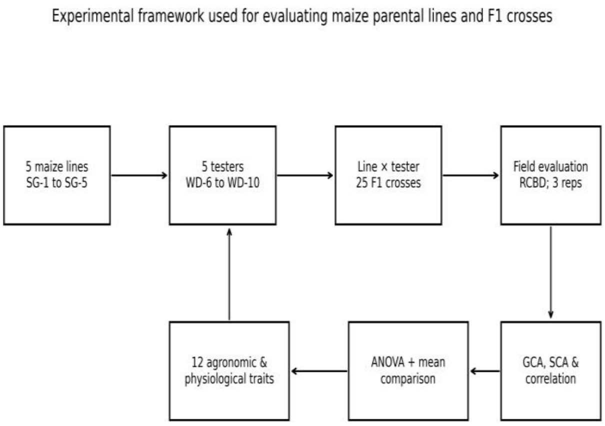
Crop management: Fertilization, irrigation, and pest management were applied uniformly according to standard regional recommendations.

All morphological, structural, and physiological measurements were taken at physiological maturity. As it was done in one place only, these results can be seen as a product of the particular environment and not necessarily ones that occur across different environments over time.

Table 1. Experimental structure

Component	Description
Lines	SG-1, SG-2, SG-3, SG-4, SG-5
Testers	WD-6, WD-7, WD-8, WD-9, WD-10 in the descriptive results and SCA table
F1 combinations	25 (5 lines × 5 testers)
Total entries	35 (10 parents + 25 F1s)
Design	Randomized complete block design
Replications	3
Plant spacing	Approximately 9 inches within rows; 30 inches between rows
Traits	12 agronomic and physiological traits

The experimental framework used in evaluating five



maize lines, five testers, and 25 F1 combinations is shown in Figure 1.

Traits recorded

Plant height (PH) was measured in centimeters from the soil surface to the top of the tassel at maturity; ear height (EH) was determined similarly but by measuring to the first ear from the soil surface. After harvesting and drying, cob length (CL) was measured. A Vernier caliper was used to record stem diameter (SD), which was then averaged for several selected plants. The number of seeds per row (SR) was counted manually. Seeds per unit weight were determined by weighing 100 seeds, using an electronic scale, which had been extracted from the husk. The number of rows on each ear was manually counted. The distance between two adjacent nodes was measured to find out internodal length (IL). Using Vernier calipers, pith diameter (PD) & ear diameter (CD) were measured, and then units were taken as per the data provided. Chlorophyll content (CC) was
computed as per the SPAD or Spectrophotometer method described in the literature, which gives content in percentage. Randomly selected green leaves were subjected to measurement with an IR thermometer for recording leaf temperature (LT).

In total, these twelve characteristics consist of those associated with plant architecture or structural features like PH, EH, SD, IL, PD; those related to ear & cob, namely CL, NR, CD; and certain grain characteristics, namely SR, HGW & CC (physiological trait). While analyzing data comprising such a diverse array of traits at the same time may seem to make things complex, simple bivariate correlation calculations can reveal trade-offs between pairs of these trait types that occur frequently enough so as not to be altogether random, thereby allowing one trait, e.g., increased biomass, to serve as a marker for another less desirable attribute(s). With all these considered, an increase in PH may be good within systems where biomass is a target but may not favorably or desirably impact individuals' objectives related to high-density planting or lodging, etc.

Statistical examination

The randomized complete block design framework was used for the analysis of variance. Combining ability was assessed through the approach of line × tester as explained by Kempthorne. Five lines and five testers had their GCA effects estimated, while 25 F1 combinations had their SCA effects estimated. To describe phenotypic associations among the measured traits, Pearson correlation coefficients were computed. It was interpreted that positive values of GCA and SCA are favorable only if they serve the purpose of breeding better. Take, for instance, a trait like leaf temperature; in this case, it could be sensible to have a low value since cool canopies are preferred.

Data integrity and nomenclature note

An important nomenclature inconsistency is present in the supplied dataset. Testers are identified as WD-6 to WD-10 in the descriptive results and SCA table, while in the tester GCA table they are referred to as WD-1 to WD-5. Nevertheless, the numerical GCA values as they were given have been kept intact and not attributed to any other tester. It is important to verify these tester labels with the original field book, seed inventory, or statistical output before submitting the journal. This is important since changing the identities of testers in the absence of original records may affect how the data is interpreted biologically.

Results

Overall genetic variation

Genetic variation in its magnitude was not the same for every trait, as revealed by analysis of variance. There were significant differences in plant height between treatments, parents, and crosses, and also a large line × tester component. However, the analysis revealed that stem diameter and number of rows per cob were less variable across some components, while ear height and cob length had great variations, denoting that there was something to differentiate in terms of plant architecture as well as ear development. Chlorophyll content and leaf temperature gave different results as indicated above, meaning that we cannot assume similar genetic control of morphological and physiological characteristics.

Table 2. Selected highest and lowest mean performances

Trait	Best reported entry	Mean	Lowest reported entry	Mean
Plant height (cm)	SG-1 × WD-6	241.33	SG-5 × WD-10	88.97
Ear height (cm)	SG-1 × WD-6	141.67	SG-4 × WD-8	45.67
Cob length (cm)	SG-1	17.00	SG-2 × WD-8	10.73
Stem diameter (cm)	WD-6	13.37	WD-8	3.13
Seeds per row	SG-1	40.33	SG-2 × WD-8	24.00
100-grain weight (g)	SG-5 × WD-8 / SG-1 × WD-9	38.33	SG-1 × WD-10	21.67
Rows per cob	SG-2 × WD-10 / SG-4 × WD-8	18.00	SG-3 × WD-6	13.33
Internodal length (cm)	SG-5 × WD-9	18.30	SG-4 × WD-10	9.40
Pith diameter	SG-1 × WD-7	25.53	SG-4 × WD-10	16.33
Cob diameter	SG-1 × WD-7	43.33	WD-10	23.23
Chlorophyll (%)	SG-3 / SG-4	49.00	SG-5 × WD-8	38.33
Leaf temperature (°C)	SG-1 × WD-9	30.67	SG-1 / SG-3	23.33

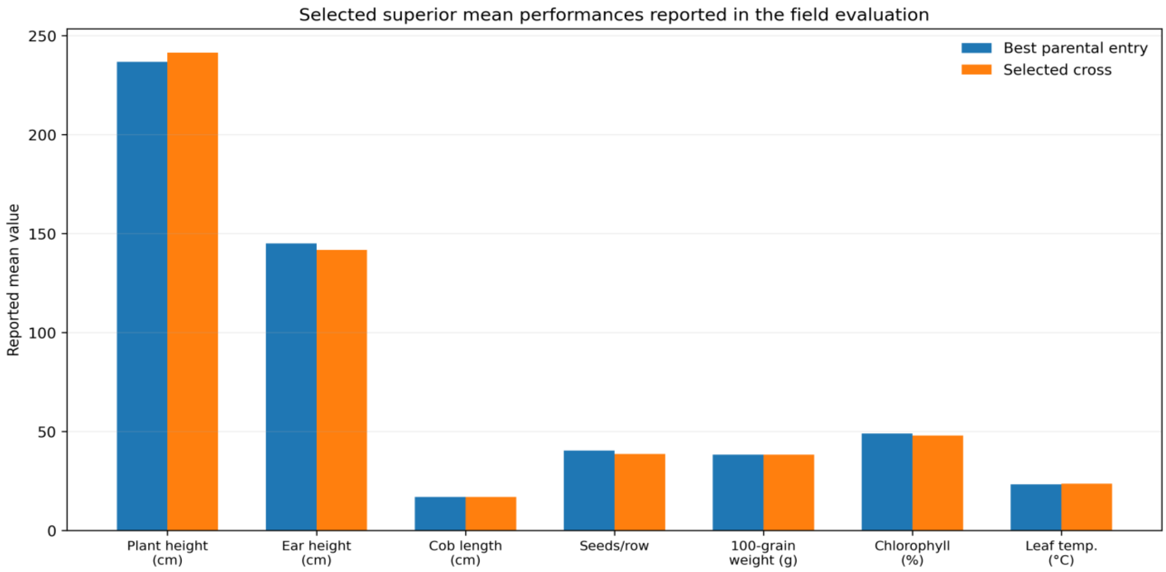


Figure 2. In the given experimental document, highlights certain parental entries along with some F1 combinations that exhibited high average performances. The values Crossbreeding is significant in hybrid breeding since it is not possible to foresee how well a cross will do based solely on the appearance of one parent. The data hence backs up the practice of combining ability analysis apart from mere per se evaluation. These studies on maize also reported variations of GCA and SCA, with some traits and populations being more influenced by additive while others by non-additive effects (Habiba et al., 2022; Dharani et al., 2024; Oladiran et al., 2026).

indicated here represent averages taken from the original text, while the characteristics remain expressed using their initial units of measurement.

Plant height

There were great variations in plant height between the parent lines and hybrids. The tallest parent line was SG-5 (236. 67 cm), while SG-4 was the shortest (185. 33 cm). Among the tester lines, WD-10 was the tallest (164. 67 cm), and WD-8 was the shortest (95. 00 cm). When combined as hybrids, some parent combinations exceeded both parents in height.

For example, SG-1 x WD-6 (241. 33) was taller than either parent. But not all combinations were like this; SG-5 x WD-10 (88. 97) was much shorter than either parent. The General Combining Ability (GCA) also showed that a parent line with high height does not necessarily translate to tall hybrid progeny, as was demonstrated by SG-5 with the highest height but negative GCA. Similarly, Specific Combining Ability (SCA) varied among crosses.

While hybrid plant height may seem straightforward to understand, there are many factors at work here; both GCA & SCA can play their parts, as well as interactions between genes from parental lines (if you like, think of them being like puzzle pieces which fit together differently depending on which partner they come from). Another thing though: tall plants don't always mean better yield; sometimes shorter ones resist lodging better – meaning they won't fall over as easily.

Ear height

There were also variations in ear height between parents and hybrids. Parental means showed that SG-5 recorded the highest value of 145. 00 cm, followed by SG-2 with 134. 00 cm, while SG-4 registered the least at 96. 83 cm. On the other hand, WD-8 had 61. 50 cm, and WD-10 had 94. 67 cm among the testers. In hybrid, SG-1 × WD-6 produced the top ear height of 141. 67 cm followed closely by SG-2 × WD-9 (140. 17 cm) and SG-3 × WD-8 (139. 17 cm). The lowest value of 45. 67 cm was observed in SG-4 × WD-8.

Ear height GCA effect was highest and positive in SG-2 (13. 51), while lowest and negative in SG-4 (−15. 09). The SCA effects were also different; for example, SG-2 × WD-7 had −50. 90, whereas SG-1 × WD-6 and SG-4 × WD-6 had 24. 13 and 26. 23 positive values, respectively. From these findings, it can be seen that both parents and crosses had an effect on ear placement. The suitability of ear position depends on the targeted production system; ears that are too high may cause lodging problems, and very low ears might not be convenient for certain types of harvesting machinery in some production systems.

Cob length

Different lengths of cobs were observed in the parents and crosses. The parental cob that was longest was from SG-1, measuring 17. 00 cm, followed by SG-3 at 16. 33 cm, and then SG-2 at 16. 20 cm, while the shortest parental value was that of SG-5 at 14. 43 cm. With regard to the testers, WD-8 recorded 8. 63 cm and WD-10 12. 57 cm. The longest hybrid cob was observed in SG-1 × WD-7, measuring 16. 90 cm, followed by 16. 43 cm in SG-3 × WD-8 and 16. 17 cm in SG-4 × WD-9. The lowest value of 10. 73 cm was seen in SG-2 × WD-8. Positive GCA effects were observed for cob length in SG-1 (1. 28) and WD-4 (1. 56), as shown in the tester GCA table. Additionally, the SCA effect of SG-1 × WD-7 was also positive (2. 10). It is evident from these data that SG-1 could have been selected to improve traits related to cob. Cobs have kernels that are borne on a surface which may be increased in size by cob length, but we should remember that grain yield is not only dependent on the length of grains. Other ear characters as well as ultimate grain yields have to be taken into account in making choices for hybrids.

Stem diameter

The pattern of variation in stem diameter was not exactly like that observed for plant height or cob length. In the analysis provided, the cross and line × tester components did not show any significant effect even though the parent and parent-versus-cross were significant. With a diameter of 8.27 cm, SG-2 had the thickest parental stem, followed by SG-1 (7. 99 cm) and then SG-5 (7. 27 cm). On the other hand, WD-6 was the thickest tester with 13. 37 cm, whereas WD-8 was the thinnest one, measuring 3. 13 cm. The hybrid mean was highest in SG-5 × WD-9 at 6. 87 cm, closely followed by SG-1 × WD-7 (6. 70 cm), while it was lowest in SG-4 × WD-8 at 2. 87 cm.

Number of seeds per row

Parental value for seeds per row was highest in SG-1 (40. 33), then SG-3 (39. 00), SG-2 (36. 00), with SG-4 having the lowest value of 31. 00. As for testers, WD-8 recorded 32. 67, while WD-6 had 24. 67 seeds per row. The maximum hybrid value of 38. 67 was seen in SG-4 × WD-9, while SG-1 × WD-7 came second with 38. 33 and SG-1 × WD-9 had 37.33. SG-2 × WD-8 registered the lowest figure at 24.00. There was a significant impact seen on this trait from treatment, parents, crosses, as well as line × tester interaction, which indicated that both parental effects and specific combinations played a role.

One-hundred-grain weight

The weight of parental lines varied between 38. 33 g in SG-1 and 26. 67 g in SG-4. Nevertheless, SG-2 and SG-3 had weights of 36. 67 and 35. 00 g, respectively. The highest tester value of 31. 67 g was recorded by WD-7, while the lowest value of 21. 67 g was recorded by WD-9. While both SG-5 × WD-8 and SG-1 × WD-9 had 38. 33 g, SG-2 × WD-6 had 36. 67 g and SG-1 × WD-10 had 21. 67 g. The GCA estimates were not consistent with per se performance: SG-4 recorded the highest positive GCA (1. 14), followed by SG-5 (0. 91) and SG-3 (0. 57), with SG-2 being negative (−1. 76). Although SG-1 had the highest parental mean, it also had a negative GCA value. This is a clear indication that one has to look at parental performance as well as combining ability in making a choice of breeding parents.

Number of rows per cob

The number of rows per cob for the parental values was as follows: 14. 67 in SG-4 and 18. 00 in SG-1, SG-2, and SG-5. On the other hand, WD-9 had 12. 67 rows while WD-6 and WD-8 had 16. 00 each. About the hybrids, 18. 00 rows were recorded in SG-2 × WD-10 and SG-4 × WD-8, while SG-3 × WD-6 had 13. 33. There were significant differences among parents, although the given analysis failed to indicate any significant differences among crosses, lines, testers, or line × tester interaction. Therefore, it can be said that the trait of row number was stable to some extent in all combinations of this material and may not easily vary with specific crossing effects like plant height or seeds per row.

Internodal length

There was a wide range in internodal length. The highest parental mean internodal length was observed in SG-3 (16. 63 cm), closely followed by SG-2 (15. 17 cm), with SG-5 having the least (13. 20 cm). On the other hand, WD-7 had 12. 87 cm, while WD-9 had 12. 57 cm. The hybrid of SG-5 and WD-9 had the longest internodes, measuring 18. 30 cm, while SG-1 crossed with WD-7 and SG-5 crossed with WD-7 recorded values of 16. 60 cm and 15. 80 cm, respectively. The shortest internodes, measuring 9. 40 cm, were recorded in SG-4 crossed with WD-10. All replications, treatments, parents, parent versus cross effects, as well as crosses and line x tester interactions were found to be significant. This means that the trait must be taken together with plant height and other factors that cause lodging and not picked independently.

Pith diameter

The largest parental pith diameter of 24. 93 cm was recorded in SG-1, and it was closely followed by SG-3 with 23. 50 cm, while SG-2 had the least value at 20. 50 cm. Among the testers, WD-8 had 19. 23 cm, and WD-7 had 15. 43 cm. It was seen that the highest hybrid value occurred in SG-1 × WD-7 at 25. 53 cm, but this was closely followed by SG-4 × WD-8 with 25. 47 cm and SG-2× WD-6 having 25. 17 cm. On the other hand, the lowest value was recorded by SG-4 × WD-10 at 16. 33 cm. This variation may be an indication that a pith-related stem structure could offer some information for selection if combined with stem diameter and plant height parameters.

Cob diameter

Cob diameter varied widely across all factors understudy namely treatments, parents, crosses, and line × tester combinations. The parental mean for cob diameter was highest in SG-1 (42. 27 cm), followed closely by SG-5 with 41. 73 cm, and SG-3 came third with a mean value of 39. 67 cm. As for the testers, WD-6 recorded the highest value (34. 37 cm), while WD-10 had 23. 23 cm. The top three values in this case were recorded in SG-1 × WD-7 (43. 33 cm), followed by SG-3 × WD-9 (42. 97 cm) and lastly SG-2 × WD-9 (41. 73 cm) for the performance of hybrids in cob diameter.

A positive GCA effect on cob diameter was recorded in SG-1 at 2. 80 as well as in SG-2 at 1. 84. In the table of tester GCA, WD-4 indicated an optimistic value of 3. 02. It had a favorable mean value for cob diameter that can be connected to its high SCA effect of 8.38, like a cross-specific genetic effect.

Chlorophyll content

The parental chlorophyll content was highest in SG-3 and SG-4 at 49. 00%, closely followed by SG-2 at 48. 67%, and lastly SG-1 at 45. 33%. It was 44. 67% for WD-9 and 44. 00% for WD-10. The maximum hybrid value of 48. 00% was obtained from SG-5 × WD-6, while SG-4 × WD-10 had 47. 67%, and the lowest value of 38. 33% was recorded by SG-5 × WD-8. Analysis of variance indicated that there were significant differences among treatments, parents, and crosses, whereas the line × tester interaction was not significant. The highest positive GCA effect was recorded in SG-5 (1. 85), followed by SG-4 (0. 65), whereas SG-3 had a negative value (−1. 62) even though its parental mean was high. Once more, it is evident that per se performance does not always rank parents in the same way as GCA.

Leaf temperature

There was variation in leaf temperature in the different replications, treatments, parents, and crosses. The lowest parental values were 23. 33 °C were recorded in SG-1 and SG-3 followed by SG-4 (24. 67 °C), SG-2 (24. 00 °C) and lastly SG-5 with 26. 67 °C. The hottest testers were WD-9 at 30. 33 °C, and then WD-6 as well as WD-10 both at 28. 33 °C. The highest hybrid value was recorded by SG-1 × WD-9 at 30. 67 °C closely followed by SG-1 × WD-10 at 30. 33 °C while the lowest value was observed in SG-4 × WD-10 at 23. 67 °C. It is possible that lower leaf temperatures mean there is a better cooling effect from the canopy when exposed to certain stresses; however, this characteristic depends on many factors such as radiation, surrounding temperature and moisture, stomatal activity, as well as moisture in the soil. As such, these readings are only initial signs and should not be taken as proof of heat resistance.

Combining ability analysis

General combining ability of lines

There were evident disparities among the five lines based on the GCA estimates (Table 3). Positive influence was observed in line SG-1 on several parameters including plant height (23. 47), ear height (7. 78), cob length (1. 28), stem diameter (0. 29), seeds per row (1. 91), internodal length (0. 62), and cob diameter (2. 80). On the other hand, it had an unfavorable impact on 100 grain weight (−0. 54), row number (−0. 82), pith diameter (−0. 58), and chlorophyll content (−0. 98). Therefore, SG-1 is of significance for the development of specific traits such as increased plant height, longer ears, more seeds per row and larger-sized cobs.

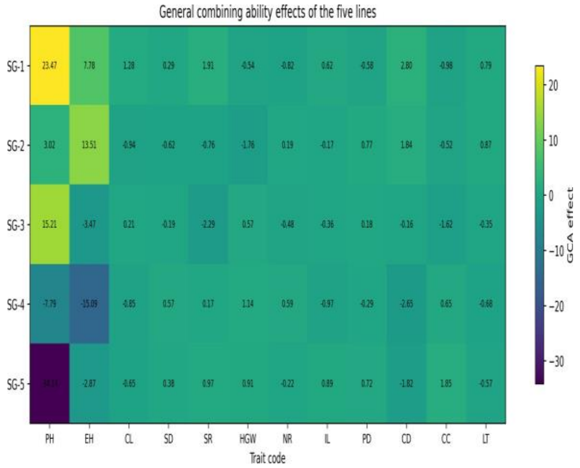


Figure 3. Overall combining ability impacts of the five lines across the 12 observed characteristics. PH, height of the plant; EH, height of the ear; CL, length of the cob; SD, diameter of the stem; SR, seeds in each row; HGW, weight of 100 grains; NR, rows on the cob; IL, length between nodes; PD, diameter of pith; CD, diameter of cob; CC, content of chlorophyll; LT, temperature of the leaf.

SG-2 had favorable GCA effects for ear height (13. 51), pith diameter (0. 77), cob diameter (1. 84), row number (0. 19), and leaf temperature (0. 87); nevertheless, it had an unfavorable effect on cob length, stem diameter, seeds per row, and 100-grain weight. This suggests that SG-2 should be utilized only for particular cross combinations and not across the board. SG-3 revealed positive GCA values for plant height (15. 21), cob length (0. 21), 100-grain weight (0. 57), as well as pith diameter (0. 18). It had unfavorable GCA values for ear height, seeds per row, row number, internodal length, cob diameter, chlorophyll content, and leaf temperature.

This shows that while SG-3 can increase height and grain weight in general, it will require careful cross selection if the number of kernels per cob or cob size itself are important parameters in that area. The analysis identified several key traits in other lines too! For instance, SG-4 positively influenced stem diameter (0. 57), seeds per row (0. 17), 100-grain weight (1. 14), row number (0. 59), other pith-related characteristics, & chlorophyll content (0. 65).

However, this line recorded decreased measures when it came to height (including both overall and at the ear), length of cobs, thickness of cobs, as well as their temperature profile via leaf test results. It means that the variety will come in handy whenever heavier grain but still relatively small, compact plant architecture were goals set on achieving.

On the other hand, SG-5 indicated positive GCA for stem thickness (0. 38), seed numbers per row (0. 97), a hundred grain weights (0. 91), as well as, internodal lengths (0. 89) and pith diameters (0. 72) but extremely negative effect on plant height with GCA value being equal minus thirty-four point one four (−34. 14) and also reduced diameter of the cobs across axis measured at one (−1. 82). Hence the observation that although parent itself tall with GCA value indicating opposite correlation with height (shortness) added something that at cross level (hybrids) may appear different than expected especially considering this one trait taken individually out context might portrayed mislead impression.

General combining ability of testers

According to the tester GCA table, WD-1 and WD-2 had positive influences on many characteristics, while WD-3 greatly reduced plant height and number of seeds in a row. On the whole, WD-4 was also seen to be advantageous. WD-4 had a favorable GCA on plant height (29. 57), ear height (5. 09), cob length (1. 56), stem diameter (0. 48), row number (0. 98), internodal length (1. 18), cob diameter (3. 02), chlorophyll (0. 32), and leaf temperature (0. 73). WD-5 showed a positive impact on stem diameter (0. 47) and chlorophyll (3. 12), but a negative effect on all the other parameters measured such as plant height, ear height, cob length, seeds per row, 100-grain weight, pith diameter, cob diameter, and Leaf Temperature. In the descriptive analysis and SCA results, WD-6 to WD-10 were used, while WD-1 to WD-5 were used in the GCA table. Therefore, confirmation is still needed from the original records for these individual tester assignments since they are provisional at this stage.

Specific combining ability

The SCA estimates were not the same for the 25 F1 combinations as shown in Table 3. The first and second cross combinations “SG-1 × WD-6” and “SG-1 × WD-7” demonstrated positive specific combining ability (SCA) effects on certain characteristics but varied with respect to others. The former exhibited positive SCA effects on plant height (37. 52) and ear height (24. 13), while the latter had positive effects on several traits including plant height, ear height, cob length, stem diameter, seeds per row, 100-grain weight, internodal length, pith diameter, and cob diameter. This cross, therefore, had one of its better-balanced SCA profiles with regard to cob and kernel-related traits, among the datase

Table 3. Selected SCA effects for representative F1 combinations

Cross	PH	EH	CL	SD	SR	HGW	NR	IL	PD	CD	CC	LT
SG-1×WD-6	37.52	24.13	0.40	0.84	1.03	-0.80	0.48	-0.55	1.52	1.21	-1.32	-3.19
SG-1×WD-7	17.90	16.98	2.10	1.62	3.76	1.53	-0.32	2.38	3.42	8.38	-1.19	-1.05
SG-2×WD-10	56.76	11.48	1.34	0.16	4.16	0.53	1.41	1.91	3.59	0.74	0.95	0.21
SG-3×WD-10	*41.01*	*24.70*	*1.62*	*0.82*	*2.03*	*4.87*	*0.08*	*2.77*	*0.84*	*-0.08*	*0.61*	*-0.32*
SG-4×WD-6	*50.99*	*26.23*	*0.96*	*0.98*	*0.43*	*-0.80*	*0.48*	*1.50*	*-0.34*	*0.81*	*-2.59*	*2.95*
SG-5×WD-8	*16.21*	*37.27*	*0.38*	*1.36*	*7.23*	*5.87*	*-0.45*	*0.95*	*2.98*	*6.78*	*-3.65*	*-0.12*

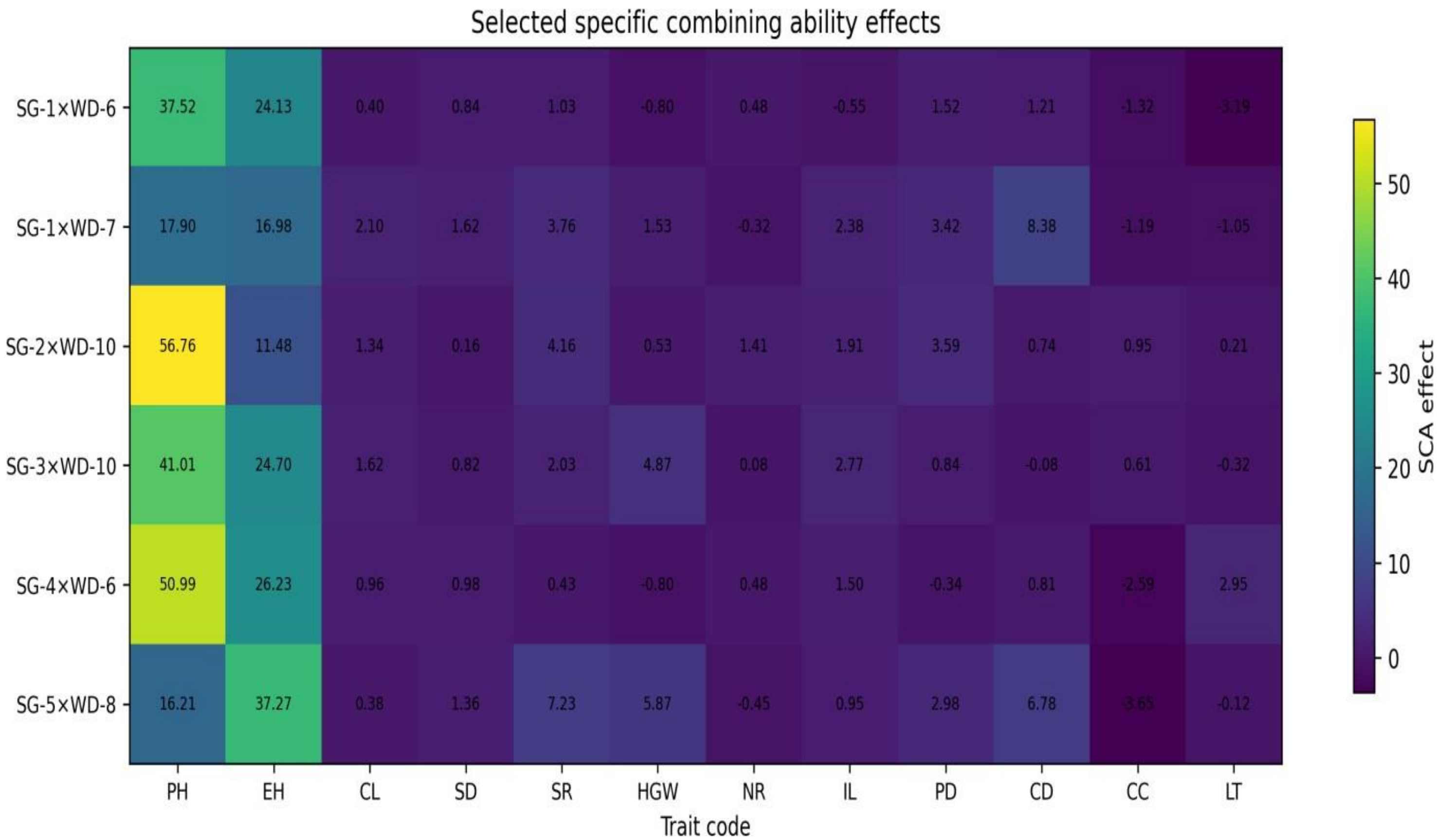


Figure 4. displays the selected SCA effects in some specific F1 combinations which were taken as representatives

The following had positive SCA effects: SG-2 × WD-10 on plant height (56. 76), ear height (11. 48), cob length (1. 34), seeds per row (4. 16), row number (1. 41), internodal length (1. 91), pith diameter (3. 59), chlorophyll (0. 95), and leaf temperature (0. 21). As such, this may be tested further in cases whereby tall plants are preferred. SG-3 × WD-10 displayed high plant height (41. 01), ear height (24. 70), cob length (1. 62), stem diameter (0. 82), seeds per row (2. 03), 100-grain weight (4. 87), internodal length (2. 77), pith diameter (0. 84) and chlorophyll (0. 61) SCA. The cross is unique for having a positive influence on both types of traits; i. e., structural and grain-related. SG-4 × WD-6 positively influenced plant height, ear height, cob length, stem diameter, seeds per row, row number, internodal length, cob diameter, and leaf temperature, but it had a negative impact on 100-grain weight, pith diameter, as well as Chlorophyll. It also exhibited positive SCA for certain structural and grain attributes, with the exception of chlorophyll, which had a negative effect. In every trait, there was no cross that outperformed the others; hence, selection ought to follow the objectives of breeding and, where feasible, through a multi-character selection index.

Correlation analysis

Phenotypic correlations gave extra details on how the traits under study related. There existed a strong positive relation between plant height and ear height (r = 0. 81). Also, as plant height increases, internodal length and ear height go up as well since they are positively related with each other (r = 0. 60, 0. 61, respectively). The correlation between cob length and plant height was moderately positive (r = 0. 54). The above relations suggest that in this material, tall plants had high ears, long internodes, as well as relatively long cobs.

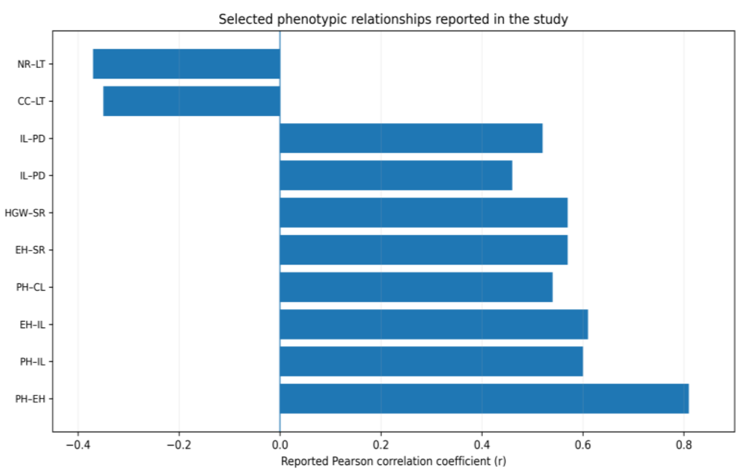


Figure 5. depicts certain observed linkages based on the analysis. It is important to note that the figure does not present any inferred or missing correlation coefficients, but only those that were explicitly provided in the text.

The ear height was seen to increase with seeds per row as depicted by the positive correlation value of 0. 57 while there was also a positive moderate correlation between 100- grain weight and seeds per row, with an r value of 0. 57. This implies that some aspects of grain size and kernel number may enhance one another, although a positive correlation does not necessarily imply that selection will act similarly on both traits. There was a negative correlation between chlorophyll content and leaf temperature (r = −0. 35), and the same trend was observed for row number versus leaf temperature (r = −0. 37). These relationships make sense from a biological point of view given that transpiration and stomatal regulation affect canopy temperature, and photosynthesis is dependent on chlorophyll. Regardless, the correlations show association alone and may not prove causality

Discussion

The positive relationships between seeds per row, ear height, and 100-grain weight have some implications for selection; however, they also indicate that one should not consider traits in isolation. Since there is a strong correlation between ear height and plant height, changing either one of them by selecting for a particular trait is possible. On the one hand, it seems that there could be some ways to improve both seeds per row and grain weight (although they are not perfectly related). These findings align with the common view that multiple interacting traits rather than a single factor determine maize yield and its components (Korsa, 2024; Mebratu et al., 2024). Combining ability analyses have gained popularity in maize breeding programs aimed at improving parental selection; this goes hand in hand with heterotic grouping and genetic diversity. Molecular diversity helps to differentiate materials at the genetic level, while line × tester analysis indicates if identified ones give favourable hybrids (Chňapek et al., 2024; Kamara et al., 2024; Guo et al., 2025). The current data substantiate this approach. Differences observed between parental means and GCA effects especially in SG-5 and SG-1 indicate that visual or per se superiority alone cannot be taken as a reliable criterion for identifying the most appropriate breeding parent.

The physiological parameters measured in the current study were important for revealing the differences among the F1 hybrids originated from the specific parents; however, the results should be interpreted carefully because physiological traits are highly influenced by changing environmental factors. The outcome derived from two physiological traits, chlorophyll content index and leaf temperature, failed to establish the potential association among the parents with the general combining ability. Furthermore, the result interpreted based on the physiological traits at a particular growth stage of a plant may not be true for the other developmental stages owing to the high sensitivity of the trait to the environment. Maize is vulnerable to abiotic stresses such as heat and drought stresses. Much research has been done in these areas, and researchers have identified that the grain yield and kernel number of maize are significantly reduced due to the effect of both stresses (Fan et al., 2024; Liu et al., 2023).

Maize breeders are working hard to combat this challenge by putting more emphasis on physiologically oriented selection along with phenotypic performance, especially under stressed environments (Mallhi et al., 2025). A review of stress physiology is imperative for breeders and, consequently, for selection for some important traits, especially if the objective is improvement of grain yield and grain yield components. The review suggests that F1 hybrid SG-1 × WD-7 can be exploited for producing extra-long ears, wide-diameter pith, thick-diameter ears, a high number of grains/ear row, with very high seed yields per plant. Similarly, other potential hybrids had specific characteristics listed previously which can be improved by crossing

them with other hybrids and by carefully selecting the right genotype, bearing in mind the target of your hybridization programme.
This study has some limitations that must be taken into account when interpreting the outcome. To begin with, the dataset provided by the supplying institution didn't contain the grain yield/ plant or per hectare grain yield that could aid in determining the hybridization transgressive segregations and grain yields, as well as the hybridizations that may result in higher or lower than mid-parental values. Without that specific piece of data, we may make reference to grain yield based on traits that are strongly correlated with grain yield. That longer cob and/or having a higher number of grains per row cannot necessarily contribute to higher grain yield. Even though longer cob and /or higher number of grains per row may contribute, at least partially, to improving grain yield for longer ears, there are other important factors for increasing grain yield; hence, longer ears may not necessarily guarantee high grain yield. The other major issue regarding the dataset provided was that it is specific to a given environment. The coming months are going to be important in terms of revealing the capability of our wheat varieties and hybrids under various environments for a variety of economically significant traits, including those examined as part of this study, combining abilities General and Specific and their interactions with the genotype x environment by applying novel methodologies using big data analysis methods that have become very popular nowadays. Some research on multi-environment trials revealed that hybrids' performance, general combining ability, and specific combining ability significantly change with the season as well as environmental differences (Hossain et al., 2026; Zhao et al., 2025). Besides being the tallest plant in the parental line, SG-5 gave an instance where its general combining ability effect (negative) on plant height didn't match up with that. This shouldn't be taken as SG-5's failure as a potential breeding parent– it really means that whether or not SG-5 is a "good" parent is highly relative & depends on what we are measuring & who we compare it to! In fact, even within the same family of traits, we noticed inconsistencies when we compared the parental means with the general combining abilities.
This was true when looking at 100-grain weight; the line with the highest parental mean was SG-1, but its estimate for GCA was negative, while SG-4 had a low parental mean but the highest positive GCA estimate. Quantitative characters are influenced by many genes as well as environmental conditions, so this discord between the parental mean & its general combining ability is not unusual.
Phenotype is what one observes and includes the effect of environment plus genes, while combining ability reflects only the general combining ability of a cross & if there is any specific combining ability. In applied breeding, therefore, we should not select parents based on their high value on one character alone from the nursery. The superior parents may not pass their superiority for that trait on to their offspring consistently, as shown by combining ability, whereas average parents may have favorable alleles to pass for that trait consistently. It is common knowledge that one cross has all the desirable traits at all times! In fact, none of the crosses evaluated excelled for all 12 characters. Such examples teach us something valuable as breeders: SG-1 x WD-7 was one such combination which caught our attention because it showed positive SCA for many ear & structural traits like row number, row length, pith diameter, and ear diameter!
SG-3 x WD-10 also had positive SCA effects on several plant and ear traits, including 100-grain weight. It would be wrong, however, to assume that any cross with positive specific combining ability on one trait is enough reason to advance such a hybrid for further evaluation! The relative importance of each character, its ideal direction, plus its environmental stability will determine how useful a particular cross is. Hence, we cannot just rule out taller plants when the focus is increased biomass, although they pose a challenge during the harvesting stage if we want high yield only. Similarly, increased leaf temperature means less effective canopy cooling, which goes against our goal if at all we want improved canopy cooling. Selection strategies based on individual traits or indices should take care of these cases better than single statistics ranking hybrids approach does.
Future researchers need to consider the physiological characteristics closely since they may account for even those variations which cannot easily be seen through analyzing an organism's shape. This study found an inverse relationship between chlorophyll content and leaf temperature, which supports the hypothesis that some genotypes may have cooling leaves due to a high level of photosynthetic activity and transpiration rate. Regardless of that, we cannot say for sure that this is a stress adaptation mechanism given by this experiment. Factors such as position in the canopy, soil water availability, and time taken for measurement have a rapid effect on leaf temperature but with slower effect on chlorophyll fluorescence that can be influenced by leaf age or nitrogen status too. In subsequent experiments, therefore, it will be imperative to follow a uniform methodology, including details of ambient conditions during data collection. If the data are correlated with grain yield, flowering time, water relations, and phenological data gathered on many occasions at different growth stages, then it will be easier to decide upon hybrids that have good physiology and can be recommended for wider adaptability. This approach would also indicate whether or not the relationships seen here would hold under different environments in the Faisalabad field experiment.
The line x tester method continues to be a valuable tool for the evaluation of parental lines and hybrid combinations. While this method is useful, it may not be able to predict all aspects of hybrid performance, especially those that are non-additive. Recent advances in breeding include the use of molecular markers, high-throughput phenotyping, and genomic prediction (GP) to evaluate hybrid performance. Zhang et al. (2022) showed that incorporating non-additive genetic effects into GP models improved predictions of hybrid performance compared to models that only included additive effects. Adak et al. (2024) found that incorporating multiple years of field-based phenotyping data into GP models also improved

predictions of grain yield and plant height. While these approaches are promising, they are not a replacement for field evaluation of hybrids. Instead, they can help breeders prioritize crosses for further evaluation. Field evaluation is especially important for grain yield because it is a complex trait that is influenced by many factors. We suggest that the next step in the study of the crosses evaluated in this study is replicated, multi-environment testing with direct measurements of grain yield. The combination of phenotypic performance, GCA and SCA, physiological measurements, and, when available, molecular data can help breeders select promising parents and hybrids for further evaluation. The study highlights a few additional points that breeders should keep in mind. The physiological measurements in this study were taken at maturity in a single environment. Differences in these measurements could reflect environmental effects (i. e., the effect of one particular environment on a given genotype) as well as genetic effects (i. e., differences among genotypes). Repeated measurements across multiple environments would allow breeders to identify genotypes that have stable physiology and good agronomic performance.

Breeding implications

Maize breeding can benefit from this research in several ways. For instance, SG-1 has a potential contribution towards improving cob length, seeds per row, and cob diameter, while SG-4 may be important in developing maize varieties with heavier grains and stronger stems. Despite having a negative GCA for plant height, SG-5 exhibited positive GCA for 100-grain weight, internodal length, pith diameter, and chlorophyll concentration. In addition, some special attention should be given to the hybrids; SG-1 × WD-7, SG-2 × WD-10, and SG-3 × WD-10, as they showed high positive SCA for various traits. Combining molecular data and line × tester information improves the prediction of hybrid performance and minimizes expensive field evaluation; hence, it is seen as an effective strategy by genomic prediction studies (Zhang et al., 2022; Adak et al., 2024).

After ensuring their repeatability, future selection indices may incorporate physiological traits. It is important to take the leaf temperature using the same procedure during the day, with low variation in radiation and leaf water potential. It is important likewise that determination of chlorophyll takes place at a given stage of development and from leaves occupying approximately the same position. This kind of standardization would help make physiological data more comparable between different environments and enhance their use in breeding.

Conclusion

There was a big difference in what was found when five maize lines, five testers, and 25 F1 crosses were studied for many traits. This shows that the average performance (Parental Means), general combining ability (GCA), and specific combining ability (SCA) should all be used when making decisions about which hybrids to choose. The line SG-1 had good general combining ability for many traits related to the cob and kernels, while SG-4 and SG-5 had positive effects on grain weight and some other important traits. Five crosses in particular stood out as having both good average performance and desirable SCA effects (SG-1 × WD-7, SG-1 × WD-6, SG-2 × WD-10, SG-3 × WD-10 and SG-5 × WD-8). This study also found strong correlations between plant height, ear height, and internodal length, while chlorophyll content was negatively correlated with leaf temperature. Such information could be useful when deciding which traits to select for indirectly, although these findings need to be confirmed under other environmental conditions. Given that different methods (parental means, GCA and SCA) gave different rankings of line performance for agronomic and physiological characters, it is important that all three methods be used prior to recommending parental lines for hybrid production. The results, however, also showed the importance of evaluating agronomic performance over several growing seasons and at multiple locations. The superior hybrids and parents reported here will be tested again in further research that involves multiple locations/seasons and grain yield as a selection trait. When preparing the manuscript for publication, there is an inconsistency in the naming of testers WD7, WD-7, and WD7.

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