

Article**Role of Molecular Marker RAPD in Determining the Genetic Divergence between Hybrids and Inbred Lines of Maize Using Full Diallel Cross**Zeyad A. Abdulhamed^{1*}, Baraa M. Abdulkareem², and Abdulsamad. H. Noaman¹¹Department of Field Crops - College of Agriculture/ University Of Anbar, Anbar. Iraq.²Accommodation Dep. University of Anbar, Iraq.*Correspondence: ag.zeyad.abdul-hamed@uoanbar.edu.iqAvailable from: <http://dx.doi.org/10.21931/RB/CSS/2023.08.03.68>**Abstract**

Field experiments were conducted during the spring and autumn seasons of 2021 in farmer's fields on the right side of the Euphrates River – Ramadidistrict / the center of Anbar Governorate. In an entire reciprocal program, four inbreds of maize (Zm1, A119, Syn-33 and A105) were used and introduced to produce 12 single hybrids. The seeds of the parents and the resulting hybrids were planted according to randomized complete block design (RCBD) at four replications to estimate the heterosis. The reciprocal hybrid Syn-33 x A105 and the reverse hybrid Syn-33 x A105 were significantly superior. They gave the highest means of grain yield (215.78, 203.10 g), grain weight (99.74 and 98.56 g), number of grains in the ear (17.4 and 16.7 grains row⁻¹) and heterosis (116.01 and 103.32%) in grain yield respectively. Randomly Amplified Polymorphic DNA (RAPD) technique and 15 primers were used. The highest genetic divergence was 0.69 between Zm1 and Syn-33 inbreds, while the highest genetic divergence between inbreds and hybrids was 0.75 between Zm1 inbred and A105 x A119 hybrid.

Keywords: Primer, Heterosis, Genetic, Polymorphic, RCR.**Introduction**

Many factors have helped to expand the cultivation of maize (*Zea mays* L.), foremost of which is that it is used in human nutrition and animal feed as well as in the manufacture of oil, starch, rubber, gums and biofuels¹. The importance of maize increases, in addition to its mentioned uses, due to the high productive capacity of the crop and its adaptation to different environmental conditions, as its cultivation is spread in different regions of the world³. One of the primary aims of most maize breeding programs is to develop high-yielding varieties that are adapted to a wide range of environmental conditions. Heterosis is one of the tools to increase the yield, which is based on increasing the accumulation of the most significant number of desirable genes in the hybrid, as individuals of the F1 of hybrids that have high heterosis, high yield and resistance to the surrounding environmental conditions during the growing season use³. Heterosis is a significant event in the history of the maize crop, which made the crop more fortunate among field crops. Heterosis will remain a critical genetic fact intended for specialists in plant breeding and genetics⁴. Therefore, plant breeders seek to

find the best hybrids by diagnosing the best parents to achieve the highest heterosis. This can be done using large numbers of pure lines to evaluate and produce the best hybrids superior in grain yield and its components. Heterosis is either positive that accompanies hybridization between pure lines or varieties that differ from each other so that the F1 shows an increase in vitality and ability to grow, or there is a reverse case where the heterosis is negative, and this is desirable in the dates of flowers and maturity to obtain early hybrids^{1,5}.stated that the heterosis results from the action of a large group of genes (polygenes), noting that the plant traits are controlled by approximately ten million genes pairs, which may work to dominance or partial dominance. Scientists have exploited the heterosis in maize and increased the genetic variations between the resulting individuals. This phenomenon means that the individuals of the F1 exceed the rate of the highest or average parents. Recently, the discovery of modern technologies has increased the knowledge about the interpretation of heterosis and how the maize interacts with the surrounding environmental conditions⁶.

Most of the plant breeders' decisions are based on the data of morphological traits in the traditional breeding programs, which makes them subject to many challenges, the most important of which is the impact of environmental conditions on these traits, which may lead to inaccurate conclusions sometimes^{7,8}.Thus, we still have an excellent opportunity to enhance traditional genetic applications with biotechnologies with some environmental independence^{9,10}. DNA markers are important over biochemical and morphological markers because they are not affected by the environment and depend on the sequence of genes or genetic material, which can detect the difference in the genetic material carried by the individual.

DNA markers are one of the most widespread and widely used markers compared with others because of their abundance. These markers depend on the apparent variances in the small DNA fragment. In the past few years, large numbers of molecular markers have become available to researchers, and the primary condition for the effectiveness of these markers is that they be polymorphic, as the chromosome that carries the mutant gene can be distinguished from the chromosome that carries the normal gene by the marker associated with that gene due to mutation. Markers are nucleotide DNA sequences in the genome that can be located and identified due to genetic variation from the rest of the DNA due to mutation. The basic structure of a specific genome site may differ according to different plant inbreds, which are called polymorphisms¹¹. Randomly Amplified Polymorphic DNA (RAPD) technique is among the critical techniques that depend on the principle of polymerization (PCR), which is characterized by being a fast, easy and low-cost technology as well as its need for small amounts of DNA and it is not needed prior information about the studied genome¹². The study aims to evaluate several inbreds of maize using the method of complete reciprocal hybridization to know the heterosis for grain yield and its components, as well as to determine the genetic fingerprint and study the genetic divergence between the inbreds through the isolation and purification of DNA using the molecular mark RAPD.

Materials and Methods

Field experiments were conducted during the spring and autumn seasons of 2021in farmer's fields on the right side of the Euphrates River – Ramadi district / the center of Anbar Governorate. Four inbreds of maize (Zm1, A119, Syn-33 and A105) symbolized 1 to 4, respectively.

Soil management operations were carried out each season according to the scientific recommendations. DAP fertilizer was added at an average of 320 Kg ha⁻¹ before planting, and 100 Kg ha⁻¹ urea was added at two equal doses, the first

when the plant reached an average height of 25 cm and the second at the beginning of the flowering stage. The field was divided into two parts. The first part was planted with the seeds of the four parental inbreds on 15 March 2021 on the lines at a rate of 8 lines for each inbred. The length of each line was 5 m, 75 cm, and the distance between plants was 25 cm. Then, the four inbred seeds were planted after 7 days of planting the first parts to ensure compatibility in flowering and to obtain vital and effective pollen grains throughout the pollination period. When the plants reached the flowering stage and the appearance of the female inflorescence and before the appearance of the silk, the female inflorescences were wrapped with paper bags to prevent open pollination and to ensure obtaining the required crosses. Also, male inflorescences were wrapped in large paper bags before 1 day of the pollination process started. After male inflorescences released the pollen grains, the pollen grains were collected the next day to be pollinated by the ready-silks. By continuing the process of bagging and pollination, it was possible to conduct all possible and complete crosses between four inbreds to obtain the F1, 16 hybrids, according to the hybridization program by the method of complete reciprocal hybridization (according to the first Griffing method, the first model) (Griffing 1956b) using a randomized complete block design at four replications. In addition, the parental inbreds were self-pollinated. The comparison experiment was conducted on 22 July 2021 in the same field, which included the parents' and the resulting hybrids' seeds.

The data were recorded for the studied traits based on an individual plant at an average of ten plants, where the traits of female flowering (day), leaf area (cm²), number of rows per ear, weight of 300 grains (g) and grain yield of individual plant (g) were studied.

Heterosis was estimated as a percentage compared with the best parents and the average of the two parents for all studied traits except female flowering, which compared with the lowest parents as follows:

$$\text{Heterobeltiosis (Heterosis) \%} = \frac{\overline{F1} - \overline{BP}}{\overline{BP}} \times 100$$

As: H = Heterosis relative to the average of the best parents.

$\overline{F1}$ = Average of F1

\overline{BP} = average of best parents

Markers of randomly amplified polymorphic DNA (RAPD)

It is one of the markers based on PCR technology. The basis of its work depends on the use of short primers with a random sequence made of ten nitrogenous bases with a high content of G-C ranging between 50-70%, as these primers find complementary locations for them on the target DNA strand. The difference in these complementary locations leads to models with a genetic fingerprint that allows any organism to be distinguished and has a distinct genetic fingerprint at the level of species or inbred, and this depends on the choice of the primer in the RAPD-PCR analysis¹² Also, these variances that appear during the test results are due to changes in the sequences of the DNA nitrogenous bases of individuals of one species due to the occurrence of mutations such as deletion and insertion in the complementary locations of the primer sequence or base substitution or the occurrence of new linkages, which leads to a change or alteration of the primer binding location with template DNA strand, resulting in a difference in the number and locations of the replication bundles between individuals.

DNA isolation of genome

Samples were taken from young maize leaves (1.5 g) of each inbred and hybrid to conduct the studying analyses in the biotechnology laboratory. The DNA of the genome was isolated from the young leaves of the sixteen maize genotypes using CTAB. There are several methods for isolating nucleic acids from plants because plants of their diversity contain different amounts of plant compounds such as proteins, polysaccharides and complex as well as nucleic acids. Thus, one method for isolating nucleic acids is to use Random Amplified Polymorphic DNA (RAPD) technology and 15 primers for all genotypes (Table 1).

No.	Nucleotide Sequence (5-3)	Primers
1	TCGTGCGAGA	OPJ18
2	CCAAGCTTCC	OPF09
3	ACGGATCCTG	OPF01
4	GAGCCCTCCA	OPG03
5	CTGGGGACTT	OPD20
6	CTCACCGTCC	VBC 3-9
7	TGCGTGCTTG	VBC 3-12
8	GTTGCCAGCC	VBC 3-15
9	AGG7CCGGTG	VBC 3-17
10	ACTTCGCCAC	VBC 3-19
11	AGGACCGGTG	VBC 3-20
12	TGGTCCGGTG	VBC 3-21
13	AGGACCGGAG	VBC 3-22
14	TGGTCCGGAG	VBC 3-23
15	AGGTCCGGAG	VBC 3-24

Table 1: Nucleotide Sequence of primers used in RAPD.

The process of isolating DNA from plants is relatively more complex than other organisms because of the thick wall surrounding the cell membrane, in addition to the fact that some plants contain a large number of phenols and polysaccharides, which are pollutants, as they sometimes precipitate with DNA to give a liquid with a high viscosity. It is an inhibitor of the PCR reaction.

Results

Table (2) results indicate significant differences between genotypes in the number of days until 50% of female flowering. The 2 and 3 inbreds took the least period for female flowering (57.70 and 58.40 days), respectively, compared with the 4, which took the most extended period (61.50 days). Regarding leaf area, the results show that the 4 inbred was significantly superior and gave the highest mean of leaf area (4412 cm²) compared with the 2 inbred, which gave the lowest mean (3582 cm²). The differences between parents were reflected in the resulting hybrids. The 2 × 3 reverse hybrid and 4 × 1 reciprocal hybrid gave the highest mean (5617 and 5167 cm²), respectively, compared with the 1 × 4 reverse hybrid, which gave the lowest mean (4084 cm²).

As for the number of rows in the ear, the 1 inbred was significantly superior and achieved the highest mean of this trait (15.40 rows ear⁻¹). Also, the 3 × 4 reciprocal hybrid and 3 × 2 reverse hybrid were significantly superior and achieved

the highest means (17.50 and 17.40 rows ear⁻¹), respectively, with the non-significant difference with five hybrids compared with 4 × 1 reciprocal hybrid, which achieved the lowest mean (15.3 rows ear⁻¹)—found¹¹ significant difference between hybrid in the number of rows per ear.

Regarding grain yield, the results show that the 1 and 4 inbreds were significantly superior and gave the highest mean of this trait (128.42 and 123.17 g). Also, the 3x2reciprocal hybrid, 2 x 3, and 1 x4 reverse hybrids had the highest means (215.78, 200.28 and 203.10 g), respectively. The superiority of these hybrids may be due to their superiority in some grain yield components and leaf area and their taking the least number of days until flowering, which allowed the hybrid sufficient period to store the metabolic products.

Genotype	Female flowering (day)	Leaf area (cm ²)	Number of rows	300 grain weight (gm)	Plant yield (gm)
1	60.60	3923	15.4	87.60	128.42
2	57.70	3582	13.7	73.50	99.89
3	58.40	3714	14.0	75.03	80.88
4	61.50	4412	14.8	89.01	123.17
1×2	55.70	4268	16.3	82.3	198.42
1×3	56.40	4719	16.6	83.18	178.52
1×4	57.80	5167	15.3	90.31	182.41
2×3	55.40	4563	16.7	98.56	215.78
2×4	59.20	4485	16.3	83.57	169.95
3×4	59.50	4128	17.5	85.78	181.78
2×1	56.20	4904	16.6	94.11	195.97
3×1	58.50	4741	15.8	92.99	179.69
4×1	56.10	4084	16.3	93.61	200.28
3×2	56.30	5617	17.4	99.74	203.10
4×2	58.10	4684	16.7	81.52	159.85
4×3	58.60	4292	16.5	90.28	190.44
LSD	1.36	607	.131	3.98	17.98

Table 2: Averages of values of parents and crosses for maize.

Heterosis

The differences between the parents' means were reflected in their hybrids for female flowering. Thus, the heterosis attributed to the lowest parents and the average of the two parents differed (Table 3). It is noticeable that the negative and significant heterosis appeared in 7 hybrids. The 1 × 4 reverse hybrid had the lowest value (-7.42%) relative to the lowest parents, while the 4 × 2 reciprocal hybrid had the highest positive heterosis.

Genotype	Female flowering (day)	Leaf area (cm ²)	Number of rows	300 grain weight (gm)	Plant yield (gm)
1×2	-3.46	11.64	5.84	-6.05	54.51
1×3	-3.42	20.29	7.79	-5.04	39.01

1×4	-4.62	17.11	-0.65	1.46	42.04
2×3	-3.98	22.86	19.28	31.36	116.01
2×4	2.60	1.65	10.13	-6.11	37.99
3×4	1.88	-6.43	18.24	-3.62	47.58
2×1	-2.43	25.00	7.79	7.43	52.60
3×1	0.17	20.85	5.60	6.15	39.92
4×1	-7.42	-7.43	5.84	5.16	55.95
3×2	-2.42	51.23	24.28	32.93	103.32
4×2	0.69	6.16	12.84	-8.41	29.78
4×3	0.34	-2.72	11.48	1.46	54.61
SE	0.86	4.81	2.02	3.99	7.63

Table 3: Heterosis of the crosses relative to the best parents of the traits studied in maize.

The calculated heterosis depends on the deviation of the F1 from the highest parents. The parents' averages varied, which may be due to the differences between the averages of the parents and their hybrids. Eleven hybrids showed positive and significant heterosis values for the number of rows per ear relative to the best parents. In contrast, one reciprocal hybrid (4×1) showed a non-significant negative value of the heterosis (-0.65%). In contrast, the 2×3 reverse hybrid had the highest positive value (24.28%), a percentage of the relative to the best parents. It concluded that there is a super-dominance of the genes responsible for showing the trait in the hybrids that gave positive heterosis. Their effect is towards increasing the number of rows per ear and the presence of partial dominance of the genes responsible for showing the trait in the hybrids that showed negative heterosis, i.e., their effect is towards reducing the number of rows per ear.

The results in Table 4 indicate that the seven hybrids gave a positive value of the heterosis relative to the best parents for the weight of 300 grains. Five hybrids were positive and significant, while two hybrids were non-significant. Their values ranged between 32.93 and 31.3% for the 2 × 3 and 4 × 1 hybrids, respectively, whereas five hybrids had a negative and significant value of the heterosis, the 2 × 4 hybrid gave the lowest negative value (-8.41%). The positive values of the heterosis indicate the super-dominance of the genes in the inheritance of the trait, and their effect is towards increasing the grain weight.

We conclude from this that the inbreds differed in their performance. The 3×2 reciprocal hybrid and 2 × 3 and 1 × 4 reverse hybrid were superior ingrain yield and gave a heterosis in the desired direction relative to the best parents in the grain yield, weight, number of grains per row and female flower traits. However, some of the parents of these hybrids were not superior in these traits. This indicates the possibility of using these superior inbreds in their crosses in breeding programs to produce superior hybrids with high heterosis to produce a high yield due to most of the traits being influenced by the super-dominance genes.

RAPD markers

The results in Table 4 show that the VBC 3-19 primer gave the highest number of bundles (32 bundles), while the OPF09 primer gave 9 bundles. The total number of the resulting bundles was 321, while the number of differentiated bundles was 283, with a percentage of 88.16%, as the VBC 3-19 and VBC 3-22 primers gave

32 and 22 differentiated bundles with a percentage of 100%, respectively, while the VBC 3-19 primer gave 5 differentiated bundles with a percentage of 77.77%. The primers were characterized by a molecular weight of 250-2700 bp.

Primers	molecular weight	Product bands	Differentiated bands	Percentage of Differentiated bands
OPJ18	500 - 2000	16	12	75.00
OPF09	550 - 1600	9	7	77.77
OPF01	250 - 1750	18	15	83.33
OPG03	660 - 2000	21	17	80.09
OPD20	400 - 2700	24	22	91.66
VBC 3-9	550 - 2200	25	19	76.00
VBC 3-12	500 – 2400	27	25	92.59
VBC 3-15	400 - 1900	15	13	86.66
VBC 3-17	450 - 2650	22	21	95.45
VBC 3-19	350 - 2500	32	32	100.00
VBC 3-20	500 – 2100	24	20	83.33
VBC 3-21	700 – 1900	20	19	95.00
VBC 3-22	450 – 1800	22	22	100.00
VBC 3-23	550 – 2400	25	23	92.00
VBC 3-24	650 - 2100	21	16	76.19
Total		321	283	
Average	250-2700		88.16	

Table 4: Primer products of total and differentiated bands and their molecular sizes.

Genetic divergence values between inbreds and hybrids of maize using RAPD technology

The genetic divergence was estimated using the NTSYS-PC version 2.1 genetic program, which depends on the similarity or difference between the genotypes. It is evident from the values of the genetic divergence (Table 5) that the values of the genetic divergence of the studied genotypes when using 15 random primers. If the genetic material is identical between two genetic structures, this indicates that the genetic divergence between them must be equal to zero. Genetic similarity represents the degree of genetic similarity between any two individuals, so it is equal when there is no genetic variation between the genotypes, and this appears when individuals participate in the general bundles and the absence of any differentiated bundles between them. Using a small number of primers reduces the interest in such results. When using a more significant number of primers due to the different locations of linkage according to the sequence of the primer, the number of associated bundles determines the genetic affinity or divergence between genotypes. The greater the number of bundles, the lower the genetic divergence. Those bundles associated with each other indicate the similarity of genetic material in the genome location of the studied genotypes, which may represent the similarity in phenotypic traits, or the similarity may be in the non-coding location, that is, those that do not have gene expression. As for the genetically divergent genotypes, they are the ones that associate at least several bundles with each other due to differences in the nucleotide sequences in the genome.

	1	2	3	4	×2 1	×3 1	×4 1	×3 2	×4 2	×4 3	×1 2	×1 3	×1 4	×2 3	×2 4	×3 4
1	0															
2	0.1 2	0														
3	0.6 9	0.6 5	0													
4	0.4 2	0.0 3	0.2 8	0												
1×2	0.2 5	0.5 0	0.1 2	0.4 2	0											
1×3	0.1 4	0.4 0	0.1 6	0.3 3	0.6 1	0										
1×4	0.4 2	0.2 8	0.5 1	0.6 6	0.2 5	0.3 3	0									
2×3	0.2 8	0.1 4	0.6 0	0.4 9	0.2 9	0.4 0	0.5 0	0								
2×4	0.7 5	0.2 2	0.5 7	0.5 0	0.3 2	0.1 1	0.5 0	0.3 7	01							
3×4	0.4 2	0.2 8	0.5 7	0.4 2	0.6 1	0.6 0	0.2 5	0.5 0	0.3 2	0						
2×1	0.4 2	0.5 0	0.2 8	0.6 6	0.6 6	0.5 7	0.4 2	0.2 8	0.3 4	0.6 6	0					
3×1	0.2 5	0.5 0	0.1 3	0.2 5	0.6 4	0.6 2	0.2 5	0.5 0	0.3 3	0.6 6	0.4 2	0				
4×1	0.1 4	0.4	0.2 7	0.3 3	0.6 0	0.2 0	0.1 4	0.1 6	0.2 5	0.3 3	0.3 3	0.3 3	0			
3×2	0.2 5	0.2 8	0.4 8	0.4 2	0.4 2	0.6 0	0.4 2	0.8 0	0.3 3	0.6 6	0.4 2	0.6 6	0.1 4	0		
4×2	0.5 0	0.3 3	0.0 5	0.2 8	0.2 8	0.4 0	0.5 1	0.1 4	0.3 7	0.2 6	0.5 0	0.2 8	0.0 1	0.2 8	0	
4×3	0.5 0	0.5 7	0.3 3	0.5 0	0.7 1	0.4 2	0.3 3	0.8 4	0.5 6	0.4 7	0.7 0	0.7 0	0.4 2	0.5 0	0.3 8	1

Table 5: Values of genetic dimensions of hybrids and inbreed line fall diallel crosses for maize by RAPD technique.

The values of the genetic divergence ranged between 0.01-0.84; the lowest genetic divergence was between 2 and 3 genotypes (0.03), and this is the highest similarity between the studied genotypes, while the most significant genetic divergence was 0.69 and 0.62 between 1 and 3 genotypes as well as 2 and 3 respectively, which is the minor genetic similarity between the two genotypes.

Cluster analysis

Cluster analysis (Fig. 1) was developed for the sixteen genotypes of maize depending on the results of similarity ratio using the UPGMA method, as the studied genotypes were placed under two main parts: The first part included 2

inbreds and seven reciprocal and inverse hybrids, whereas the second part contained three and five reciprocal and inverse hybrids. The 2 and 3 inbreds were the most widely divergent, while 1 and 3 inbreds were the most similar.

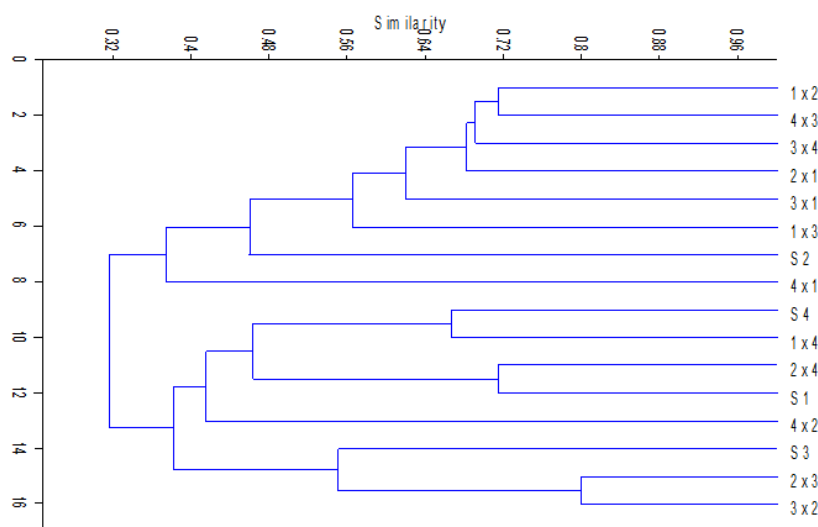


Figure 1: The dendrogram illustrated the four hybrids and inbred line fall diallel cross of maize based on the results of 321 bands from RAPD data using (UPGMA).

Discussion

The differences between parents were reflected in the resulting hybrids. The 3x2 hybrid took the least period for male flowering (55.40 days) with non-significant differences between the three reciprocal and reverse hybrids. Similar results were found by ^{13,14}.

These results agree with ¹⁵, who showed that some inbreds and hybrids were significantly superior in leaf area.

According to the research data, the 4 and 1 inbreds were significantly superior in the weight of 300 grains (89.01 and 87.60 g), respectively. Also, the 2 × 3 reverse hybrid and 3 × 2 reciprocal hybrid gave the highest means of 300-grain weight (99.74 and 98.56 g), respectively, compared with the 2 × 4 reverse hybrid, which gave the lowest mean grain (81.52 g). Similar results were obtained by ^{16,17}.

These results indicate a super-dominance of genes towards the early emergence of female inflorescences in hybrids that gave negative heterosis values. In contrast, the hybrids that gave positive values of heterosis had the effect of genes towards the delay in flowering, especially the partial dominance of genes. These results agree with those who found positive and negative heterosis relative to the lowest and average parents in maize.

The genetic divergence between the parents led to giving the hybrids positive and negative heterosis for the leaf area, as the 8 hybrids showed positive and significant heterosis relative to the best parents, the highest reached 51.23% for the 2 × 3 reverse hybrid. In contrast, the 1 × 4 reverse hybrid gave the lowest heterosis relative to the best parents (-7.43%). The effect of the super-dominance of the genes for the highest parents and the effect of partial dominance for the lowest parents is the reason for the presence of positive and negative values for the heterosis. The results agree with ¹⁶.

The effect of super-dominance of the genes for the best parents and partial dominance of the lowest parents is the reason for the presence of positive and negative heterosis values. Similar results were obtained by⁶.

Regarding the grain yield, the results indicate a positive heterosis relative to the best parents in all hybrids, the highest being 116.01% and 103.32% for the 3×2 reciprocal hybrid and 2×3 reverse hybrid, respectively, which shows the dominance of the super-dominant genes action in the inheritance of the grain yield trait. These results agree with^{6,19}.

It is noted from the same Table that the lowest genetic divergence between hybrids and inbreds was 0.75 in the 1 inbred, and 4 × 2 hybrid can be exploited to produce triple hybrids. In contrast, the highest genetic divergence was 0.84 in the 3 × 2 reciprocal hybrid and 3 × 4 reverse hybrid. This could help the plant breeder to produce double hybrids, while the genetic divergence values for the other genotypes ranged between those values (Table 6). Similar results were obtained by^{15,20,21,22,23}.

Moreover, it is evident from these results that there is a degree of genetic similarity or difference between studied genotypes, and this is due to two reasons; the first is that the difference between the five genotypes may be due to the difference in the phenotypes, and this is the result of the environment influence, which is reliable in many breeding programs. In contrast, the second may result from a high genetic similarity between the genotypes, which contradicts the first, which depends on the phenotypes due to the similarity in the non-coding location of the genes²⁵. The yield components in the genotypes of maize according to the climatic stage and some morphological traits, especially the number of rows per ear and the weight of the grain, can be independently inherited from the environment. Molecular markers may provide information about the history and biology of the genotypes but not necessarily reflect what can be observed in planting. These results agree with many researchers who used molecular markers, especially^{11,16,25,26} RAPD.

Conclusion

We conclude that some inbreds were characterized by high grain yield, and the reciprocal hybrid (A105 × Syn-33) and the reverse hybrid (Syn-33 × A105) were the best as a result of giving the highest grain yield due to their superiority in the yield components, in addition to giving them the highest heterosis based on the best parents and a high genetic divergence characterized them. On the other hand, the traits that were characterized by the highest values of productivity and heterosis can be improved by selection. In contrast, the traits that were characterized by the lowest values can be improved by hybridization.

References

1. Abdulhamed, Z.A.; Abas SA and Abed A.A. Studying some genetics in maize by line × tester analysis. *International Journal of. Agricultural and. Statistical Sciences*. Vol. 16, Supplement 1, pp. **2020a**.1421-1426.
2. Bisen, P.; Dadheech A.; Namrata ON and Meena R.K. Exploitation of heterosis in single cross hybrids of quality protein maize (*Zea mays* L.) for yield and quality traits. *Int. J. of Bio-resource and Stress Management*, **2017a**. 8(1):12-19.
3. McLean- Rodriguez, F.D.; Camacho- Villa TC; Alamekinders C.J.M.; Pe. ME; Dell M. Acqua and Costich D.E. The abandonment of maize landraces over the last 50 years in Morelos, Mexico: a tracing study using a multi-level perspective. *Agric. Human Values*. **2019**. 36: 651-668.

4. Abdulhamed. Z. A., Abas S. A., Noaman A.H. and Abood N.M. Review the role of plant breeding in developing drought-tolerant maize genotypes in Iraq. Interval IOP Conference Series: Earth and Environmental Science, **2021d**, 904(1), .01210.
5. Bisen, P.; Dadheech A.; Namrata, ON and Meena R.K. Exploitation of heterosis in single cross hybrids of quality protein maize (*Zea mays* L.) for yield and cultivars of maize. PhD thesis. College of Agriculture, University of Baghdad, Iraq. AS: **2017b** .130.
6. Abd . MS; Abdulhamed Z.A. and Ghadir MA Response of maize hybrids and inbred to yield and its components under irrigation interval. . IOP. Conf. Seri.:Earth Environ. Sci. . **2021b** .904 01 2003.
7. Abdulhamed, Z.A.; Noaman A.H.; Mohammed Y.A. and Abood N. M. The Genetic Parameters Of Several Oats Cultivars Under The Influence Of The Biofertilizer (Humiforte) Spraying ..*Journal of Physics. Conf. Series* .**2019**. 1294. 092040.
8. Abd H. S.; Abdulhamed Z. A. and Ghadir MA Estimation of genetic parameter by using full diallel cross in maize under different irrigation interval . IOP Conf. Seri.:Earth Environ.Sci. **2020a**. 904 .012054.
9. Farhan, M. B.; Abdulhamed Z.A.; Noaman A.H. and Abod N.M. Determination of genetic distance among bread wheat *Triticum aestivum* L. genotypes, using issr markers. *J. Plant Archives*. **2019**. 19(1): 455-459.
10. Amoon, M.H. and Abdul Hamed Z.A. Determination genetic diversity of inbred lines and hybrids of maize using ISSR technic. *Iraqi J. of Agri. Sci*. **2020**. 51(1) :269-277.
11. Fadel. AA; Abdulhamed Z.A. and Yousif H.A. RAPD technique to determine the genetic divergence of barley genotypes. IOP. Conf. Seri.:Earth Environ. Sci. **2022**. 761 012077.
12. Al-Obaidi, S.S.M. Morphological and Molecular Evaluation of Genetic Variation for Number of Maize *Zea mays* L. Inbred Line. Ph.D. Dissertation. Dept. of Field Crop Sci. College of Agric. Univ. of Anbar. Iraq. pp. **2018**.141.
13. Mukesh, K.M.; Kamalvanshi V.; Kushwaha S. and Sen C. Optimization of Resources use on Irrigated and Rain-Fed Farms of Eastern Uttar Pradesh: Sens Multi-Objective Programming (MOP) METHOD. International. *Journal of Agricultural and Statistical. Sciences*. **2019**. 15 (1). 183-186.
14. Abdulhamed ,Z.A.; Abod N.M. and Noaman A.H. Genetic Path Analysis and Correlation Studies of Yield and Its Components of Some Bread Wheat Varieties. IOP Conf. Ser.: Earth Environ. Sci. **2020**.761 012066.
15. Abdulhamed. Z. A.; Abas S.A. and Kosaj K.I. Genetic and Molecular Variations Using The Molecular Marker RAPD in Barley Yield .Int. Agriculture. Stat. Sci.**2022** .18, Supplement 1.
16. Abdulhamed, Z.A.; Abdulkareem B.M. and Noaman A.H. Efficiency of ISSR markers detect genetic and molecular variation between barley genotypes. International. *Journal of Agricultural and Statistical. Sciences*. **2021b**. 17, Supple. 1.
17. Abdulhamed. Z.A.; Abas S.A.; Noaman A.H. and Abood N.M. Genetic Performance of Inbred and Hybrids of Maize under Irrigation Interval IOP Conference Series: Earth and Environmental Science, **2021c**. 904(1), 012001.
18. Wuhiab, K. M.; Hadi B. H. and W. A. Hassan. Hybrid vigor, heterosis, and genetic parameters in maize by diallel cross analysis. *Inter. J. Appl. Agri. Sci*, **2016**. 2(1): 1-11.
19. Dhoot, M.; Dubey R. B.; Ameta K. D.; Dhoot R.; Komar R. and Badaya V.k. Estimating heterosis for grain yield and architectural traits in yellow seeded maize (*Zea mays* L.). *Int. J. curr. microbio. App. Sci*. **2017**. 6(7): 4536- 4542.
20. Vivodik, M.; Balazova Z.; Galova Z. and Petrovicova L. Genetic diversity analysis of maize (*Zea mays* L.) using (SCoT) markers . *J. Microbiol. Biotechnol. Food Sci* .**2017**. 6: 1170-1173.
21. Dar, T.H.; Shakeel R. and Verma S. Comparative germplasm characterization of maize (*Zea mays* L.) in Rajouri region of PirPanjal Himalaya J& K (India), based on morphological and ISSR Markers. *J. Crop Sci. Biotechnol*. **2018**. 21:43-55.
22. Sadek, M.S.E. and Ibrahim S.D. Genetic relationships among maize inbred lines as revealed by stars codon targeted (SCoT) analysis. *J. Innov. Pharm*. **2018**. 5:103-107.
23. Abdulhamed. Z. A., A.O. Alfalahi. and N.M. Abood. **2020**. Riboflavin and cultivars affecting genetic parameters in maize (*Zea mays* L.). AIP Conference proceedings. 2290.020020. <https://doi.org/10.1063/5.0027367>

24. Soliman. ERS; EL-Shazly H.H.; Borner A. and Badr A. Genetic diversity of a global collection of maize genetic resources in relation to their subspecies assignments, geographic origin, and drought tolerance. *J. Breeding Science* **2021**. 71:313-325.
25. Baktash. FA; and Abdel Al-Hameed Z.A. Molecular variation between of maize inbreds. *The Iraqi Journal of Agriculture Sciences*. **2015**. 46(3):291-299.
26. Badr, A.; HH; El-Shazly, R.A. Tarawneh and A. Borner Screen for drought tolerance in maize (*Zea mays* L.) Germplasm using germination and seedling traits under simulated drought conditions. *J. Plants*. **2020**. 9:565.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023

Citation: Abdulhamed, Z.A.; Abdulkareem, B.M.; Noaman, A.H. Role of Molecular Marker RAPD in Determining the Genetic Divergence between Hybrids and Inbred Lines of Maize Using Full Diallel Cross. *Revis Bionatura* 2023;8 (3) 68. <http://dx.doi.org/10.21931/RB/CSS/2023.08.03.68>