

Topcross Analysis of Genetic Variability and Heterosis in Maize (*Zea mays* L.)

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Abstract

The research took place in 2024 on maize (*Zea mays*) in Al-Khalidiyah, Iraq during the autumn season, and aimed to identify variations in heterosis, as well as field performance and genetic diversity, of multiple inbred lines and their hybrids produced through top-crosses. The experiment involved seven inbred lines (S1 to S7), with line S1 being the male parent crossed with the remaining six inbred lines to produce six hybrids. Experimental treatments were arranged in a randomized complete block design (RCBD) with three replications, the experimental plot approximately 2.5 m × 2.5 m. Plants were spaced at 75 cm between rows and 25 cm between plants, giving a plant density of about 53.333 plants ha⁻¹. Eight morphological traits had been recorded and were statistically different among the treatments, and the LSD test showed that many of the hybrid plants had greater yields than their respective parents. The hybrid S1-S5 expressed positive and statistically significant mid-parent heterosis for most of the measured traits. When analyzing genetic variance, broad heritability estimates indicate that many types are influenced more by genetic factors than the environment. Simple correlation coefficient analysis found that yield had a positive significant relationship with grain per ear, grain per row, rows per ear and leaf area on yield. Cluster analysis classified the genotypes into three main clusters, reflecting substantial genetic

diversity and suggesting the usefulness of these hybrids in maize breeding programs aimed at improving yield related traits.

Keywords: Top cross, Maize, *Zea mays* L., Inbred line, Hybrid, Heterosis.

1. Introduction

Maize (*Zea mays* L.) Maize ranks as one of the world's most essential crops along with wheat and rice and has great importance economically and nutritionally [1]. In addition to its many uses for humans and animals, maize is also extremely valuable to the food and agricultural industries [2,3]. In addition, maize is essential raw material for many globally significant industries, including the production of ethanol (biofuel) as a replacement for fossil-based automobile fuels, and alternative plastics, dyes, etc[3]. Although maize has been grown more extensively in Iraq, there are still many issues with productivity levels that are far below global averages due mostly to the limited number of maize varieties being grown, as well as not taking full advantage of genetically diverse resources available.

The availability of genetic variation amongst inbred crops greatly impacts their ability to improve genetically. Selecting these best population types for breeding and developing hybrids has a strong impact on crop productivity, due to the amount of variation available. [4,5] Variation is the difference in some or all of an

organism's characteristics. Variation can be influenced by both genetics and environmental circumstances and accumulate results in many variations about a specific characteristic. Genetic variation specifically refers to the difference in gene sequences from individual to individual within a population. Allelic variation is the basis for genetic variation, which can be expressed as differences in phenotype with respect to the same characteristic. Genetic variation is an important source of genetic diversity, which describes the extent of variation present within a population. Genetic variation plays a fundamental role in maize breeding programs by providing the diversity required for the selection of superior genotypes. Hybridization facilitates the exploitation of heterosis and genetic diversity, contributing to genetic improvement and increased yield potential in maize [6].

Topcross is one of the primary techniques used for research of heterosis, or hybrid vigor, and estimation of genetic parameters, due to the high reliability of its identification of superior lines which may have the potential for production

of high-yielding hybrids. In addition, topcross hybrids also provide a means of reducing time and costs associated with breeding programs because the use of topcrosses requires fewer self-pollinations and a reduced amount of area is needed to produce and multiply both lines allowing the hybrids to reach the market quicker than fully inbred lines and with similar yield levels [7,8].

This research aimed to evaluate the field performance and genetic

variability and differences found in inbred maize lines and their corresponding hybrids by using the top cross method. Other objectives included the examination of other parameters such as correlation coefficients and cluster analysis for each trait being studied to determine which genotypes show the most potential for future breeding programs..

2. Materials and Methods

Seven inbred lines of maize (*Zea mays* L.) were utilized in the topcross breeding, during the Fall of 2024 in Al Khalidiya, Iraq. The purpose of this study is to assess field performance, heterosis, as well as genetic variability among both groups. The genetics used in this study consisted of seven inbred lines (S1, S2, S3, ... S7); the first strain (S1) was used as a parent (tester) because it showed good agricultural performance and wide genetic diversity compared to other pure strains, to create six single-cross hybrid lines (Topcrosses) with each of the remaining six. A total of thirteen treatments were used (seven inbred lines and six hybrids)

The study utilized a randomized complete block design (RCBD)

consisting of three replications. Treatments were arranged in rows spaced apart by 0.75 m between rows and 0.25 m between plants to create experimental units measuring 2 by 3 m in size. A random, but uniform selection of ten guarded plants was made from each of the experimental units for measurement of eight different morphological and yield traits; these included: height of the plant (cm), total leaf area (cm²) as calculated using the equation - Total leaf area = (plant height) x (average maximum width of leaf) x 0.75 [9], chlorophyll content (SPAD), number of rows per ear of grain (row-1), number of grains per row of grain (row-1), number of grains per ear of grain (row-1), weight of 200 grains (g), and total plant yield (g).

The methods for data analysis included ANOVA and comparisons of treatment means by the least significant difference (L.S.D.) test at $P < 0.05$. The mid heterosis was calculated and that data have been reported. In addition to the analysis of variance, genetic variance (σ^2_g) and phenotypic variance (σ^2_p), and broad sense heritability ($H^2_{b.s}$) were estimated for all studied traits. The

3. Results and Discussion

ANOVA Table (1) has been completed for all traits with significant differences (0.05) for leaf area, number of rows per ear, number of grains per row, number of grains per ear, 200 grain wt, and plant yielding ability indicating sufficient genetic variability of studied genotypes exploited in breeding programs to improve these traits. The remaining traits plant height and SPAD (chlorophyll) did not demonstrate significant differences in the general F-test; however, mean comparisons via the LSD test indicated significant differences for some of the genotypes tested. Observed results indicate that the LSD test, as compared to the general F-test was more sensitive when detecting pairwise differences of means. Additionally, low mean square

characteristics of all traits studied were further evaluated by calculating the correlation coefficient of each trait in relation to other traits using the least squares method as described by [10]. Cluster analysis was then used to group inbred lines and hybrids according to their morphological and yield characteristics using Ward's method..

error (MSe) values for the majority of the traits indicated the experimental design was accurate and the tools utilized to measure the genotypes were reliable, providing greater validity to the estimates of genetic parameters and heterosis tests.

Selecting an appropriate tester parent is critical in developing high-yielding maize hybrids. Prior research has previously demonstrated the importance of this selection process [11,12,13]. In addition, when examining the role of genetic diversity and line interaction are also important in developing and selecting better genotypes to improve key agronomic traits such as ear length and width, [14,15] found that genetic diversity and line interaction are critical parts of the improvement and selection process[16].

Table 1: Analysis of variance (ANOVA) showing mean squares (MS) for the studied traits in maize

S. V.	O. d. f.	Plant Height (cm)	Leaf Area (cm ²)	Chlorophyll (SPAD)	Rows No. Per Ear (Row Ear ⁻¹)	Grains No. Per Row (Grain Row ⁻¹)	Grains No. Per Ear (Grain Ear ⁻¹)	Weight of 200 Grain (g)	Plant Yield (g)
R	2	36.49	724594.82*	10.89	2.08	8.49	2539.72	367.14*	925.82
Treatments	12	438.47	1216408.44*	30.66	7.25*	53.86*	26531.56*	74.30	1461.72*
Error	24	173.68	144186.07	12.17	1.94	11.32	2287.05	79.52	424.62

* Significant at 0.05 probability level.

The means of the evaluated characteristics are shown in Table two along with the least significant difference (LSD) for a 0.05 probability level, indicating significant variation in both inbred lines and hybrids. Of the hybrids evaluated, hybrid 1×5 had the highest mean for height at 215.67 cm, leaf area at 6147.92 cm², and number of rows per ear (18.67 rows ear⁻¹); this was reflected in its highest yield of 142.63 g. In addition, inbred line S7 was significantly greater than all other inbreds for the number of grains per

row (34.67 grains row⁻¹), which contributed to its high yield of 153.6 g. The hybrid that had the highest 200-grain weight (65.48 g) was hybrid 1×3. Of the inbreds evaluated, S1 and S3 had the highest SPAD (chlorophyll) values of 60.1 and 60, respectively, indicating that they were the most efficient in conducting photosynthesis. This evidence is in line with previous findings[14,17,18,19], where it was shown that F1 hybrids differed substantially from their parent inbreds..

Table 2: Means of the studied traits and LSD (0.05) values for maize inbred lines and their hybrids (Topcross).

Genotype	Plant Height (cm)	Leaf Area (cm ²)	Chlorophyll (SPAD)	Rows No. Per Ear (Row Ear ⁻¹)	Grains No. Per Row (Grain Row ⁻¹)	Grains No. Per Ear (Grain Ear ⁻¹)	Weight of 200 Grain (g)	Plant Yield (g)
S1	201.67	4078.33	60.1	15	24.67	367.67	50.27	92.91
S2	193.67	4219.33	50.7	14	22	308	53.68	84.62
S3	176.33	4855.17	60	17	25	422.67	49.78	107.34
S4	172.67	4975	53.03	14	25.67	357.33	51.20	92.92
S5	206	4412.5	52	15.33	25.67	391.33	48.69	96.20
S6	193.67	5470.83	58.2	15	29	433.33	57.94	132.18
S7	186.33	5887.5	55.8	16.67	34.67	574.67	52.98	153.6
1×2	197.67	4382.17	55.57	12.67	28.67	373.33	51.09	87.37
1×3	192.67	4886.5	55.07	16	22.67	358	65.48	117.70
1×4	190.33	4692.92	58.5	14.67	27	394	52.87	103.39
1×5	215.67	6147.92	59.13	18.67	34	634.67	45.10	142.63
1×6	186	5485.42	54	15.33	34	519.33	49.58	128.36
1×7	178.67	4855	52.57	16.67	26.33	437.33	54.37	118.31
LSD (0.05)	22.21	639.92	5.88	4.65	5.67	80.6	15.03	34.73

The heterosis estimates for mid-parent hybridization of the traits under study are provided in Table 3. Hybrid 1*5 displays a superior level of many of the traits, achieving the highest level of positive heterosis in plant height

(5.8%), leaf area (44.81%), chlorophyll content (5.50%), number of rows per ear (23.08%), number of grains per row (35.10%), number of grains per ear (67.24%) and grain yield (50.85%). Therefore, hybrid 1*5

exhibits a substantial additive genetic advantage that could be utilized by plant breeders to enhance productivity. Hybrid 1*3 also exhibited relatively large positive heterosis levels for 200-grain weight (30.90%), making this hybrid particularly remarkable for that trait .

The study's conclusions indicate that heterosis is greater and has a positive

effect on traits related to productivity, therefore indicating that the hybrids could provide potential benefits in genetic improvement programs. Previous publications have all demonstrated that heterosis and genetic variation among lines were an important basis for improving yield traits [14,18,19,20] as indicated by the results from the current study.

Table 3: Mid-Parent Heterosis (%) for the studied traits of maize inbred lines and their hybrids (Topcross).

Hybrid	Plant Height (cm)	Leaf Area (cm ²)	Chlorophyll (SPAD)	Rows No. Per Ear (Row Ear ⁻¹)	Grains No. Per Row (Grain Row ⁻¹)	Grains No. Per Ear (Grain Ear ⁻¹)	Weight of 200 Grain (g)	Plant Yield (g)
1×2	0	5.62	0.30	-12.64	22.86	10.51	-1.71	-1.57
1×3	1.94	9.40	-8.30	0	-8.72	-9.41	30.90	17.56
1×4	1.69	3.67	3.42	1.15	7.28	8.69	4.21	11.27
1×5	5.8	44.81	5.50	23.08	35.10	67.24	-8.85	50.85
1×6	-5.9	14.89	-8.71	2.22	26.71	29.67	-8.36	14.06
1×7	-7.9	-2.57	-9.29	5.26	-11.24	-7.18	5.30	-4.01
SE	9.32	26.85	2.47	0.98	2.38	33.82	6.31	14.57

As shown in Table 4, the estimates of the genetic parameters for the traits studied in maize were significantly different from the estimates of the genetic parameters for the same traits reported by other researchers who

worked with maize. Many of the traits (i.e., the number of rows per ear, 73.24%; the number of grains per row, 78.98%; the number of grains per ear, 91.38%; and the yield of the plant, 70.95%) exhibited high broad

sense heritability coefficients which indicate that there is more genetic variance than environmental variance in these traits and therefore the majority of the phenotypic variability in these traits is due to genetic differences among individuals, indicating that these traits can be improved through selection programs for traits with high broad-sense heritability. On the other hand, leaf area (43.58%), showed low to moderate heritability, reflecting a larger environmental influence. Notably, the initial negative heritability estimate for the 200 grain weight, was constrained to zero (0.00%), had low broad-sense heritability coefficients which indicate that there are large amounts of environmental variance when

compared to genetic variance for these traits and their phenotypic expression is influenced predominantly by environmental factors and therefore, any attempt to improve these traits through selection would be difficult. Plant height (60.39%) and chlorophyll content (60.29%) had moderate broad-sense heritability coefficients indicating that these traits have similar amounts of genetic and environmental variance .

Overall, yield-related traits had relatively high heritability estimates, especially the number of grains and yield of plant material. Thus, these two traits should be used as primary selection criteria for maize breeding. The results from the current research support the findings made by [14,18,19,21,22] .

Table 4: Estimates of genetic parameters for the studied traits in maize.

Traits Genetic Parameters	Plant Height (cm)	Leaf Area (cm ²)	Chlorophyll (SPAD)	Rows Per Ear (Row ⁻¹)	No. Grains No. Ear (Grain Row ⁻¹)	No. Grains No. Row (Grain Ear ⁻¹)	Weight of 200 Grain (g)	Plant Yield (g)
σ^2G	88.26	37121.45	6.16	1.77	14.18	8081.52	0.00	345.7
σ^2E	173.68	144186.07	12.17	1.94	11.32	2287.05	79.52	424.62
σ^2P	146.15	85183.47	10.22	2.42	17.95	8843.87	24.77	487.24
$H^2.b.s\%$	60.39	43.58	60.29	73.24	78.98	91.38	0.00	70.95

Table 5 shows the simple correlation coefficients among the studied traits in maize. The results showed that plant yield was strongly positively

correlated with the number of grains per ear (0.87), the number of grains per row (0.76), the number of rows per ear (0.72), and leaf area (0.92).

These findings suggest that these traits can be considered as key selection indicators for improving grain yield. In contrast, 200-grain weight showed a negative correlation with plant yield. Plant height also did not show a significant correlation with yield, indicating that this trait does not represent a direct selection indicator of productivity. Strong positive correlations were also observed among some yield components, such as the relationship between the number of grains per row and the number of grains per ear (0.90), and the relationship between the number of rows and the number of grains per

ear (0.73), reflecting the genetic and functional overlap between these traits.

The possibility of improving a trait depends on the presence and type of correlation, therefore, traits with high heritability can contribute significantly to yield improvement. similar results were obtained by [23,24]. To better illustrate the results, correlation coefficients were also represented graphically in a heatmap (Figure 1), where strong positive associations appeared in dark red, while negative associations were displayed in blue.

Table 5: Simple correlation coefficients between the studied traits in maize.

Traits	Plant Height (cm)	Leaf Area (cm ²)	Chlorophyll (SPAD)	Rows No. Per Ear (Row Ear ⁻¹)	Grains No. Per Row (Grain Row ⁻¹)	Grains No. Per Ear (Grain Ear ⁻¹)	Weight of 200 Grain (g)	Plant Yield (g)
Plant Height (cm)	1							
Leaf Area (cm ²)	0.03ns	1						
Chlorophyll (SPAD)	0.21ns	0.23ns	1					
Rows No. Per Ear (Row Ear ⁻¹)	0.15ns	0.64*	0.34ns	1				
Grains No. Per Row (Grain Row ⁻¹)	0.18ns	0.81**	0.23ns	0.73**	1			
Grains No. Per Ear (Grain Ear ⁻¹)	0.24ns	0.89**	0.32ns	0.73**	0.90**	1		

Ear ⁻¹)								
Weight of 200 Grain (g)	-0.25ns	-0.11ns	-0.15ns	-0.16ns	-0.42ns	-0.41ns	1	
Plant Yield (g)	0.07ns	0.92**	0.27ns	0.72**	0.76**	0.87**	0.06ns	1

Note: ns = not significant, * = significant at the 0.05 probability level, ** = significant at the 0.01 probability level.

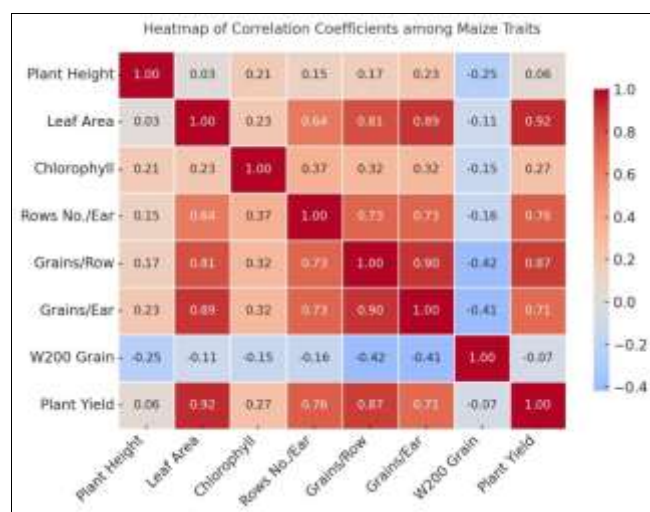


Figure 1: Heatmap of correlation coefficients among the studied traits in maize genotypes.

The study of genetic relationship trees among genotypes is important for improving yield and quality, as using genetically diverse parents increases the likelihood of obtaining high heterosis [18]. Cluster analysis (Figure 2), performed using Ward's method and Euclidean distance, divided the studied maize genotypes into three main groups:

1. Group I: included line S6 and hybrid 1×6 along with line S7 and hybrid 1×5, indicating their

genetic proximity and similarity in the studied traits.

2. Group II: comprised lines S3 and S4 with hybrids 1×3, 1×4, and 1×7, showing close genetic relationships and integration in growth and yield traits.
3. Group III: consisted of lines S1, S2, and S5 with hybrid 1×2, reflecting their genetic closeness and relative divergence from the other groups.

The differences in the genetic divergence of these groups demonstrate that the gene diversity present is a resource that can be used in maize breeding programs to select genetically separate parents for creating superior hybrids. Previous research[25,26] has established that genotypes can be placed into phenotype groups according to variation in phenotypic traits

considered for their phenotyping. Also, [27] have shown that using genetically varied parental lines that have distinct traits enhances the heterosis seen when hybridizing the respective lines. Finally, as stated by [28], cluster analysis based on Euclidean distance is a valid technique for utilizing the genetic and phenotypic variance seen when utilizing these two methods..

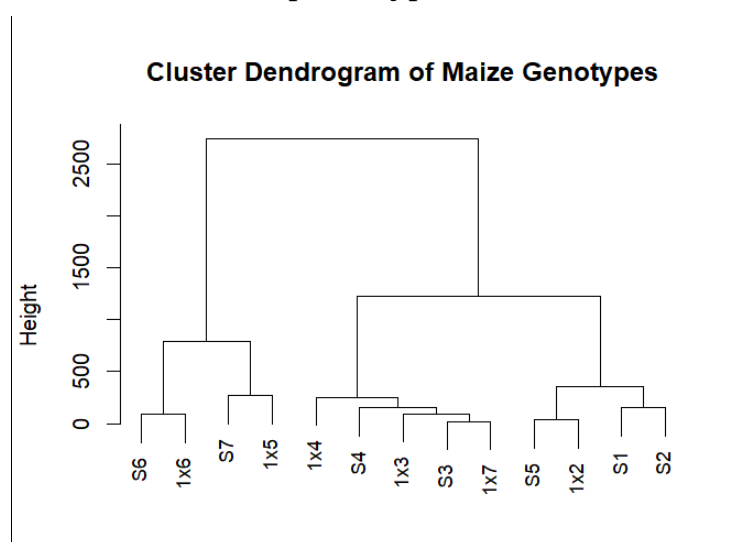


Figure 2: Cluster dendrogram of maize inbred lines and their hybrids based on studied traits using ward's method and Euclidean distance.

4. Conclusions

In this study, we observed that genetic differences exist between the inbred lines and their hybrids for a majority of the traits measured. These differences create considerable opportunity to use them in plant breeding programs. The hybrid 1x5 had very good basic yield traits, but particularly for yield components. Therefore, it represents a very

important genetic resource for increasing productivity of maize. The estimated amount of heterosis associated with the yield components, as well as the broad-sense heritability for yield-related traits, such as the number of grains per ear and yield from the plant, indicates that direct selection is an effective means of improving those traits. Yield

components (number of grains per ear; number of grains per row; number of rows per ear; and leaf area) are also good indicators of yield for purpose of making selection for yield increase, as indicated by simple correlation analysis. The cluster

analysis indicates that the three main clusters of genotypes studied will be a good starting point for plant breeders who plan to use genetically divergent parents to develop hybrids with higher amounts of heterosis in the future..

5. References

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