GENETIC DISTANCE OF INBRED LINES AND PREDICTION OF MAIZE SINGLE-CROSS PERFORMANCE USING RAPD AND SSR MARKERS

A. A. EL-HOSARY[1], M. EL. M. EL-BADAWY[1] AND Y. M. ABDEL-TAWAB[2]

1. *Agron. Dept., Fac. of Agric. Moshtohor, Benha Univ., Egypt*
2. *Seed Technology Dept., Field Crops Research Institute, Agric. Research Center, Giza, Egypt*

Maize (*Zea mays* L.) is considered as the third cereal crop after rice and wheat all over the world for production and consumption. In addition to its use as a human food, it is also utilized as a poultry and livestock feed and also as a fodder. Maize breeding programs are based on the development and selection of outstanding hybrids from inbred lines. Developing and selecting inbred lines for *per se* performance is quite easy, although time consuming. It is not possible to predict hybrid performance from inbred parent performance because of the high level of dominance for the grain-yield trait. Thus, hybrid performance is evaluated from extensive yield traits that are costly and time consuming. Moreover, the large number of possible hybrids produced from a relatively small number of inbred parents does not allow the evaluation of all hybrids (Smith, 1986; Hallauer *et al.*, 1988; Hallauer, 1990; Bernardo, 1994; Lanza *et al.*, 1997). The use of genetic markers to assess the genetic divergence among pairs of inbred lines has been suggested as a means to over-come these drawbacks, allowing the prediction of single-cross hybrid performance. Random amplified polymorphic DNA, RAPD, is a class of genetic markers (Welsh and McClelland 1990; Williams *et al.*, 1990). This technique uses the polymerase chain reaction (PCR) to generate random amplified fragments of DNA with 10-mer primers. The advantages of RAPD markers include the ease and rapidity of analysis, the availability of a large number of primers and the very small amount of DNA required for analysis (Welsh and McClelland, 1990; Williams *et al.*, 1990). Simple sequence repeats, SSR, is another type of molecular markers (Davies, 1993). Senior and Heun (1993) have reported that SSR loci provide a high level of polymorphism in maize. SSR analysis presents the potential advantages of reliability, reproducibility, discrimination; standardization and cost-effectiveness over RFLP analysis (Smith *et al.*, 1997). Senior *et al.* (1998) have reported that SSR analysis using high quality agarose gels can conveniently assess the genetic diversity of maize inbred lines.

The objectives of our study were (1) to estimate general and specific

Egypt. J. Genet. Cytol., 35:209-224 , July, 2006.

combining ability and their interaction with sowing dates and to find out the best parental lines and the prospective single crosses to be used in hybrid maize breeding programs, (2) to distinguish among the maize inbred lines using RAPD and SSR analysis, (3) to evaluate the genetic divergence of maize inbred lines, and (4) correlate single-cross performance and heterosis to the genetic divergence of the parental lines.

MATERIALS AND METHODS

Field experiments

Eight inbred lines were used as parents in this study. Moshtohor P_1 (102-G), P_2 (313-B), P_3 (210-2), P_4 (2060), P_5 (102), P_6 (1200), P_7 (302-1) and P_8 (T2) were developed at the Department of Agronomy, Fac. of Agric. at Moshtohor, Benha Univ. by Prof. Dr. A. A. M. El-Hosary. In 2004 season, the eight inbred lines were split planted in May 15th, 25th and June 5th to avoid differences in flowering time and to secure enough hybrid seeds. A half diallel set of crosses was carried out. In 2005 season, two experiments were undertaken in two different planting dates (May 18th and June 12th) at the Agricultural Research and Experimental Station of the Fac. of Agric., Moshtohor. Each experiment included the 28 crosses along with S.C.10 (check variety). A randomized complete block design with three replications was used. Each plot consisted of two ridges of five meters length and 70 cm width. Hills were spaced at 25 cm with three kernels per hill on one side of the ridge. The seedlings were thinned to one plant per hill. The cultural practices were followed as usual for ordinary maize field in the area. Random sample of 20 guarded plants in each plot were taken to evaluate silking and tasseling dates (days) in 50% of the plant silked or tasseled, plant height (cm), ear height (cm), maturity date (days) in physiological matured, ear husk, no. of ears/plant ear length (cm), ear diameter (cm), no. of kernels/row, no. of rows/ear, ear weight, 100-kernel weight and grain yield/plant which was adjusted for 15.5% moisture.

DNA extraction

Leaf tissue from each genotype was collected from 5-7 days old germinated seedlings. Equal quantities of leaf tissue from eight seedlings of each line were bulked, lyophilized, and ground with a mortar. Genomic DNA was isolated and extracted using the mi-Plant Genomic DNA Isolation Kit (Metabion).

RAPD-PCR

Amplifications were conducted with 10-mer primers from Operon Technologies Inc. (Alameda, Calif., USA). All PCR reactions were performed as reported by Williams *et al.* (1990), with minor modifications, using 25 ng of DNA. Controls were made by replacing DNA with water. Reaction mixtures (25 µl) contained 0.2 µM of primer, 2.0 units of Taq DNA polymerase, 2.5 µl of 10 x supplied buffer, 0.2 mM of each dNTP, and 2.5 mM of $MgCl_2$. The amplifications were carried out in a PTC 200 DNA

Thermal Cycler. DNA denaturation was done at 94°C for 4min., followed by 36-cycle amplification (94°C, 30 sec.; 36°C, 1 min.; 72°C, 2 min.) and by a final extension step at 72°C for 10min. Amplification products were separated by electrophoresis on 1.2% agarose gels, stained with ethidium bromide, and photographed under UV light.

SSR-PCR

Based on chromosome loci, we chose 10 SSR primers from maize DB and assayed their discriminatory power. The reactions were carried out in a DNA Thermal Cycler (PTC 200). Each 25 µl PCR reaction consisted of 2.5x PCR buffer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2 units of Taq DNA polymerase, 1 µl of each primer, and 3 µl (25 ng) of DNA. The amplification conditions were 94°C for 4min; 36 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min; and a terminal extension step at 72°C for 10 min. Amplification products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and photographed under UV light. DNA fragment sizes were determined by comparisons with the 100 bp + 1.5 kb DNA Ladder.

Data analysis

The experimental obtained data was statistically analyzed for analysis of variance using computer statistical program MSTAT-C. General and specific combining ability estimates were estimated according to Griffing (1956) diallel cross analysis designated as method 4 model I for each experiment. The combined analysis of the two experiments was carried out whenever homogeneity of variance was detected (Gomez and Gomez, 1984). Heterosis was expressed as the percentage deviation of the F_1 mean performance from S.C.10 mean. The obtained data of SSR and RAPD analysis were entered in a computer file as binary matrices where 0 stands for the absence of a band and 1 stands for the presence of a band in each individual sample. Similarity coefficients between a pair of inbred lines were calculated according to Jaccard (1908). A dendrogram tree was constructed by the UPGMA clustering algorithm from the SAHN option of NTSYS-PC version 2.1(Rohlf, 2000).

RESULTS AND DISCUSSION

The analysis of variance for ordinary analysis over the two experiments for all traits is given in Table (1). Planting dates mean squares were significant for all traits except ear husk, ear length, ear diameter, number of rows/ear and number of kernels/row. Moreover, the mean performance of all traits was much higher in the early planting date than that of the late planting date. The high mean performance in the early planting date may be due to the prevailing of favorable environmental conditions. Therefore, first planting date seemed to be non-stress environment. Such results are in good agreement with those reported by Frey and Maldonado

(1967), Roy and Murty (1970), Nawar and Khamis (1983), El-Hosary (1988) and Sedhom (1994).

Hybrid mean squares were highly significant for all traits. Significant hybrids by planting dates mean squares were detected for all traits except for no. of rows/ear. Such result indicated that the performance of genotypes differed from one planting date to anther. For the exceptional trait (no. of rows/ear), insignificant interaction between hybrids and planting dates was obtained revealing that the response of hybrids had nearly similar in magnitude at two the planting dates.

The mean square associated with general and specific combining ability was significant for all traits except ear diameter and ear length, respectively, revealing that both additive and non-additive types of gene action were involved in determining the performance of single-cross progeny. To determine the genetic effects of greatest importance, GCA/SCA ratio was computed. With the exception of ear diameter, high values which largely exceed the unity were detected, indicating that the largest part of the total genetic variability associated with these traits was a result of additive and additive by additive types of gene action. For ear diameter, however, it showed the lowest GCA/SCA ratio, indicating that greatest role of the non-additive type of gene action in the expression of this trait (Table 1). Significant interaction mean square between planting dates and general combining ability was detected for tasseling, silking and maturity dates, plan and ear heights, ear length, no. o kernels/row, ear weight and grai yield/plant. Whereas, significant interac tion mean squares between planting date and SCA were obtained for tasselin silking and maturity dates, plant and ea heights, ear husk, no. of ears/plant, no. o kernels/row, ear weight, 100-kerna weight and grain yield/plant.

It is fairly evident that ratios fo SCA X planting dates/SCA was muc higher than ratios of GCA X plantin dates/GCA for all traits except no. o kernels/row. Such results indicated tha non additive effects were much mor influenced by the different planting date than the additive genetic ones in thes traits under test. This conclusion is in we agreement with that reported by Gilbe (1958). For the exceptional case the rati of GCA X planting dates/GCA was highe than ratio of SCA X planting dates/SC revealing that additive and additive additive types of gene effects were muc more influenced by planting dates (Tabl 1).

Estimates of general combinin ability effects ($\hat{g}_i$) for individual inbre lines over two experiments are presente in Table (2). High positive values woul be of interest for all traits in questio except; silking, tasseling and maturit dates, plant and ear heights, where hig negative ones would be useful from th breeder point of view. The parental inbre line P_1 behaved as the best combiner fo tasseling, silking and maturity dates, an

plant and ear heights, meanwhile it was on the average in the rest traits. The parental inbred lines P_2 and P_7 gave poor combiners for all the studied traits.

Moshtohor P_1 seemed to be good combiner for; tasseling, silking and maturity dates, plant and ear heights and ear husk. On the contrarily, it expressed either significantly negative or non appreciable positive $\hat{g}_i$ effects for the rest traits. The parental line P_4 expressed good combiner for no. of ears/plant and no. of rows/ear. The inbred line P_5 appeared to be one of the good combiner for; maturity date, ear husk, number of ears/plant, no. of kernels/row, ear weight and grain yield/plant. The parental inbred line P_6 gave the desirable $\hat{g}_i$ effects for; tasseling and maturity dates, ear weight/plant and grain yield/plant, meanwhile, it was on the average for other traits. Also, the inbred line P8 expressed good combiner for tasseling, silking dates and ear husk. These results indicated that these parental inbred lines possess favourable genes and that improvement in yield may be attained if they are used in a hybridization program.

Specific combining ability effects (S_{ij}) for the studied twenty-eight combinations were computed for all the studied traits (Table 3). The most desirable inter- and intra-allelic interactions were presented by the combinations; P_1xP_7, P_1xP_8, P_2xP_6, P_3xP_6, P_4xP_5 and P_4xP_8 for tasseling date, P_1xP_7, P_1xP_8, P_2xP_6, P_2xP_7, P_3xP_6, P_4xP_5, P_4xP_8 and P_5xP_8 for silking date, P_1xP_7, P_2xP_4, P_3xP_8 and P_4xP_8 for maturity date; P_1xP_5, P_2xP_6, P_2xP_8, P_3xP_5, P_3xP_8, P_4xP_6 and P_5xP_7 for plant and ear heights, P_1xP_3, P_1xP_8, P_2xP_3, P_2xP_6, P_2xP_7, P_3xP_5, P_4xP_5, P_5xP_8 and P_6xP_7, for ear husk, P2xP4, P_2xP_5, P_4xP_8, P_5xP_6 and P_6xP_8 for no. of ears/plant, P_2xP_6, P_5xP_8 and P_7xP_8 for no. of kernels/row, P_2xP_4 and P_5xP_8 for 100-kernel weight and P_1xP_7, P_2xP_5, P_3xP_6, P_4xP_8 and P_6xP_8 for ear weight and grain yield/plant, also, the five combinations expressed significant heterotic effects relative to S.C.10 mean value for grain yield over two experiments (Table 4). The cross P_2xP_5 had the highest values for both SCA and heterotic effects followed by crosses P_5xP_6, P_6xP_8, P_4xP_8. These hybrids surpassed the check hybrid with average value (22.69%). Hence it could be concluded that these crosses offer good possibility for improving grain yield of maize. Also, the most considerable heterosis was generally detected from combinations involving parental inbred lines that are very diverse in origin and widely different in their mean performance (Table 4). The average of heterotic effects ranged from 32.27% to -24.44%. Also, the crosses P_1xP_2, P_1xP_6, P_1xP_7, P_2xP_4, P_2xP_6, P_2xP_7, P_3xP_4, P_3xP_5, P_3xP_6, P_4xP_7, P_5xP_7, P_5xP_8, P_6xP_7 and P_7xP_8 had insignificant useful heterotic effects in these crosses revealed that a hybrid program based in these material may be useful for testing under different locations and years. Many investigators reported high heterosis for yield of maize (Nawar *et al.*, 1992, Mohamed, 1993, Sedhom, 1994; El-Hosary and El-Badawy, 2005).

Hybrid performance

The mean performances of the 28 hybrids and S.C.10 for all of the studied traits in the combined analysis of the two planting dates are presented in Table (4). For tasseling and silking dates, the two crosses P_1xP_8 and P_3xP_6 gave the earliest hybrids in the combined analysis of the two planting dates. However, the crosses P_1xP_7 and P_3xP_8 for maturity date. Earliness, if found in corn, is favourable for escaping destractive injuries caused by (*Sesami cretica* led), (*Chilo agamemnon* Blės) and (*Purausta nubilalis* Hub).

For plant height, the crosses P_1xP_5, P_1xP_7 and P_3xP_5 gave the lowest values. While, the crosses P_1xP_5, P_3xP_5 and P_3xP_8 expressed the lowest values for ear height. For ear husk, the average ranged from 6.66 (P_1xP_3) to 3.33 (P_1xP_2). The most crosses surpassed the check hybrid S.C.10 for this trait.

Regarding ear length and ear diameter, the cross P_5xP_6 had the lowest mean value. For no. of ears/plant, the crosses P_2xP_4 and P_5xP_6 showed the highest values, while the crosses P_1xP_8, P_2xP_6, P_3xP_8, P_5xP_8 and the check hybrid S.C.10 gave the lowest no. of ears/plant. Concerning no. of rows/ear, the cross P_4xP_6 gave the highest value, but without significant superiority over the crosses P_1xP_2, P_3xP_4 and P_4xP_8. For no. of kernels/row, the average ranged from 47.37 (P_5xP_8) to 38.02 (P_2xP_8). Regarding ear weight and grain yield/plant, the crosses P_2xP_5 and P_5xP_6 significantly surpassed the highest mean values. While, the cross P_4xP_5 gave the lowest value fo 100-kernel weight.

RAPD-PCR analysis

Twelve 10-mer primers were pre screened for the ability to detec polymorphism in eight maize inbred line The five primers that presented the highe degree of polymorphism were selecte (Table 5). These primers produced a tot of 24 reproducible bands, from which 2 (91.7%) were polymorphic. An average o 4.8 bands per primer was obtained, range from 156 to 1086 bp. The least number o polymorphic bands was detected fo primer A11 (zero out of 1 amplifie band), while the largest number o polymorphic bands was detected fo primers B06 (7 out of 7 amplified bands and B07 (7 out of 7 amplified bands) (Fig 1). However, 5 bands were commo (monomorphic) for all genotypes.

The specific markers for maiz inbred lines are shown in Tables (5 and 6) Thirteen out of 24 (54.2%) bands wer found to be useful as genotype-specifi markers. The largest number of specifi markers was scored for inbred lines 3, and 5 (3 markers), while the lowe number (1 marker) was scored for inbre lines 2 and 6. A number of 11 positiv specific markers were scored for th presence of unique bands for a give genotype, while 2 negative specifi markers were scored for the absence of common band. In the meantime, th largest number of genotype-specifi markers was generated by primer B01 (markers), while the primer A11 does no

produce any specific markers. The primers B07 and B12 generated the least number of specific markers (2 markers). In conclusion, 4 out of 5 primers used allowed high distinction among the genotypes.

SSR analysis

Ten SSR primers produced 24 alleles among eight maize inbred lines, and the allele number for the SSR loci ranged from 1 to 6, with the mean allele being 2.4, and the PIC values for the SSR loci ranged from 0.15 to 0.83 for the primers phi069 and nc003, respectively, (Fig. 2), with the mean PIC being 0.31 (Table 7). The mean PIC value in this study was lower than that determined by Smith *et al.* (1997) (0.62), Senior *et al.* (1998) (0.59) and Enoki *et al.* (2002) (0.69). This probably resulted from using low number of SSR primers and that gave low number of alleles and low discriminatory power. Di-repeat SSR loci gave a higher mean allele (3) and mean PIC (0.46), and tri-, tetra-repeat SSR loci gave lower mean allele (2-1.5) and mean PIC (0.08). The highest mean PIC value of the di-repeat SSR loci is consistent with the results obtained before by Smith *et al.* (1997), Senior *et al.* (1998) and Enoki *et al.* (2002).

Genetic similarity

The results of genetic similarity are shown in (Table 8). The lowest genetic similarity (0.47) was observed between the two inbred lines 4 and 6, while the highest genetic similarity (0.77) was scored between the two inbred lines 2 and 7. Cluster analysis classified the 8 inbred lines into three main clusters (Fig. 3). The first main cluster consisted of four inbred lines (P_1, P_2, P_7 and P_4), while the second main cluster included the three inbred lines (P_3, P_6 and P_8) and the third cluster included one inbred line (P5). The first main cluster subdivided into two sub-clusters. One of them included the inbred line P_1 only, while the other combined the two inbred lines 2 and 7 with a genetic similarity (0.77). The second main cluster subdivided into subclusters, also. One of them included the inbred line P_3 only, while the other included the two inbred lines P_6 and P_8 with a genetic similarity (0.72).

Genetic distances and single cross performance

The relationship of average grain yield and genetic distances GD was estimated, the correlation coefficients produced low value (0.07) between for all hybrids meanwhile, the correlation coefficient between cluster 1 (inbred lines 1, 2 and 7) and cluster 3 (inbred line 5) was (0.33) moderately. The high of grain yield and heterosis was 240.7 and 32.27, respectively, produced from crossed between inbred line 5 (cluster 3) and inbred line 2 (cluster 1). Also, the cross between inbred line 5 (cluster 3) and inbred line 4 gave the third high hybrid for grain yield and specific combining ability and heterosis. Meanwhile, the cross between inbred line 5 (cluster 3) and inbred line 6 (cluster 2) had second high

hybrid for grain yield and heterosis. On the contrary, in some hybrids between high genetic distances had the lower grain yield or heterosis. A particular use of genetic markers is the prediction of hybrid performance. Although genetic distances between parents were significantly related with hybrid performance, the estimates of GD did not consistently identify the best crosses. This is similar to results already published by Godshalk *et al.* (1990), Melchinger *et al.* (1990) and Ajmone Marsan *et al.* (1998).

There are many potential reasons for the finding of low correlation between GD and hybrid performance. One is that linkage should exist between genes controlling that trait measured and the markers used to estimate genetic distances in order for high correlations to occur (Melchinger *et al.*, 1990; Bernardo, 1992; Ajmone Marsan *et al.*, 1998). In addition, some of the marked chromosome regions could be more important than others in their contribution to F1 yield performance and heterosis. From this point of view, current investigations designed to map QTLs affecting grain yield and related traits confirm that the magnitude of genetic effects for any single QTL contributing to these traits considerable, ranged from 5 to 25% of the phenotypic variance (Stuber *et al.*, 1992; Ajmone Marsan *et al.*, 1995). Inadequate genome coverage and different levels of dominance are other reasons suggested for the low correlation between GD and hybrid performance. Ajmone Marsan *et al.* (1998) suggested that AFLPs are able to detect a larger number of polymorphisms in a more efficient was in comparison to RFLPs or SSRs due to the much higher number of loci assayed in a single multiplex PCR reaction.

The results demonstrated low or moderate correlation between RAPDs and SSRs based genetic distance and maize single cross grain yield, using inbred lines white maize germplasm. The results assess the potentiality of the RAPD and SSR technology for characterizing at the molecular level and for generating unique fingerprint for each inbred lines. This could have great impact in plant improving programs, particularly, of important crops such as maize.

SUMMARY

The Half diallel cross between eight inbred lines of maize (*Zea mays* L.) were evaluated under two different planting (D) dates, for 14 quantitative characters. Planting dates mean squares were significant for most traits. Hybrid mean squares were highly significant for all traits. Significant hybrids by planting dates mean square values were detected for most traits. The mean square associated with general and specific combining ability was significant for all traits except ear diameter and ear length, respectively. The magnitudes of the ratios of GCA/SCA revealed that the additive and additive x additive types of gene action were the most important expressions for most traits. Significant interaction mean square between planting dates and both types of combining ability were

detected for most traits. The ratios for SCA x D/SCA were much higher than ratios of GCA x D/GCA for all traits except no. of kernels/row. The inbred line P_5 appeared to be one of the good combiner for; maturity date, ear husk, number of ears/plant, no. of kernels/row, ear weight and grain yield/plant. The cross P_2xP_5 had the highest values for both SCA and heterotic effects followed by crosses P_5xP_6, P_6xP_8, P_4xP_8 for grain yield. These hybrids surpassed the check variety with average value (22.69%). Five random arbitrary primers used were generated a total of 24 bands, from which 22 (91.7%) were polymorphic. Thirteen out of 24 (54.2%) bands were found to be useful as genotype-specific markers. Ten SSR primers produced 24 alleles among eight maize inbred lines, and the allele number for the SSR loci ranged from 1 to 6, with the mean allele being 2.4, and the PIC values for the SSR loci ranged from 0.15 to 0.83, with the mean PIC being 0.31. The lowest genetic similarity (0.47) was observed between the two inbred lines 4 and 6, while the highest genetic similarity (0.77) was scored between the two inbred lines 2 and 7. The correlation coefficient between cluster 1 (inbred lines 1, 2 and 7) and cluster 3 (inbred line 5) was (0.33) moderately. The high of grain yield and heterosis was 240.7 and 32.27, respectively, produced from crossed between inbred line 5 (cluster 3) and inbred line 2 (cluster1). Also, the cross between inbred line 5 (cluster 3) and inbred line 4 gave the third high hybrid for grain yield and specific combining ability and heterosis. The results assess the potentiality of the RAPD and SSR technology for characterizing at the molecular level and for generating unique fingerprint for each inbred lines. This could have great impact in plant improving programs, particularly, of important crops such as maize.

REFERENCES

Ajmone Marsan, P.; P. Castigjoni; F. Fusari; M. Kuiper and M. Motto (1998). Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet., 96: 219-227.

Ajmone Marsan, P.; G. Monfredini; W.F. Ludwing; A.E. Melchinger; P. Franceschini; G. Pagnotto and M. Motto (1995). In an elite cross of maize, a major quantitative traits locus controls one-fourth of the genetic variation for grain yield. Theor. Appl. Genet., 90: 415-424.

Bernardo, R. (1992). Relationship between single-cross performance and molecular marker heterozygosity. Theor. Appl. Genet., 83: 628-634.

Bernardo, R. (1994). Prediction of single-cross performance using RFLPs and information from related hybrids. Crop Sci., 34: 20-25.

Davies, K. (1993). Of mice and men (and cows and cats). Nature (London), 361: 478.

El-Hosary, A. A. (1988). Heterosis and combining ability of ten maize inbred lines as determined by diallel crossing over two planting dates. Egypt. J. Agron., 13: 13-26.

El-Hosary, A. A. and E. El. M. El-Badawy (2005). Heterosis and combining ability in yellow corn (*Zea mays* L.) under two nitrogen levels. The 11th Conference of Agronomy, Agron. Dept., Fac. Agric., Univ., Nov. 15-16, 2005: 89-98.

Enoki, H.; H. Sato and K. Koinuma (2002). SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theor. Appl. Genet., 104: 1270-1277.

Frey, K. J. and U. Maldonado (1967). Relative productivity of homogenous and heterogeneous oat cultivars in optimum and sub-optimum environments. Crop Sci., 7: 532-535.

Gilbert, N. E. G. (1958). Diallel cross in plant breeding. Heredity, 12: 470-492.

Gomez, K. N. and A. A. Gomez (1984). Statistical Procedures for Agricultural Research. John.Wiley and Sons. Inc., New York, 2nd ed.

Godshalk, E.B.; M. Lee and K.R. Lamkey (1990). Relationship of restriction fragment length polymorphisms to single-cross hybrid performance of maize. Theor. Appl. Genet., 80: 273-280.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Aus. J. Biol. Sci., 9: 463-493.

Hallauer, A. R. (1990). Methods used in developing maize inbreds. Maydica, 35: 1-16.

Hallauer, A. R.; W. A. Russel and K.R. Lamkey (1988). Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and Corn Improvement. American Society of Agronomy, Inc Madison Wisconsin, USA, 3rd ed, pp 463-564.

Jaccard, P. (1908). Nouvelles researchers sur la distribution florale. Bull. Soc. Vaudoise Sci. Nat., 44:223-270.

Lanza, L. L. B.; C. L. de Souza Jr; L. M. M. Ottoboni; M. L. C. Vieira and A. P. de Souza (1997). Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theor. Appl. Genet., 94: 1023-1030.

Mohamed, A. A. (1993). Effect of nitrogen fertilization levels on the performance and combining ability of maize hybrids. (*Zea mays* L.). Ann. of Agric. Sci. Ain shams Univ. Cairo, 38: 531-549.

Melchinger, A. E.; M. Lee; K. R. Lamkey; A. R. Hallauer and W. L. Woodman (1990). Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. Theor. Appl. Genet., 80: 488-496.

Nawar, A. A.; A. N. M. Khalil and Sh. A. El-Shaarka (1992). Heterosis and genetic effects of maize grain yield under three nitrogen fertilizer rates. Minofiya, J. Agric. Res., 17: 1753-1762.

Nawar, A. A. and M. N. Khamis (1983). Influence of date of planting of the expression of general and specific combining ability in maize. Minofiya, J. Agric. Res., 7: 85-100.

Rohlf, F. J. (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1 Exeter Software, Setauket, N.Y.

Roy, N. N. and B. R. Murty (1970). A selection procedure in wheat for stress environment. Euph., 19: 509-521.

Sedhom, S. A. (1994). Genetic analysis of diallel crosses in maize (*Zea mays* L.) over two years. Ann. Agric. Sci. Moshtohor, 32: 9107.

Senior, M. L. and M. Heun (1993). Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome, 36: 884-889.

Senior, M. L.; M. M. Murohy; M. M. Goodman and C. W. Stuber (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci., 38: 1088-1098.

Smith, O. S. (1986). Covariance between line *per se* and testcross performance. Crop Sci., 26: 540-543.

Smith, J. S. C.; E. C. L. Chin; H. Shu; O. S. Smith; S. J. Wall; M. L. Senior; S. E. Mitchell; S. Kresovitch and J. Ziegle (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor. Appl. Genet., 95: 163-173.

Stuber, C. W.; S. E. Lineoln; D. W. Wolff; T. Helentjaris and E. S. Lander (1992). Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics, 132: 823-839.

Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrarily primers. Nucleic Acid Res., 18: 7213-7218.

Williams, J. K. F.; A. R. Kubelik; K. G. Livak; J. A. Rafalki and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18: 6531-6535.

Table (1): Mean squares and combining ability for the studied traits over the two planting dates.

Trait / S.O.V	d.f.	Tasseling date	Silking date	Plant height	Ear height	Maturity date	Ear husk	Ear length (cm)	Ear diameter (cm)	No. of ears/ plant	No. of rows/ ear	No. of kernels /row	Ear weight /plant (g)	100-Kernel weight	Grain yield/plant (g)
Dates	1	3565.93**	3375.05**	7647.75**	14180.91**	6562.50**	0.02	1.99	0.11	0.38**	0.04	24.20	33031.68**	50.38**	22498.33**
Blocks/D	4	1.53	6.71**	271.64*	72.11	5.32	2.88**	0.88	0.25	0.004	0.53	9.29	456.19	4.68	283.52
Hybrid	27	39.70**	42.07**	2292.62**	1256.25**	40.59**	5.84**	4.27**	0.23*	0.04**	2.11**	35.25**	5447.66**	12.23**	4176.27**
Hybrid x D	108	1.47	42.13**	760.37**	828.87**	19.13**	0.72**	3.21*	0.21*	0.004	0.60	25.91**	2032.18**	6.00*	1610.16**
Error	7	28.96**	28.09**	1547.49**	693.60**	27.20**	1.32**	2.71**	0.08*	0.01**	0.25	5.83**	671.16**	2.02*	63.27
G.C.A	20	7.73**	9.10**	490.06**	322.55**	8.75**	0.16	1.36**	0.07	0.002	0.19	2.13	85.18	1.13	1.82
S.C.A	7	25.11**	19.76**	238.79**	373.55**	9.50**	0.26**	0.97	0.07	0.002	0.17	1.92	2.17	2.07	0.25
G.C.A x D	20	14.09**	12.04**	258.59**	242.25**	5.28**	0.11	0.62	0.04	0.88	1.45	3.89	0.23	0.30	0.47
S.C.A x D	108	0.49	0.28	30.77	10.63	0.90	2.82	2.79	1.0	0.10	0.16	0.91	0.48	0.63	
Error		3.75	3.09	3.16	2.15	3.11	0.04	0.51	1.0	0.90	1.32	0.46	0.61		
G.C.A/S.C.A		0.86	0.70	0.15	0.54	0.34	0.20	0.90	1.0	1.32					
GCAxD/GCA		1.82	1.32	0.53	0.75	0.60									
SCAxD/SCA															

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Table (2): General combining ability effects for all the studied traits over the two planting dates.

Trait / Inbred line	Tasseling date	Silking date	Plant height	Ear height	Maturity date	Ear husk	Ear length (cm)	Ear diameter (cm)	No. of ears/ plant	No. of rows/ ear	No. of kernels / row	Ear weight/ plant (g)	100-Kernel weight	Grain yield/plant (g)
P_1	-2.00**	-2.26**	-17.92**	-12.16**	-1.39**	0.17	0.37	-0.10	-0.05**	0.10	-1.20	-20.52**	0.19	-17.52**
P_2	2.36**	1.97**	9.19**	2.24	1.31**	-0.72**	0.40	-0.08	0.003	0.12	-0.65	-0.47	0.72	1.15
P_3	-1.37**	-1.37**	-16.42**	-11.39**	-1.67**	0.83**	-0.13	-0.02	-0.05**	-0.02	-0.83	-12.87**	0.17	-13.89**
P_4	1.39**	1.22**	6.90**	-0.34	2.78**	-0.25*	-0.84**	0.03	0.05**	0.65**	-0.43	-2.96	-1.58**	1.66
P_5	0.06	0.05	5.00*	3.74	-0.81*	0.28*	0.58	0.03	0.03*	-0.29	2.37**	29.52**	-0.28	24.14**
P_6	-0.72**	-0.20	2.80	5.81**	-0.89*	-0.47**	0.07	0.15	0.02	0.16	0.21	14.88**	0.78	10.30**
P_7	1.36**	1.69**	-1.57	5.38**	0.14	-0.44**	-0.47	-0.01	0.02	-0.57**	-0.75	-8.48*	-0.19	-5.10
P_8	-1.11**	-1.09**	12.02**	6.73**	0.53	0.61**	0.02	0.002	-0.02	-0.17	1.28*	0.90	0.19	-0.75
LSD5%(gi)	0.53	0.40	4.21	2.48	0.72	0.25	0.60	NS	0.03	0.31	1.11	7.01	0.81	6.04
LSD1%(gi)	0.70	0.54	5.59	3.29	0.95	0.33	0.79	NS	0.04	0.42	1.47	9.30	1.07	8.01
LSD5%(gi-gi)	0.80	0.61	6.37	3.74	1.09	0.38	0.90	NS	0.05	0.47	1.67	10.60	1.22	9.13
LSD1%(gi-gi)	1.07	0.81	8.45	4.97	1.44	0.51	1.20	NS	0.06	0.63	2.22	14.06	1.62	12.11

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Table (3): Specific combining ability effects for all the studied traits over the two planting dates.

Trait / Hybrid	Tasseling date	Silking date	Plant height	Ear height	Maturity date	Ear husk	Ear length (cm)	Ear diameter (cm)	No. of Ears/plant	No. of rows/ear	No. of kernels/ row	Ear weight/ plant (g)	100-Kernel weight	Grain yield/plant (g)
$P_1 \times P_2$	1.97**	2.29**	17.73**	15.28**	0.30	-0.85**	0.39	-0.05	0.04	0.62	2.03	14.99	1.18	11.68
$P_1 \times P_3$	2.00**	1.79**	15.67**	7.58**	-0.23	0.93**	0.44	0.01	0.02	-0.28	1.18	3.99	-0.77	4.46
$P_1 \times P_4$	0.94	1.04*	-1.65	-4.63	1.16	-0.32	-0.66	-0.05	-0.08*	-0.31	0.80	-11.29	0.15	-10.92
$P_1 \times P_5$	1.11	1.37**	-21.75**	-12.22**	0.74	-0.52	-1.23	-0.20	-0.02	0.08	-0.65	-30.77**	-2.49**	-24.56**
$P_1 \times P_6$	-0.11	-0.88	-4.22	-5.95*	-1.01	-0.43	0.70	0.08	0.02	-0.29	-1.40	10.77	1.46	11.43
$P_1 \times P_7$	-4.03**	-3.27**	-5.68	-7.02*	-4.37**	0.21	0.51	0.33	0.05	0.08	-1.22	28.60**	0.43	20.80**
$P_1 \times P_8$	-1.89**	-2.33**	-0.11	6.96*	3.41**	0.98**	-0.15	-0.12	-0.04	0.11	-0.74	-16.29*	0.04	-12.90
$P_2 \times P_3$	-0.69	-0.10	2.23	16.09**	0.41	0.82**	-0.92	-0.02	0.04	-0.02	-0.16	-7.53	-0.13	-4.80
$P_2 \times P_4$	0.25	-0.35	-4.43	-3.54	-2.20*	0.07	0.46	0.01	0.10*	-0.54	-0.68	1.46	1.96*	2.90
$P_2 \times P_5$	-0.92	-0.85	23.81**	6.71*	-0.12	-0.46	0.53	0.30	0.10*	0.57	-1.35	39.11**	-0.02	37.92**
$P_2 \times P_6$	-2.64**	-3.27**	-9.50*	-9.52**	0.30	0.79**	0.53	-0.16	-0.13**	0.10	3.07*	-7.50	-0.74	-9.44
$P_2 \times P_7$	-0.39	-1.33**	0.38	0.08	0.27	0.60*	-0.50	0.06	-0.10*	0.28	2.33	5.68	-0.77	2.06
$P_2 \times P_8$	2.42**	3.62**	-30.22**	-25.11**	1.05	-0.96**	-0.49	-0.13	-0.05	-1.00**	-5.24**	-46.21**	-1.49	-40.33**
$P_3 \times P_4$	1.44*	1.15*	4.18	5.76*	3.44**	-0.99**	0.68	0.18	-0.01	0.02	0.82	5.07	0.68	8.14
$P_3 \times P_5$	0.11	-0.19	-23.75**	-18.58**	-0.31	0.65*	-0.14	-0.19	-0.01	0.28	-1.03	-21.14**	-0.96	-21.67**
$P_3 \times P_6$	-2.94**	-2.60**	3.62	0.60	-0.40	-0.93**	0.57	0.05	-0.02	0.24	0.07	24.06**	0.65	25.48**
$P_3 \times P_7$	0.14*	-0.66	7.99	5.87	1.24	-0.79**	0.50	-0.18	0.02	-0.51	-0.60	-7.42	0.96	-11.00
$P_3 \times P_8$	-0.06	0.62	-9.94*	-17.31**	-4.15**	0.32	-0.12	0.16	-0.04	0.27	-0.28	2.97	-0.43	-0.62
$P_4 \times P_5$	-1.61*	-1.60**	8.43	9.13**	-0.92	0.90**	0.15	0.21	-0.06	0.05	0.26	4.00	0.12	2.61
$P_4 \times P_6$	1.00	0.65	-21.37**	-13.61**	-1.51	0.15	-0.21	0.15	-0.06	0.56	-0.29	-51.43**	-2.10*	-51.88**
$P_4 \times P_7$	0.58	1.42**	-0.58	-0.76	1.80*	0.46	-0.21	-0.48**	0.01	-0.09	0.48	12.52	-0.79	15.18*
$P_4 \times P_8$	-2.61**	-2.30**	15.41**	7.64**	-1.76*	-0.27	-0.21	-0.01	0.09*	0.32	-1.39	39.66**	-0.02	33.97**
$P_5 \times P_6$	1.33*	2.15**	14.20**	11.48**	1.74*	-0.71*	-1.08	-0.13	0.10*	-0.59	0.48	21.66**	0.26	13.10
$P_5 \times P_7$	1.08	0.92*	-13.43**	-9.26**	-0.12	-0.57*	1.13	0.05	0.01	-0.04	-0.30	-8.41	1.23	-4.90
$P_5 \times P_8$	-1.11	-1.80**	12.48**	12.73**	-1.01	0.71*	0.62	-0.05	-0.12**	-0.34	2.58*	-4.47	1.85*	-2.51
$P_6 \times P_7$	1.36*	2.34**	8.10	6.51*	-0.20	1.01**	-0.65	0.03	-0.03	-0.19	-3.84**	-26.44**	-0.32	-16.62*
$P_6 \times P_8$	2.00**	1.62**	9.17	10.49**	1.08	0.12	0.13	-0.02	0.12	0.17	1.91	28.88**	0.79	27.92**
$P_7 \times P_8$	1.25*	0.56	3.21	4.59	1.38	-0.90**	0.22	0.18	0.04	0.47	3.15*	-4.55	-0.74	-5.54
LSD5%(sij)	1.18	0.89	9.32	5.48	1.59	0.56	NS	0.35	0.07	0.69	2.45	15.51	1.78	13.37
LSD1%(sij)	1.56	1.19	12.37	7.27	2.11	0.74	NS	0.46	0.09	0.92	3.25	20.58	2.37	17.73
LSD5%(sij-sik)	1.80	1.37	14.24	8.37	2.43	0.85	NS	0.53	0.10	1.06	3.74	23.70	2.73	20.42
LSD1%(sij-sik)	2.38	1.81	18.89	11.11	3.22	1.13	NS	0.70	0.14	1.41	4.97	31.43	3.62	27.09
LSD5%(sij-ski)	1.61	1.22	12.74	7.49	2.17	0.76	NS	0.47	0.09	0.95	3.35	21.20	2.44	18.27
LSD1%(sij-ski)	2.13	1.62	16.90	9.93	2.88	1.01	NS	0.63	0.12	1.26	4.44	28.11	3.23	24.23

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Table (4): Mean performance of the crosses and S.C. 10 for all the studied traits and Heterosis relative to S.C. 10 for grain yield over the two planting dates.

Trait / Hybrid	Tasseling date	Silking date	Plant height	Ear height	Maturity date	Ear husk	Ear length (cm)	Ear diameter (cm)	No. of Ears/plant	No. of rows/ear	No. of kernels/ row	Ear weight/ plant (g)	100-Kernel weight	Grain yield/ Plant (g)	Heterosis for grain yield/plant (g)
$P_1 \times P_2$	63.00 BD	65.00 CE	312.0 EF	178.0 DF	102.0 DH	3.333 J	19.08 AB	3.917 BD	1.100 DF	14.43 AB	42.82 BG	199.4 EH	32.50 AC	172.8 FH	-5.04
$P_1 \times P_3$	59.33 JL	61.17 JL	284.3 JL	156.7KM	98.50 K	6.667 A	18.60 AC	4.033 AD	1.033 FG	13.40 CJ	41.78 DH	176.0 IK	30.00 CI	150.6 IK	-17.27**
$P_1 \times P_4$	61.00 FH	63.00 GI	290.3 IK	155.5 LM	100.3 GK	4.667 DF	17.65 AD	3.883 CD	1.067 EG	13.48 BJ	43.16 BG	170.7 JK	29.17 FI	150.7 IK	-17.18**
$P_1 \times P_5$	59.83 GJ	62.17 IJ	268.3 M	152.0 LN	98.50 K	4.000 FJ	19.07 AB	4.283 AC	1.150 BE	13.57 BI	40.24 FI	183.7 HJ	27.83 HI	159.6 HJ	-12.32**
$P_1 \times P_6$	57.83 L	59.67 MN	283.7 KL	160.3 IL	96.17 L	4.667 DF	18.33 AD	4.375 AC	1.133 CE	13.20 EJ	39.42 GI	184.6 GJ	27.83 HI	181.7 EG	-0.15
$P_1 \times P_7$	56.00 M	57.33 O	277.8 LM	158.8 JM	96.17 L	6.500 A	18.17 AD	3.933 BD	1.000 G	13.63 BI	41.97 CH	169.5 JK	31.17 BG	183.2 EG	0.68
$P_1 \times P_8$	55.67 M	57.33 O	297.0 GJ	174.2 EG	104.3 BC	6.500 A	18.17 AD	4.017 AD	1.100 DF	13.68 BI	40.98 FI	203.5 EH	31.50 BF	240.7 A	32.27**
$P_2 \times P_3$	61.00 FH	63.50 FH	298.0 GI	179.6 CF	101.8 DH	5.667 BC	17.27 BD	4.100 AD	1.267 A	13.83 BG	40.87 FI	273.6 A	30.83 BG	179.5 FG	-1.36
$P_2 \times P_4$	64.67 A	65.83 BC	341.0 A	185.3 BD	102.2 CH	3.833 GJ	19.43 A	4.393 AB	1.000 G	13.98 BF	45.26 AD	212.4 DF	31.17 BG	175.6 FH	-3.50
$P_2 \times P_5$	62.17 DF	64.17 DG	305.5 FH	171.2 FH	102.5 BG	4.333 DH	18.92 AB	4.058 AD	1.033 FG	13.43 BJ	43.55 BF	202.2 EH	30.17 BI	137.6 K	-24.40**
$P_2 \times P_6$	59.67 HK	61.50 JK	311.0 EF	180.3 CE	103.5 BE	4.167 EI	17.35 BD	3.933 BD	1.033 FG	12.55 J	38.02 I	159.8 K	29.83 CI	173.4 FH	-4.71
$P_2 \times P_7$	64.00 AC	65.33 CD	294.0 HK	156.5KM	104.7 AB	3.667 HJ	17.85 AD	4.333 AC	1.100 DF	14.25 AD	42.19 CG	194.7 FI	29.67 DI	166.1 GI	-8.73
$P_2 \times P_8$	64.33 AB	67.50 A	297.7 GI	166.7 GJ	106.3 A	4.333 DH	17.63 AD	3.967 BD	1.083 DG	13.57 BI	43.15 BG	200.9 EH	29.33 EI	199.4 CE	9.57
$P_2 \times P_9$	62.17 DF	64.00 EG	267.8 M	146.4 N	99.00 IK	6.500 A	18.23 AD	4.325 AC	1.067 EG	13.98 BF	42.08 CG	231.5 BD	32.00 BE	147.5 IK	-18.94**
$P_3 \times P_4$	59.50 HK	61.50 JK	293.0 HK	167.7 GI	98.83 JK	4.167 EI	18.43 AC	4.333 DH	1.100 DF	12.50 J	42.08 CG	176.7 IK	31.33 BG	160.6 HJ	-10.84*
$P_3 \times P_5$	58.83 N	59.50 HK	293.0 HK	172.5 EH	101.5 EH	4.333 DH	16.82 CD	3.933 BD	1.000 G	13.68 BI	42.80 BG	196.5 EI	30.33 BH	205.9 CD	13.15**
$P_3 \times P_6$	55.67 M	58.83 N	293.0 HK	172.5 EH	101.5 EH	6.500 A	17.69 AD	4.283 AC	1.000 G	13.68 BI	44.84 AE	236.0 BC	28.67 GI	137.6 K	-24.40**
$P_3 \times P_7$	60.83 FI	62.67 HI	293.0 HK	150.7MN	96.50 L	6.500 A	17.82 AD	4.417 AB	1.133 CE	14.01 BF	42.12 CG	166.0 JK	27.50 I	189.3 DF	3.99
$P_3 \times P_8$	58.17 KL	61.17 JL	288.7 IL	150.7MN	102.8 BF	5.667 BC	16.95 CD	4.483 A	1.133 CE	14.97 A	42.12 CG	206.5 EG	27.83 HI	212.4 BC	16.70**
$P_3 \times P_9$	60.50 GI	62.67 HI	323.3 CE	185.2 BD	102.2 CH	4.167 EI	16.40 D	3.683 D	1.200 AC	13.58 BI	41.92 CH	243.0 B	29.00 FI	225.1 AB	23.66**
$P_4 \times P_5$	62.33 DF	64.67 CF	291.3 IK	164.5 HK	102.6 A	4.500DG	16.89 CD	4.167 AD	1.233 AB	14.40 AC	42.08 CG	271.5 A	31.17 BG	191.7 DF	5.31
$P_4 \times P_6$	64.00 AC	67.33 A	307.8 FG	176.9 DF	106.5 A	4.833 DE	16.89 CD	4.200 AC	1.267 A	12.88 GJ	45.70 AC	218.1 CE	31.17 BG	198.4 CE	9.02
$P_4 \times P_7$	58.33 JL	60.83 KL	337.3 AB	186.7 BC	103.3 BE	4.833 DE	17.50 AD	4.200 AC	1.267 A	12.70 IJ	43.95 AF	231.4 BD	32.17 BD	166.1 GI	-8.74
$P_4 \times P_8$	61.33 EG	65.00 CE	325.0CD	193.7 AB	101.8 DH	3.833 GJ	17.27 BD	4.017 AD	1.100 DF	13.83 BG	40.87 FI	273.6 A	30.83 BG	179.5 FG	-1.36
$P_4 \times P_9$	63.17 AD	65.67 C	293.0 HK	172.5 EH	101.0 FI	4.000 FJ	17.94 AD	4.100 AD	1.267 A	13.83 BG	40.87 FI	273.6 A	30.83 BG	179.5 FG	-1.36
$P_5 \times P_6$	58.50 JL	60.17 LM	332.5 AC	195.8 A	100.5 GK	6.333 AB	16.88 CD	4.325 AC	1.133 CE	13.77 BH	46.03 AB	250.1 B	32.17 BD	215.0 BC	-8.72
$P_5 \times P_7$	62.67 CE	66.83 AB	312.3 EF	190.3 AB	100.8 FJ	4.833 DE	18.15 AD	4.283 AC	1.233 AB	13.33 DJ	46.30 AB	193.3 FI	29.67 DI	166.1 GI	
$P_5 \times P_8$	60.83 FI	63.33 GI	327.0 BD	195.7 A	102.5 BG	5.000 CD	17.70 AD	4.317 AC	1.150 BE	12.50 J	44.07 AF	213.4 DF	34.83 A	182.0 EG	
$P_6 \times P_7$	60.83 FI	63.33 GI	316.7 DF	189.3 AB	103.8 BD	4.000 FJ	19.35 A	4.050 AD	1.000 G						
$P_6 \times P_8$	62.17 DF	64.17 DG	339.0 AB	187.7 AC	100.2 HK	3.500 IJ									
S.C. 10	61.33 EG	63.00 GI													

Table (5): Number of amplified fragments and specific markers of eight maize inbred lines based on RAPD analysis.

Primer	Sequence 5→3	TAF	PB	Inbred lines 1 AF	1 SM	2 AF	2 SM	3 AF	3 SM	4 AF	4 SM	5 AF	5 SM	6 AF	6 SM	7 AF	7 SM	8 AF	8 SM	TSM
A11	CAATCGCCGT	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0
B01	GTTTCGCTCC	5	5	1	0	1	0	1	2	3	2	2	1	1	0	1	0	1	0	5
B06	TGCTCTGCCC	7	7	1	0	1	0	2	1	3	0	3	2	2	1	1	0	2	0	4
B07	GGTGACGCAG	7	7	3	0	1	0	4	0	3	0	1	0	4	0	3	0	6	2	2
B12	CCTTGACGCA	4	3	3	0	1	1	2	0	4	1	3	0	2	0	2	0	2	0	2
Total		24	22	9	0	5	1	10	3	14	3	10	3	10	1	8	0	12	2	13

TAF = Total amplified fragment, PB= Polymorphic bands, AF = Amplified fragment, SM = Specific markers, including either the presence or absence of a band in specific inbred, TSM = Total no. specific markers across inbred lines.

Table (6): Genotype specific markers in eight maize inbred lines resulting from RAPD analysis.

Inbred line	Marker type		Total
	Positive	Negative	
1	0	0	0
2	0	B12-276	1
3	B01-619; B06-176	B01-374	3
4	B01-320, 263; B12-492	0	3
5	B01-547; B06-244, 215	0	3
6	B06-849	0	1
7	0	0	0
8	B07-214, 156	0	2
Total	11	2	13

Table (7): Allele number and PIC values for SSR loci found in eight maize inbred lines.

SSR locus	Genomic location	Repeat class	No. alleles	MW (bp)	Inbred lines 1	2	3	4	5	6	7	8	PIC
phi002	1.08	-	2	84	-	+	+	+	+	+	+	+	0.61
				77	+	-	-	-	-	-	-	-	
nc003	2.06	2	6	123	-	+	-	-	-	-	-	-	0.83
				105	+	-	+	+	-	-	+	-	
				97	-	-	-	-	+	+	-	+	
				69	+	-	+	-	+	+	+	+	
				64	-	+	-	+	-	-	-	-	
				49	-	-	-	-	+	-	-	-	
phi046	3.08	4	1	104	+	+	+	+	+	+	+	+	-
phi026	4.04	2	2	125	+	-	+	+	-	-	-	+	0.38
				81	+	+	+	+	+	+	+	+	
phi069	5.04	3	3	177	+	+	+	+	+	-	+	+	0.15
				70	+	+	+	+	+	+	+	+	
				46	+	+	+	+	+	-	+	+	
phi081	6.07	6	3	130	-	-	+	-	-	+	-	-	0.56
				97	+	+	+	+	+	+	+	+	
				70	-	+	-	+	+	-	+	-	
phi049	7.07	3	1	144	+	+	+	+	+	+	+	+	-
bngl666	8.06	2	3	133	+	+	+	+	+	+	+	+	0.61
				96	-	-	+	-	-	+	-	+	
				74	-	-	-	-	-	-	-	+	
phi032	9.04	4	2	214	+	+	+	+	+	+	+	+	-
				87	+	+	+	+	+	+	+	+	
phi054	10.04	2	1	98	+	+	+	+	+	+	+	+	-

Table (8): Genetic similarity based on Jaccard's coefficient for eight inbred lines in maize revealed by RAPD and SSR.

Inbred line	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
P_1	1.00							
P_2	0.54	1.00						
P_3	0.63	0.56	1.00					
P_4	0.66	0.64	0.56	1.00				
P_5	0.59	0.57	0.50	0.58	1.00			
P_6	0.58	0.50	0.59	0.47	0.55	1.00		
P_7	0.71	0.77	0.72	0.68	0.67	0.60	1.00	
P_8	0.69	0.62	0.70	0.61	0.60	0.72	0.72	1.00

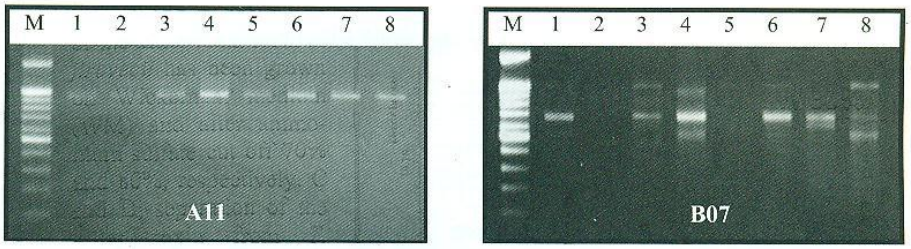

Fig. (1): Agarose gel (1.2%) in TAE buffer stained with ethidium bromide showing RAPD-PCR polymorphism of DNA for eight maize inbred lines P_1, P_2, P_3, P_4, P_5, P_6, P_7 and P_8 (Lanes 1-8, respectively) using random primers (A11 and B07). M refers to 100 bp DNA Ladder.

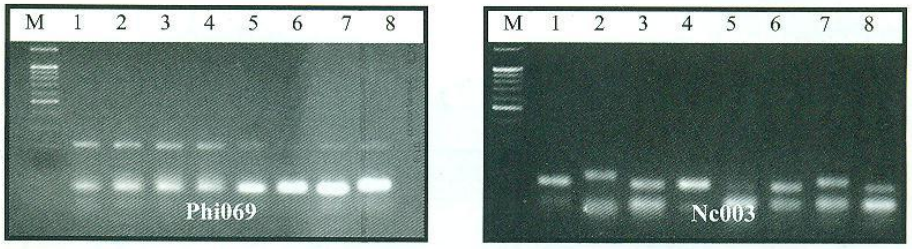

Fig. (2): Agarose gel (2%) in TAE buffer stained with ethidium bromide showing SSR-PCR polymorphism of DNA for eight maize inbred lines P_1, P_2, P_3, P_4, P_5, P_6, P_7 and P_8 (Lanes 1-8, respectively) using SSR primers (phi069 and nc003). M refers to 100 bp DNA Ladder.

Fig. (3): Dendrogram of the genetic distances among the eight maize inbred lines based on RAPD and SSR analysis.