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IDENTIFICATION AND INTERRELATIONSHIP OF YIELD RELATED TRAITS THROUGH DNA FINGERPRINTING IN ZEA MAYS

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ABSTRACT

Zea mays is an important grain crop grown throughout the world for its grain and fodder used as food for human and feed for animals. A study was conducted in the Centre of Excellence in Molecular Biology, University of the Punjab Lahore Pakistan during 2011-14. The aim of the study was to find out genetic diversity in maize germplasm, for this purpose 14 inbred lines were selected and 6 best inbred lines were crossed to produce F_1 hybrids. The results revealed that the higher heritability, genetic advance and significant correlation was found for traits like 100-grain weight, grain yield, leaf area, plant height, seeds per row, seed rows per cob, cob weight, cob length, cob diameter and cobs per plant for parent inbred lines and F_1 hybrids. It was found from results that the F_1 crosses 590×510, 210×460 and 470×135 performed better for most of the studied yield related traits. It was concluded that these traits played an important role in the grain yield of maize and hence the selection on the basis of these traits may be helpful to improve grain yield and production of maize. The Four RAPD primers (R-8, R-15, S-13 and S-19) were used for molecular assessment of true hybrids and parents for RAPD analysis. Patterns of inheritance of RAPD fragments (bands) were noted and seven types of RAPD fragments were identified. Clustering was performed and dendrograms were generated using RAPD data. The adopted pattern of molecular analysis and multiplication is an ideal way of exploiting variability which usually arises during the hybridization of two parental lines.

Keywords: ***Zea mays*, RAPD, genetic diversity, grain yield, F_1 hybrid, dendrogram, DNA fingerprinting**

INTRODUCTION

Maize (*Zea mays* L.) is the most widely grown cereal in the world. In terms of productivity, it is also a leading cereal of the world. Maize is a short duration, tropical plant but it is being successfully grown in temperate, tropical and sub-tropical regions of the world. Maize is grown twice in a year. Maize is a diploid species having 20 chromosomes. It is monoecious plant having staminate flowers in the tassel and pistillate flowers located midway on the stalk [1]. Ovules are 95 % cross pollinated and 5% self-pollinated where principal pollinating agent in cross-pollination is wind. Maize is indigenous to Americas and for the native Americans, it has been principal food grain. Its grain constitutes about 9.7396 % grain protein, 4.85 % grain oil, 9.4392 % grain crude fibre, 71.966 % grain starch, 11.77 % embryo while fodder contains 22.988 % acid detergent fibre, 51.696% neutral detergent fibre, 28.797 % fodder cellulose, 40.178 % fodder dry matter, 26.845 % fodder crude fibre, 10.353 % fodder crude protein and 9.095 % fodder moisture [2-4]. About 8000 years ago, maize was domesticated and in its wild form, it is no more available [5]. Maize is used as food for humans and feed for livestock and poultry. It is used as raw material in food, medicine and textile industries for the manufacturing of corn oil, corn flakes, dextrose and textile dyes [6]. Molecular Markers have played significant role in genetic research and plant breeding program. Recently a new class of molecular markers known as Random Amplified Polymarphic DNA (RAPD) was developed for the scanning of genome for genetic changes and it has been reported that molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors and are easily accessible to breeders and biotechnologists. PCR based assays are considered to meet both the technical and genetic requirements for the characterization of plant genetic resources. The RAPDs is used for the characterization of plant species, and distribution of variability within their gene pools [7, 8]. The prescribed study was conducted keeping in view the objectives as (1) maintenance of inbred lines and the development of F_1 hybrids, (2) conformation of hybrids through RAPD, (3) evaluation of inbred lines and hybrids for various grain yield traits and (4) identification of best performance F_1 hybrids. The patterns of RAPD fragments could be classified into seven types: Type I fragments shared bands in both parents, and offspring; Type II fragments shared bands in male and female parents; Type III fragments shared bands in male parent and offspring; Type IV fragments shared bands in female parent and offspring; Type V fragments were presented in the male parent only; Type VI fragments were present in the female parent only; Type VII fragments were present in offspring only [9].

MATERIALS AND METHODS

Genetic analysis

RAPD analyses of six parents and their respective hybrids were used for genetic analysis.

DNA extraction

DNA was extracted using the CTAB method, with modifications according to the laboratory set up. Fresh leaf tissue (100 mg) from each plant form each treatment was ground to a fine powder in the presence of liquid nitrogen using chilled mortar and pestle. The leaf tissue powder was transferred into 1.5 ml eppendorf tube followed by the addition of 0.5 ml of DNA extraction buffer (Appendix F) and 0.1 ml of 20 % SDS. The mixture was thoroughly stirred and incubated at 65 ^{o}C for 10 min and then kept at room temp for 10-15 min, 0.5 ml of 5 M potassium acetate was added, the tubes were shaked gently and were kept at ice for 20 min. After centrifugation at 10,000 rpm for 10 min, the aqueous phase was pipetted out carefully into a fresh tube. The DNA was precipitated by adding equal volume of chilled isopropanol. Precipitated DNA was pelleted by centrifugation at 14,000 rpm for 5 min, the pellet was air dried and dissolved in 700 µl of autoclaved distilled water. Single stranded RNA was digested with RNase 10 µl of 10 mg/ml was used for 30 min at 37 °C. After adding 500 µl of chloroform isoamylalcohol (24:1) subjected to a centrifugation at 14000 rpm for 3 min, DNA being in the aqueous phase was shifted into fresh tube and was precipitated by adding 10 µl of 3 M sodium acetate and equal volume of isopropanol. After pelleting the DNA by centrifugation at 14000 rpm for 5 min, the pellet was washed with 70 % ethanol. The pellet was air dried completely and was re-suspended in suitable amount of distilled autoclaved water.

Photometric quantification

The DNA was quantified by taking absorption in low EC autoclaved distilled water at 260 nm. Absorption of 1 OD (optical density) corresponds to 50 µg/ml dsDNA. Using this factor, the concentration of an unknown sample was calculated via the measured extinction and dilution factor.

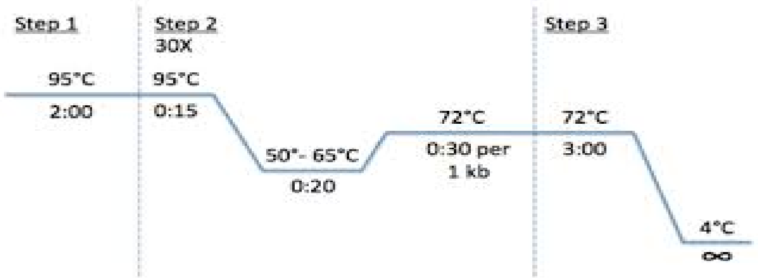
Quantification of DNA by agarose gel

DNA was also quantified by electrophoresis (3 V/Am) in 0.8 % agarose gels (w/v) by comparison to known concentration (100 ng/µl) of lamda phage DNA. The DNA was visualized by ethidium bromide staining and DNA solutions were then diluted to 100 ng/µl for PCR reaction (Figure I).

Randomly amplified polymorphic DNA (RAPD) amplification conditions and analysis

PCR amplifications were performed in ABI Thermocycler, with modifications. The autoclaved water in a final volume of 25 µl. The reactions were subjected to 40 cycles, after an initial denaturation at 95 °C for 3 min. Each cycle consisted of 1 minute at 95 °C, 1 minute at 38 °C and 2 min at 72 °C with a final 10 min extension at 72 °C. Amplicons were analyzed by electrophoresis in 1% Agarose gel with 1X TBE buffer (Trisma Base 10.8 g/l, Boric Acid reaction mixture contained 10 mM tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dNTPs, 25 µM primer, 350 ng – 375 ng template DNA, 2 units of Taq polymerase, and an ultra pure low EC 5.5 g/l, 0.5 M EDTA (pH 8.0) 4 ml/l). A DNA ladder (100 bp, DNA ladder Plus, ready to use, Fermentas life sciences) was used as molecular weight marker. The gels were photographed under UV light, using a Gel-Documentation 2000 system (UVP).

Figure I: Shows the temperature conditions for PCR



RAPD data evaluation

The amplification of DNA samples were repeated three times and only reproducible bands were considered for analyses. Each amplified product was scored across 3 F_1 hybrids and their respective parents. For this purpose four 10-mer primers were selected. Bands were scored as present (+) or absent (-) for each treatment, very weak bands were not included in the analyses.

A pair wise similarity matrix was constructed using the DICE similarity index SD=2a /(2a + b + c), where 'a' is number of shared bands between a pair of plants 'X' and 'Y'; 'b' is the number of bands present in plant 'X' only and 'c' is the number of bands present in 'Y' only.

Similarity estimates were analyzed using Unweighted Pair Group Method Arithmetic Averages (UPGMA) provided by Mega 2.1

software and the resulting cluster were represented as dendrograms.

Development of F_1 hybrids and statistical analysis of data

The inbred lines, 77, 510, 530, 590, 410, 460, 680, 600, 640, 210, 570, 82, 135 and 470 were grown 4 times in the field during crop both spring and autumn growing seasons of 2011-12 and 2012-13 following randomized complete block design in three replications to , seeds per row, cob diameter, tassel emergence days, No. lateral tassel branches, 50% silk emergence days, cob weight and seed rows per cob was recorded for inbred lines. The F_1 hybrids 77×570, 77×680, 210×82, 210×460, 590×510, 210×530, 210×600, 210×640, 470×135, 470×570, 470×680, 590×82, 590×410,210×510, 590×530 and 560×600 were sown in the field following randomized complete block design in three replications during autumn crop season of 2013-14 to evaluate the F_1 hybrids. The data was recorded for various traits *viz*., cob length, days taken to maturity, exertion above flag leaf, 50% tassel emergence, grain yield, 100-grain weight, internode diameter, internode length, leaf area, leaf length, leaf width, plant height, peduncle length, seed thickness, seed width, seed length, tassel stalk length, cobs per plant, seeds per row, cob diameter, No. lateral tassel branches, produce self seeds while in spring season of 2013-14 the lines were crossed to produce F_1 hybrids. The data of following traits *viz*., cob length, days taken to maturity, exertion above flag leaf, 50% tassel emergence, grain yield, 100-grain weight, internode diameter, internode length, leaf area, leaf length, leaf width, plant height, peduncle length, seed thickness, seed width, seed length, tassel stalk length, cobs per plant 50% silk emergence days, cob weight and seed rows per cob. The data was statistically analyzed by using analysis of variance technique [10].

RESULTS

RAPD analysis

RAPD analysis of three F1 hybrids and their respective parents were performed with 8 decamer primers. A total of 25 amplified reproducible fragments produced from 4 random decamer primers. The number of fragments per primer ranged from minimum 5 in R-8 to maximum 7 fragments in S-13 and S-19. The total size of the amplified products varied from 250bp to 2800bp. All the 4 primers were found to be polymorphic and gave an average of 68% polymorphism (Table 1.1). R-8 primer produced 5 fragments (Figure), R-15 primer 6 fragments (Figure), S-13 primer produced 7 fragments (Figure), S-19 produced 7 fragments

(Figure), 60% fragments of R-8 primer, 83% fragments of R-15, 57% fragments of S-13 and 71% fragments of S-19 were found to be polymorphic (Table 1.1).

The RAPD fragments reproduced were also grouped into seven types according to presence and absence of bands (Table 1.1). Type II, III, and V fragment were not found in 590 × 510; type IV and VI bands in 470 × 135 and type VII fragments in 210×460. In the hybrid combinations of 210×460, bands of offspring revealed only 55.6% shared bands with parents. The hybrid 470×135 and 590×510 revealed 47.6% and 54.5% bands shared with parents, respectively (Figure II, III, IV, V, Table 1.2).

Figure II. RAPD Profile Generated Using R-8 Primer

Lane1	100 bp DNA ladder plus, Fermentas Life Sciences
Lane 2	210
Lane 3	460
Lane 4	210 x 460
Lane 5	470
Lane 6	135
Lane 7	470 x 135
Lane 8	590
Lane 9	510
Lane 10	590 x 510
Lane 11	Negative Control

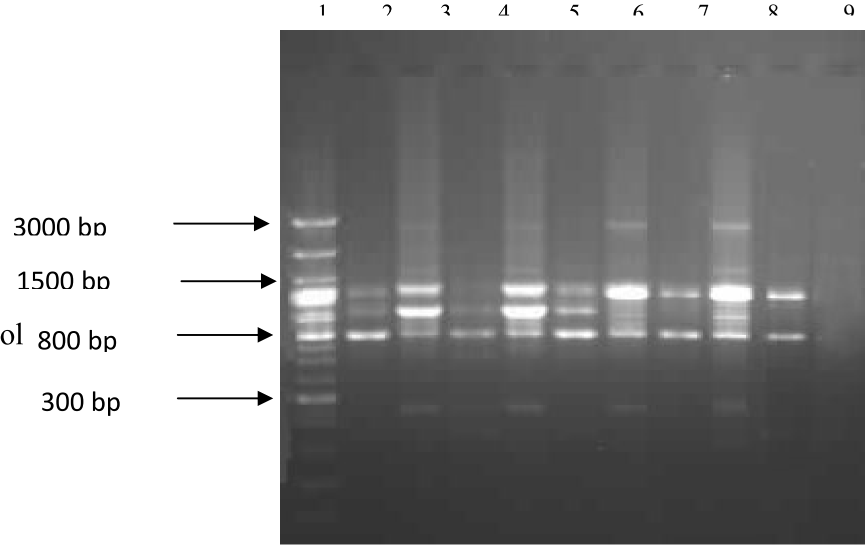


Figure III. RAPD Profile Generated Using R-15 Primer

Lane1	100 bp DNA ladder plus, Fermentas Life Sciences
Lane 2	210
Lane 3	460
Lane 4	210 x 460
Lane 5	470
Lane 6	135
Lane 7	470 x 135
Lane 8	590
Lane 9	510
Lane 10	590 x 510
Lane 11	Negative Control

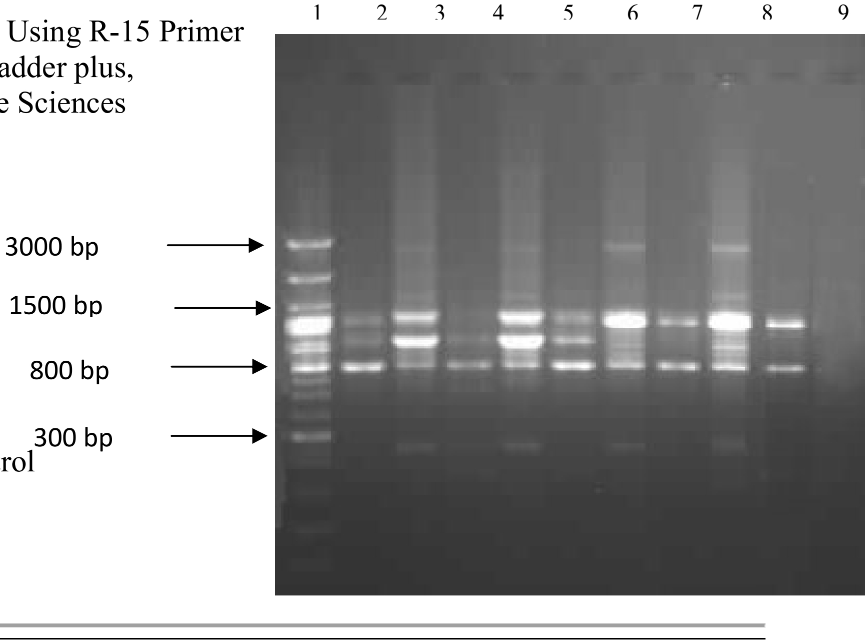


Figure IV. RAPD Profile Generated Using S-13 Primer

Lane1 100 bp DNA ladder plus, Fermentas Life Sciences
Lane 2 210
Lane 3 460
Lane 4 210 x 460
Lane 5 470
Lane 6 135
Lane 7 470 x 135
Lane 8 590
Lane 9 510
Lane 10 590 x 510
Lane 11 Negative Control

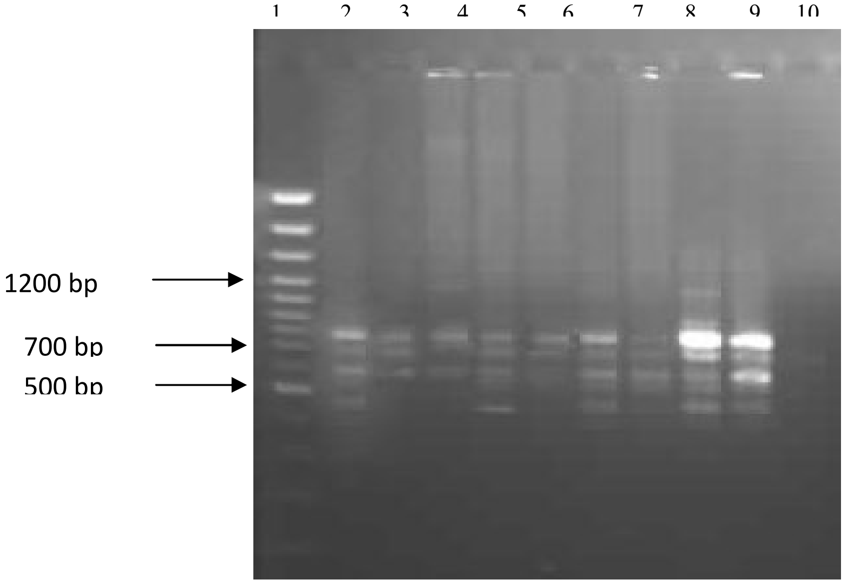


Figure V. RAPD Profile Generated Using S-19primer

Lane1 100 bp DNA ladder plus, Fermentas Life Sciences
Lane2 210
Lane3 460
Lane4 210 x 460
Lane5 470
Lane6 135
Lane7 470 x 135
Lane8 590
Lane9 510
Lane10 590 x 510
Lane11 Negative Control

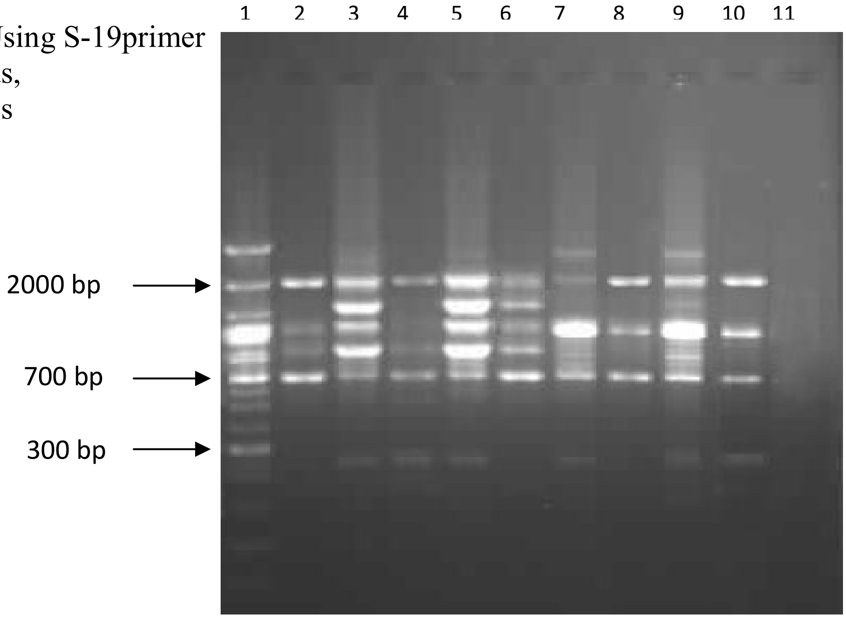


Table 1.1: Description of Four Decamer Primers Used for RAPD Analysis

Sr. No.	Primer	Sequence 5'-3'	Resolved Bands	Scored Bands	Monomorphic Bands	Polymorphic Bands	Fragment Size of Scored Bands
1	R-8	-CCCGTTGCCT-	5	5	2	3 (60%)	300-1800
2	R-15	-GGACAACGAG-	6	6	1	5 (83%)	250-2800
3	S-13	-TTCAGGGTGG-	7	7	3	4 (53%)	450-1050
4	S-19	-GAGTCAGCAG-	7	7	2	5 (71%)	250-2700
				25	8	17 (68%)	

Monomorphic Bands **Bands present in all samples**
Polymorphic Bands **Bands present in some samples, not in all**

Table 1.2: Types of RAPD Fragments Identified From the Three F1 Hybrids

Type of Fragments	Property of Fragments			RAPD Fragments		
	Male	Female	Offspring	210 x 460	470 x 135	590 x 510
				No. (%)	No. (%)	No. (%)
I	+	+	+	10 (55.6)	10 (47.6)	12 (54.5)
II	+	+	-	3 (16.7)	3 (14.3)	0 (0)
III	+	-	+	1 (5.5)	4 (19)	0 (0)
IV	-	+	+	1 (5.5)	0 (0)	2 (9.1)
V	+	-	-	1 (5.5)	2 (9.5)	0 (0)
VI	-	+	-	2 (11.1)	0 (0)	7 (31.8)
VII	-	-	+	0 (0)	2 (9.5)	1 (4.5)
Total				18	21	22

+/- Indicate presence/absence of band respectively.

CLUSTER ANALYSIS

Cluster analysis was done on the basis of similarity coefficient generated from RAPD data among the three F1 hybrids and their respective parents. Similarity indices of R-8 primer ranged from 0.571 to 1.00; R-15 primer from 0.333 to 0.888; S-13 Primer from 0.727 to 0.888 and S-19 primer from 0.571 to 1.00; all the analysis were grouped into one cluster and similarity indices found in the range of 0.714 to 0.912. Data presented in dendrogram shown in Figure VI based on DICE's similarity indices was generated through RAPD-PCR analysis using R-8 primer. The dendrogram has shown the similarity indices data in the form of two groups of genetically related genotypes. 470 × 135, 590 × 510, 510, 210 and 460 fall in 1st group while 210 × 460, 590, 470 and 135 fall in 2nd group. Data presented in dendrogram shown in Figure VII based on DICE's[10] similarity indices was generated through RAPD-PCR analysis using R-15 primer. The dendrogram has expressed the similarity indices date in the form of two groups 210 x 460, 470 × 135, 590, 460 and 470 fall in 1st group. While 510, 590 × 510 and 135 fall in other group. 210 is representing unique genetic identity/ polymorphism in response to R-15 primer. Data presented in dendrogram shown in Figure VIII based on DICE's similarity indices was generated through RAPD-PCR analysis using S-13 primer. The dendrogram has expressed the similarity indices date in the form of four groups. 135, 510 and 470 fall in group-1; 210 × 460 and 590 × 510 fall in group-2; 460 and 470 × 135 fall in group-3; 210 and 590 fall in group-4. Data presented in dendrogram shown in Figure IX based on DICE's similarity indices was generated through RAPD-PCR analysis using S-19 primer. The dendrogram has expressed the similarity indices data in the form of three groups of genetically related genotypes. Grande x 470, 590 × 510 and 210 fall in group-1; 460, 210 × 460 and 135 fall in group-2; similarly 470, 590 and 510 placed in group-3. Data presented in dendrogram shown in Figure X based on DICE's similarity indices was generated through RAPD-PCR analysis using R-8, R-15, S-13 and S-19 primer. The dendrogram has expressed the similarity indices data in the form of two groups of genetically related genotypes. 210 × 460, 510, 135, Grande × 470 and 590 × 510 showed genetic similarity therefore placed in group-1; similarly 590 and 470 in group-2; 460 and 210 showed unique genetic identity/polymorphism and did not fall in any group.

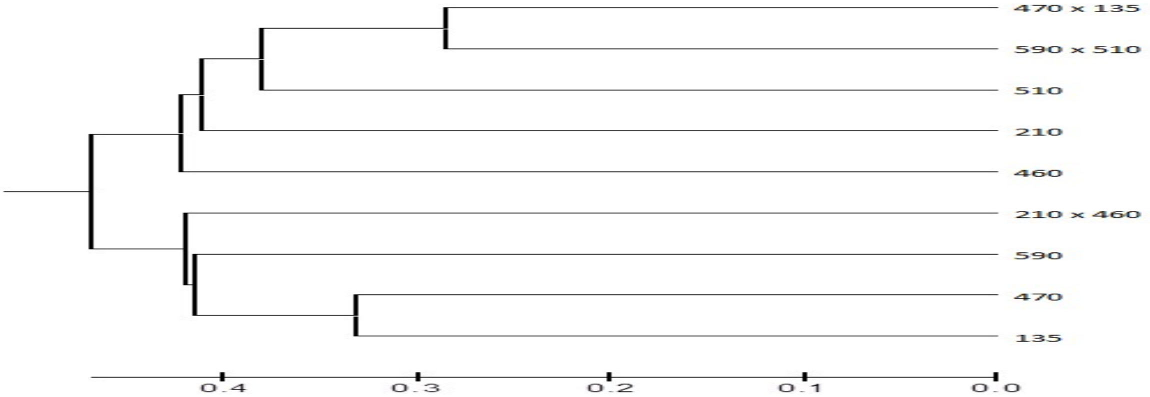


Figure VI. Dendrogram generated from the five RAPD fragments, using R-8 primer among 3 F1 hybrids and their respective parents. RAPD fragments obtained were visually scored as either present (+) or absent (-). Genetic distances were calculated by sample matching method and clustering was performed by UPGMA, using a programme provided by the Mega 2.1.

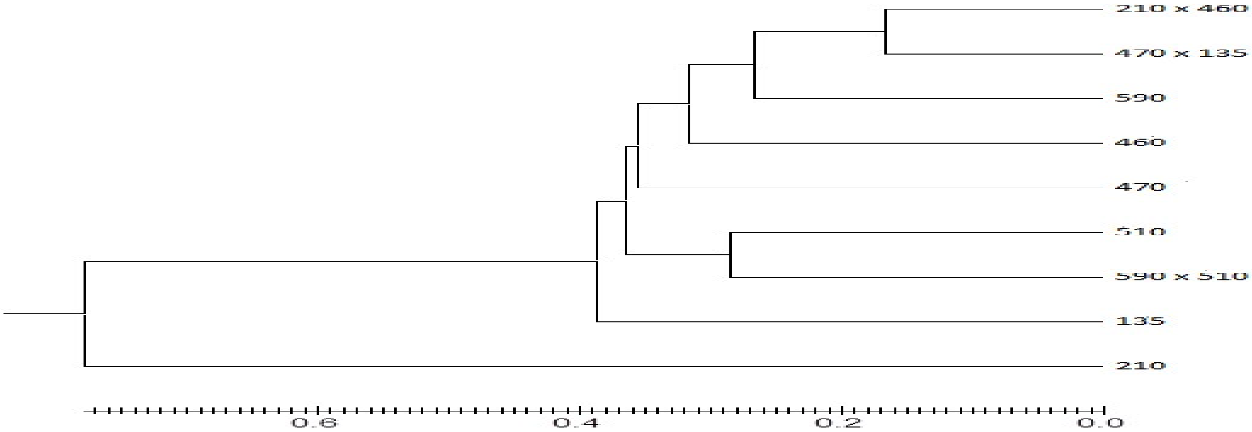


Figure VII. Dendrogram generated from the six RAPD fragments, using R-15 primer among 3 F1 hybrids and their respective parents. RAPD fragments obtained were visually scored as either present (+) or absent (-). Genetic distances were calculated by sample matching method and clustering was performed by UPGMA, using a programme provided by the Mega 2.1.

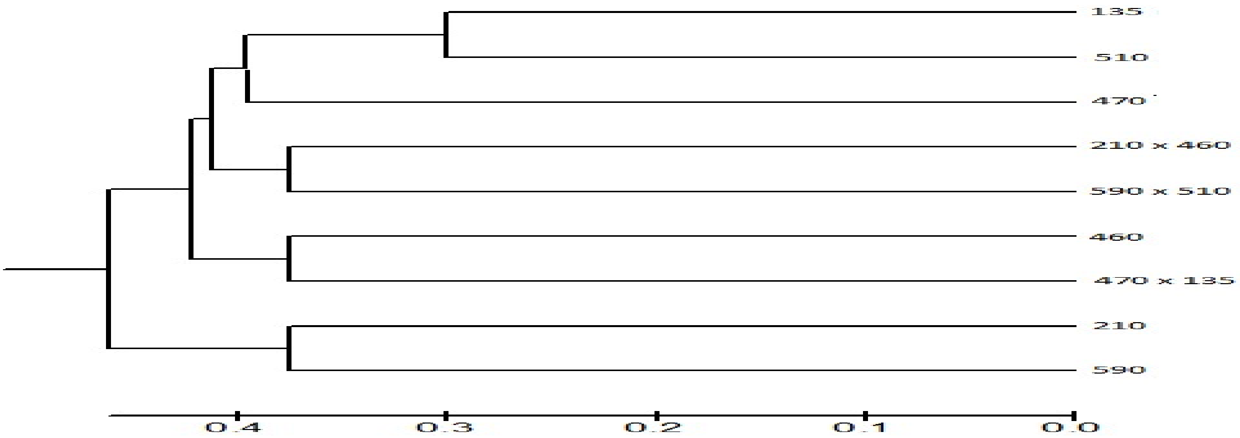


Figure VIII. Dendrogram generated from the seven RAPD fragments, using S-13 primer among 3 F1 hybrids and their respective parents. RAPD fragments obtained were visually scored as either present (+) or absent (-). Genetic distances were calculated by sample matching method and clustering was performed by UPGMA, using a programme provided by the Mega 2.1.

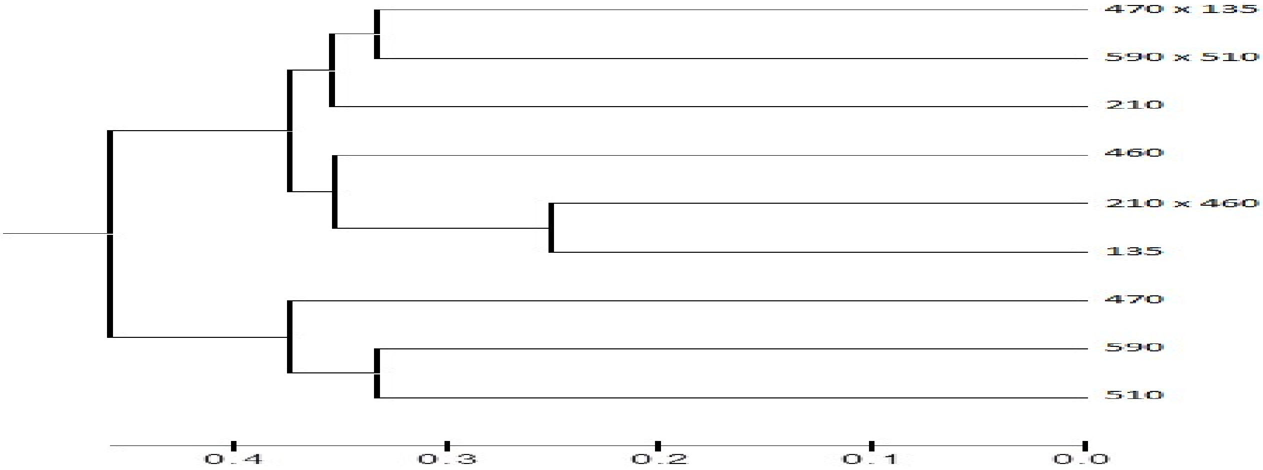


Figure IX. Dendrogram generated from the seven RAPD fragments, using S-19 primer among 3 F1 hybrids and their respective parents. RAPD fragments obtained were visually scored as either present (+) or absent (-). Genetic distances were calculated by sample matching method and clustering was performed by UPGMA, using a program provided by the Mega 2.1.

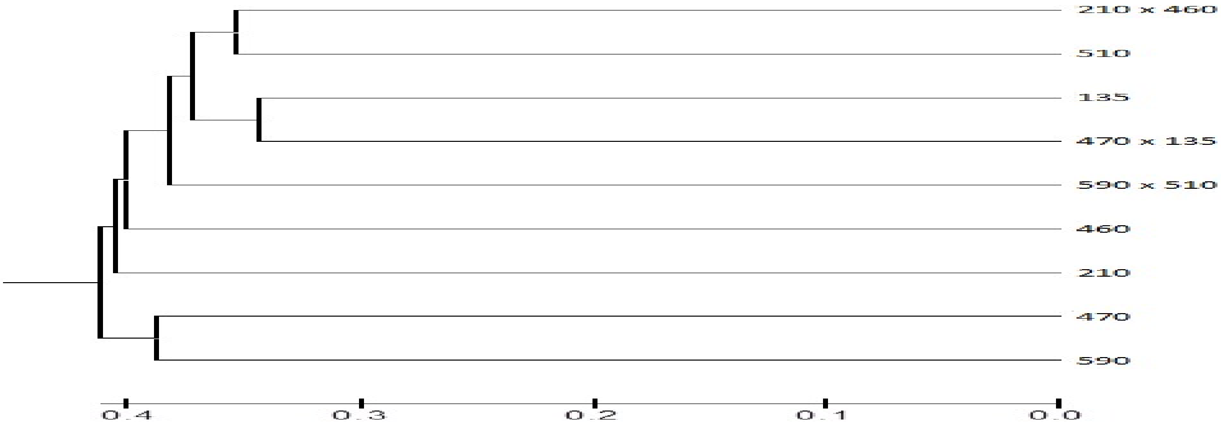


Figure X. Dendrogram generated from the twenty five RAPD fragments, using R-8, R-15, S-13, and S-19 primers among 3 F1 hybrids and their respective parents. RAPD fragments obtained were visually scored as either present (+) or absent (-). Genetic distances were calculated by sample matching method and clustering was performed by UPGMA, using a programme provided by the Mega 2.1.

2. Yield related traits evaluation of inbred lines and F_1 hybrids

It was persuaded from tables 1, 1a, 5 and 5a that significant differences were reported for all studied traits of maize. The results revealed (Table 3) that higher tassel lateral branches were found for inbred line 77 (18.120) and 590 (17.120) while lowest was found for inbred line 82 (10.120). Higher cob length was recorded for inbred line 600 (30.120cm) and inbred line 510 (29.120cm) while lowest was for inbred line 210 (21.120cm), higher number of days taken for maturity was found for inbred line 510 (124.12) while lowest for inbred line 680 (104.12), higher exertion flag leaf length was recorded for inbred line 210 (54.12cm) while lowest for inbred line 135 (42.12cm), maximum days were taken for 50% silk emergence were reported for inbred line 510 (53.12) while lowest for inbred line 570 (44.12) and higher grain yield was found for inbred line 570 (1504.1kg/acre) while lowest was found for inbred line 470 (1054.1 kg/acre). The results persuaded (Table 7 and 7a) that higher days to maturity was taken by cross 77×570 (120.49) while lowest days were taken by 590×410 (90.486). The cross takes higher number of days to maturity indicated its late maturing character while less days taking cross showed early maturing character. Higher cob length (28.486cm, 26.486cm), cob diameter (6.1856 cm, 5.8857 cm), 100-grain weight (38.486 g, 36.486 g), seeds per row (50.486, 49.486), cobs per plant (2.6856, 2.5856), grain yield (3583.5 kg/acre, 3474.5 kg/acre), leaf width (11.486 cm, 11.485 cm), leaf area (507.31 cm^2, 515.17 cm^2) and cob weight (240.49 g, 220.49 g) for crosses 210×460 and 470×135 respectively. Higher peduncle length (22.486 cm) was recorded for 210×530, higher tassel lateral branches (16.486) for 210×600, seed rows per cob (16.486, 14.486) for 210×460 and 590×510 respectively, higher 50% silk emergence days (51.486) was recorded for the cross 210×600 and 590×82. Higher tassel emergence days (56.486, 54.486) was recorded for 590×510 and 470×135 respectively, leaf length (65.486 cm), tassel length (50.486cm), tassel stalk length (18.486cm) and internode length (23.486 cm) and internode diameter (4.6856 cm) for cross 590×510. Higher plant height (172.49 cm, 168.49 cm) was recorded for 210×600 and 470×135 respectively. The results in table 3a showed that higher 100-grain weight was recorded for inbred line 640 (30.12 g) and 82 (29.12 g) while lowest was for inbred line 210 (22.12 g). The higher internode length was recorded for inbred line 210 (19.12cm) and 470 (19.12cm) but 14.12 cm was lowest for inbred line 77, higher internode diameter was estimated for inbred line 640 (7.32 mm) and 82 (7.12 mm) while inbred line 410 showed 6.12 mm at lowest level, higher leaf length, leaf width and leaf area was recorded for inbred line 210 (89.12 cm^2; 13.12 cm^2 and 577.87 cm^2) respectively while lowest was found for inbred line 77 (59.12 cm^2; 11.12 cm^2 and 292.87 cm^2). It was revealed from results indicated in table 3b that higher peduncle length was reported for inbred line 530 (24.12cm), 680 (24.12 cm) and 640 (24.12 cm) while inbred line 470 (20.12cm) showed lowest peduncle length, higher seeds rows per cob was found for inbred line 210 (18.12), 280 (18.12) and 410 (18.12) but lowest for inbred line 680 (13.12), higher seed thickness was recorded for inbred line 135 (5.42 mm) while lowest was for 77 (4.12 mm), higher seed width was recorded for inbred line 510 (12.52 mm) and 530 (12.32 mm) but lowest for 210 (9.92 mm), higher days taken to silk emergence was showed be inbred lines 210, 510 and 590 (54.12) while lowest was for inbred line 570 (46.12). Higher number of seeds per row was recorded for inbred line 570 (47.12), 410 (46.12) and 680 (46.12) while lowest was reported for inbred line 590 (39.12). It was shown from table 3c that higher tassel stalk length was for inbred line 77 (14.12 cm) and 470 (14.12 cm) but lowest for 135 (10.62 cm), higher cobs per plant were for inbred lines 135, 210, 570 and 590 (2.00) respectively while lowest was found for inbred line 680 (1.00), higher cob diameter was found for inbred line 280 (5.62 cm), 201 (5.52 cm) and 410 (5.32 cm) while lowest was recorded for inbred line 77 (4.32cm), higher seed length was shown by inbred line 82 (16.12 mm) while lowest by 590 (13.12 mm), higher number of days taken to tasseling was recorded for inbred line 77 (53.12) and 470 (50.12), lowest for inbred line 135 (42.12), higher cob weight was reported for inbred line 570 (129.12 g), 280 (127.12 g) and 680 (126.12 g), lowest for inbred line 210 (102.12g), higher plant height was reported for inbred lines 210 (239.12 cm), 470 (224.12 cm) and 280 (214.12 cm), lowest plant height was found for inbred line 77 (134.12 cm). It was persuaded from results given in table 2 that heritability for all recorded traits was recorded from 25.890 % to 99.903 %. Higher heritability was reported for grain yield (99.903 %), leaf area (99.729 %), plant height (98.231%), seed rows per row (97.587 %), days to tasseling (99.80 %), cob weight (96.575 %), internore diameter (92.866 %) and seeds per row (86.978%) while lowest for cob diameter (51.883 %), seed thickness (43.317 %), cobs per plant

(25.890%) and seed length (58.287%). Higher heritability suggested that the selection of maize inbred lines for the development of hybrids may be helpful on the basis of higher heritability traits. The grain yield may be improved through the selection of inbred lines showed higher performance for grain and grain related traits. Genetic advance was recorded from range 7.942 % to 60.527 %. Higher genetic advance was reported for internode diameter (60.527%), tassel lateral branches (28.894%), leaf area (30.591%), plant height (27.685%), peduncle length (22.887%), seed rows per cob (32.940%), seed width (39.118%) and tassel stalk length (34.321%) but lowest for 50% silk emergence days (7.979 %), days taken to maturity (9.072 %), exertion flag leaf length (9.154 %), seed thickness (9.209 %) and days to silk emergence (7.942 %). It was shown from the results (Table 6) that broad sense heritability for hybrid studies was recorded from 24.964 % to 99.954 %. Higher heritability was recorded for grain yield (99.954 %), tassel stalk length (89.106 %), tassel length (97.836 %), plant height (99.548 %), internode length (89.88 %), leaf width (95.685%), leaf length (90.454 %), leaf area (88.542 %), 50% tassel emergence days (91.976 %), 50% silk emergence days (88.938 %), seeds per row (91.128 %), seed rows per cob (97.334 %), cobs per plant (90.85 %) and cob diameter (81.74 %). Genetic advance was recorded from 2.736% to 77.465%. Higher genetic advance was estimated for cobs per plant (77.465%), tassel stalk length (20.641%), internode length (36.41%) and cob diameter (49.619%).

It was revealed form results (Table 4) that strong and significant correlation was found for exertion flag leaf length with tassel lateral branches, leaf width, tassel stalk length and tassel emergence days. Cob length was strongly and significantly correlated with internode length, internode diameter, peduncle length, seed thickness, seed width, seed per row, cob diameter, leaf width and 100-grain weight. 100-grain weight was significantly and strongly correlated with seeds rows per cob, seed width, seeds per row, tassel stalk length, seed length and cob diameter. Grain yield strongly and significantly correlated with most of its contributing traits.

Table 1: Analysis of variance for various morphological and grain yield traits in maize

Source	DF	Tassel Lateral branches	Cob length	Days taken to maturity	Exertion flag leaf length	50% Silk emergence	Grain Yield	100-grain weight	Internode Diameter	Internode Length	Leaf area	Leaf Length	Leaf Width	Plant Height
Genotypes	13	19.8516*	21.906*	117.973*	36.7129*	24.396*	52874.2*	18.044*	16.974*	6.8571*	18831.9*	216.676*	16.9744*	2693.41*
Error	28	1.1704	1.7014	6.1174	6.0744	3.0044	17	2.7544	0.4238	0.9744	17	16.134	1.0602	16.07

* = Significant at 5% probability level

Table 1a: Analysis of variance for various morphological and grain yield traits in maize

Source	DF	Peduncle Length	Seed rows per cob	Seed thickness	Seed Width	Days to Silk emergence	Seeds per row	Tassel stalk Length	Cob per plant	Cob diameter	Seed length	Days to Tasseling	Cob weight
Genotypes	13	35.6209*	29.132*	0.63445*	23.1387*	19.7802*	54.234*	23.988*	0.39011*	1.2544*	16.9742*	26.1264*	248.852*
Error	28	3.0414	0.2381	0.19269	1.2037	1.0713	2.578	2.0294	0.19048	0.29621	3.2693	0.0174	2.9074

* = Significant at 5% probability level

Table 2: Estimations of genetic components for various morphological and grain yield traits in maize

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h^2bs%	GA%
Tassel Lateral branches	19.8516*	13.906	6.2271	66.917	7.397	72.935	1.1704	29.0112	84.178	28.894
Cob length	21.9066*	27.231	6.7350	49.732	8.436	55.661	1.70	24.9960	79.832	14.944
Days taken to maturity	117.973*	109.48	37.2851	58.357	43.403	62.963	6.1177	23.6388	85.904	9.072
Exertion flag leaf length	36.7129*	48.513	10.2128	45.882	16.287	57.942	6.0744	35.3852	62.704	9.154
50% Silk emergence	24.3956*	49.263	7.1304	38.045	10.1348	45.357	3.0044	24.6955	70.355	7.979
Grain Yield	52874.2*	1277.7	17619.067	371.345	17636.067	371.524	17	11.5347	99.903	18.223
100-grain weight	18.044*	26.834	5.09653	43.581	7.851	54.0901	2.7544	32.0383	64.916	11.896
Internode Diameter	16.9744*	6.5629	5.516867	91.685	5.9407	95.1414	0.4238	25.41168	92.866	60.527
Internode Length	6.8571*	16.977	1.9609	33.986	2.9353	41.581	0.9744	23.9573	66.804	11.831
Leaf area	18831.9*	453.39	6271.633	371.924	6288.633	372.428	17	19.3637	99.729	30.613
Leaf Length	216.676*	79.049	66.8473	91.959	82.981	102.457	16.134	45.1775	80.557	16.292
Leaf Width	16.9744*	12.063	5.30473	66.314	6.3649	72.6389	1.0602	29.6460	83.343	30.591
Plant height	2693.41*	187.69	892.4477	218.057	908.515	220.012	16.067	29.2581	98.231	27.685
Peduncle Length	35.6209*	22.334	10.8598	69.7313	13.9012	78.893	3.0414	36.9023	78.121	22.887
Seed rows per cob	29.132*	16.334	9.6313	76.788	9.8694	77.731	0.2381	12.0735	97.587	32.940
Seed thickness	0.63445*	4.8129	0.1472	17.4915	0.3399	26.576	0.19269	20.0090	43.317	9.209
Seed Width	23.1387*	11.241	7.3117	80.6502	8.5153	87.036	1.2037	32.7233	85.864	39.118
Days to Silk emergence	19.7802*	50.977	6.2363	0.34976	7.3076	0.0530	1.0713	14.496	85.339	7.942
Seeds per row	54.234*	43.763	17.219	62.726	19.797	67.258	2.578	24.271	86.978	15.519
Tassel stalk Length	23.988*	12.241	7.320	77.327	9.349	87.392	2.029	40.717	78.293	34.321
Cob per plant	0.390*	1.547	0.067	20.742	0.257	40.765	0.190	35.093	25.890	14.893
Cob diameter	1.254*	5.141	0.319	24.924	0.616	34.603	0.296	24.003	51.883	13.896
Seed length	16.974*	15.120	4.568	54.967	7.838	71.997	3.269	46.500	58.287	18.940
Days to Tasseling	26.126*	46.477	8.703	43.273	8.720	43.316	0.017	1.935	99.800	11.129
Cob weight	248.852*	116.91	81.982	83.740	84.889	85.212	2.907	15.770	96.575	13.357

* = Significant at 5% probability level, Mean Sum of Squares (MS), Grand mean (GM), Genotypic variance (GV), Genotypic coefficient of variance (GCV %), Phenotypic variance (PV), Phenotypic coefficient of variance (PCV %), Environmental Variance (EV), Environmental coefficient of variance (ECV %), Broad sense heritability (h^2bs %), Genetic advance (GA)

Table 3: Mean significance differences among different maize inbred lines for various crop yielding traits

Lines	Tassel Lateral branches	Lines	Cob length	Lines	Days taken to maturity	Lines	Exertion flag leaf length	Lines	50% Silk emergence	Lines	Grain Yield
77	18.120A	600	30.120 A	510	124.12 A	210	54.120 A	510	53.120A	570	1504.1 A
590	17.120AB	510	29.120 AB	410	119.12 AB	590	54.120 A	590	53.120A	510	1404.1 B
470	16.120ABC	410	28.120 ABC	570	114.12 BC	640	50.62AB	210	52.120AB	530	1404.1 B
570	15.120ABC	570	28.120 ABC	590	112.12 C	77	50.120AB	410	51.120ABC	600	1404.1 B
410	14.120ABC	640	28.120 ABC	77	111.12 C	600	50.120AB	530	51.120ABC	680	1384.1 C
530	14.120ABC	82	27.120ABCD	210	109.12 CD	530	49.120ABC	82	50.120ABCD	135	1304.1 D
640	14.120ABC	280	27.120ABCD	470	109.12 CD	570	49.120ABC	600	50.120ABCD	410	1304.1 D
680	14.120ABC	680	27.120ABCD	640	109.12 CD	680	49.120ABC	280	49.120ABCD	82	1254.1 E
280	13.120ABC	530	26.120ABCD	82	104.12 D	470	48.12ABCD	640	49.120ABCD	280	1254.1 E
600	13.120ABC	135	25.120ABCD	135	104.12 D	410	47.120BCD	135	48.120ABCD	640	1204.1 F
135	12.120ABC	590	24.120ABCD	280	104.12 D	510	47.120BCD	470	47.120ABCD	210	1154.1 G
510	12.120ABC	470	23.120BCD	530	104.12 D	280	45.120BCD	77	46.120 BCD	590	1154.1 G
210	11.120 BC	77	22.120CD	600	104.12 D	82	43.120CD	680	45.120 CD	77	1104.1 H
82	10.120 C	210	21.120 D	680	104.12 D	135	42.120 D	570	44.120 D	470	1054.1 I

Table 3a: Mean significance differences among different maize inbred lines for various crop yielding traits

Lines	100-grain weight	Lines	Internode Diameter	Lines	Internode Length	Lines	Leaf Length	Lines	Leaf Width	lines	Leaf area
640	30.120 A	640	7.3200 A	210	19.120 A	210	89.120 A	210	13.120 A	210	577.87A
82	29.120 A	82	7.1200 A	470	19.120 A	590	87.120 AB	680	13.120 A	680	557.62B
135	28.120 AB	590	7.1200 A	135	18.120 AB	680	86.120 AB	590	12.620 AB	590	533.25C
280	28.120 AB	77	6.6200 AB	280	18.120 AB	470	84.120 AB	280	12.120 AB	470	484.12D
530	28.120 AB	570	6.6200 AB	510	18.120 AB	600	84.120 AB	410	12.120 AB	600	484.12D
570	28.120 AB	280	6.5200 AB	410	17.120 ABC	510	83.120 AB	470	12.120 AB	510	478.12DE
510	27.120 AB	600	6.5200 AB	530	17.120 ABC	410	82.120 BC	510	12.120 AB	410	472.12 E
590	27.120 AB	680	6.5200 AB	600	17.120 ABC	640	82.120 BC	600	12.120 AB	640	472.12E
600	27.120 AB	470	6.4200 ABC	680	17.120 ABC	570	81.120 BC	640	12.120 AB	570	437.25F
410	26.120 AB	210	6.3200 ABC	82	16.120 C	280	76.120 CD	82	11.620 ABC	280	436.12F
680	26.120 AB	530	6.3200 ABC	570	16.120 C	530	73.120 DE	135	11.620 ABC	82	386.62 G
77	24.120 AB	510	6.2200 ABC	590	15.120 CD	82	72.120 DE	570	11.620 ABC	530	376.72 H
470	24.120 AB	135	6.1200 ABC	640	15.120 CD	135	67.120 E	530	11.320 ABC	135	358.50I
210	22.120 B	410	6.1200 ABC	77	14.120 D	77	59.120 F	77	11.120 ABC	77	292.87J

Table 3b: Mean significance differences among different maize inbred lines for various crop yielding traits

Lines	Peduncle Length	Lines	Seed rows per cob	Lines	Seed thickness	Lines	Seed Width	Lines	Days to Silk emergence	Lines	Seeds Per Row
530	24.120 A	210	18.120 A	135	5.4200 A	510	12.520 A	210	54.120 A	570	47.120 A
640	24.120 A	280	18.120 A	82	5.3200 A	530	12.320 A	510	54.120 A	410	46.120AB
680	24.120 A	410	18.120 A	410	5.3200 A	82	12.120 A	590	54.120 A	680	46.120AB
82	23.120 AB	82	17.120 AB	280	5.1200 A	135	12.120 A	410	53.120 AB	82	45.120ABC
510	23.120 AB	135	17.120 AB	510	5.1200 A	600	12.120 A	530	52.120 ABC	280	45.120ABC
570	23.120 AB	590	17.120 AB	680	5.1200 A	640	12.120 A	600	52.120 ABC	530	45.120ABC
135	22.120 AB	510	16.120 ABC	530	4.9200 AB	280	11.120 AB	82	51.120 ABC	135	44.120ABC
280	22.120 AB	530	16.120 ABC	570	4.9200 AB	410	11.120 AB	280	51.120 ABC	510	44.120ABC
410	22.120 AB	570	16.120 ABC	600	4.8200 ABC	680	11.120 AB	640	51.120 ABC	640	44.120ABC
600	22.120 AB	600	16.120 ABC	210	4.3200 BC	470	10.420 ABC	77	49.120 ABC	210	42.120ABC
210	21.120 AB	640	16.120 ABC	590	4.3200 BC	77	10.120 ABC	135	49.120 ABC	470	42.120ABC
590	21.120 AB	470	15.120 ABC	640	4.3200 BC	570	10.120 ABC	470	49.120 ABC	600	42.120ABC
77	20.120 ABC	77	14.120 C	470	4.2200 BC	590	10.120 ABC	680	47.120 BC	77	40.120 BC
470	20.120 ABC	680	13.120 D	77	4.1200 C	210	9.9200 C	570	46.120 C	590	39.120 C

Table 3c: Mean significance differences among different maize inbred lines for various crop yielding traits

Lines	Tassel stalk Length	Lines	Cob per plant	Lines	Cob diameter	Lines	Seed length	Lines	Days to Tasseling	Lines	Cob weight	Lines	Plant Height
77	14.120 A	135	2.000 A	280	5.620 A	82	16.120 A	77	53.120A	570	129.12 A	210	239.12A
470	14.120 A	210	2.000 A	210	5.520 A	135	16.120 A	470	50.120AB	280	127.12 AB	470	224.12B
210	13.120 AB	570	2.000 A	410	5.320 AB	510	16.120 A	210	49.120ABC	680	126.12AB	280	214.12C
510	12.420 ABC	590	2.000 A	590	5.320 AB	530	16.120 A	590	49.120ABC	410	124.12ABC	510	209.12CD
530	12.120 ABC	280	1.667AB	135	5.220 ABC	600	16.120 A	510	46.120BCD	82	123.12ABC	135	204.12DE
570	12.120 ABC	410	1.667 AB	530	5.220 ABC	640	16.120 A	530	46.120BCD	135	121.12BC	410	199.12EF
590	12.120 ABC	600	1.667 AB	600	5.220 ABC	280	15.120 B	600	46.120BCD	530	119.12 CD	680	194.12 F
640	12.120 ABC	82	1.333 AB	82	5.120 ABC	410	15.120 B	640	46.120BCD	640	119.12CD	530	184.12 G
680	12.120 ABC	470	1.333 AB	510	5.120 ABC	570	15.120 B	410	45.120BCD	510	114.12DE	600	179.12GH
410	11.820 C	510	1.333 AB	570	5.120 ABC	680	15.120 B	570	45.120BCD	600	114.12DE	82	174.12HI
82	11.620 C	530	1.333 AB	640	5.120 ABC	77	14.120 AB	680	45.120BCD	470	109.12EF	590	169.12 I
600	11.620 C	640	1.333 AB	470	4.920 C	470	14.120 AB	82	44.120BCD	77	104.12FG	570	154.12 J
280	11.320 C	77	1.000 B	680	4.820 C	210	13.120 C	280	43.120CD	590	104.12FG	640	149.12 J
135	10.620 D	680	1.000 B	77	4.320 D	590	13.120 C	135	42.120D	210	102.12 G	77	134.12 K

Table 4: Correlation among various morphological and crop yielding traits in maize

Traits	Branches	CL	DTM	EFL	FTD	GY	HGW	IND	IL	LA
CL	0.5667*									
DTM	0.4958*	0.459*								
EFL	0.7655*	0.4126*	0.4521*							
FTD	0.5353*	0.6235*	0.5319*	0.6341*						
GY	0.7498*	0.4808*	0.8401*	0.9484*	-0.0525					
HGW	0.6541*	0.8982*	0.3363*	0.4339*	0.6607*	0.8712*				
IND	0.8481*	0.7899*	0.4646*	0.7216*	0.7686*	-0.0034	0.87*			
INL	0.6643*	0.708*	0.4286*	0.5819*	0.7568*	0.0465	0.7082*	0.8834*		
LA	-0.0418	0.0617	0.1565	0.4111*	0.2103*	0.8055*	-0.1133	0.0481	0.1909	
LL	0.2553*	0.4068*	0.3765*	0.6036*	0.4691*	0.7035*	0.2429*	0.3833*	0.4848*	0.9065*
LW	0.8147*	0.77*	0.4834*	0.7513*	0.7876*	0.7061*	0.806*	0.9804*	0.9297*	0.2021*
PH	-0.1638	-0.0261	0.0517	-0.02	0.3006*	-0.1002	-0.1256	0.05	0.483*	0.4801*
PL	0.704*	0.8886*	0.4155	0.6029*	0.7145*	0.2854	0.9255*	0.9318*	0.8404*	0.0642
SRPC	0.6768*	0.7365*	0.4845	0.6129*	0.8644*	0.9073*	0.8118*	0.912*	0.8901*	0.0783
ST	0.2295*	0.7375*	0.2439	-0.0154	0.4542*	0.5354*	0.6808*	0.5364*	0.6089*	-0.0968
SW	0.7346*	0.8688*	0.4374	0.5795	0.7962*	0.9469*	0.9112*	0.9569*	0.8947*	-0.0228
Silk	0.5917*	0.6295*	0.5543*	0.6904*	0.9912*	-0.0955	0.652*	0.7993*	0.7752*	0.2127*
SPR	0.5675*	0.8573*	0.4009*	0.3792*	0.5325*	0.9047*	0.8378*	0.8118*	0.8117*	0.0153
TSL	0.8894*	0.6625*	0.5317*	0.7758*	0.717*	-0.1281	0.7178*	0.9579*	0.8768*	0.0533
CPP	-0.1716	-0.1224	-0.017	-0.0279	0.0272	0.7746*	-0.0927	-0.1262	-0.0386	0.167
CD	0.7088*	0.7904*	0.4445*	0.6641*	0.8471*	0.9823*	0.8422*	0.94*	0.9327*	0.1755
SL	0.7276*	0.8963*	0.4255*	0.5351*	0.7288*	0.7148*	0.9277*	0.9457*	0.8745*	-0.0754
TED	0.8679*	0.3471*	0.5033*	0.846*	0.5925*	-0.3851	0.4173*	0.7688*	0.6288*	0.0486
CW	0.1425	0.6746*	0.0434	-0.1602	0.0452	0.9294*	0.6252*	0.3498*	0.3441*	-0.1259

LL	LW	PH	PL	SRPC	ST	SW	Silk	SPR	TSL	CPP	CD	SL	TM
0.5028*													
0.4321*	0.2008												
0.3966*	0.9182*	0.0426											
0.4012*	0.9117*	0.2507	0.8431*										
0.122	0.5469*	0.2379*	0.6815*	0.6055*									
0.3247*	0.9378*	0.1115	0.9598*	0.8929*	0.6678*								
0.4739*	0.8196*	0.2798*	0.7236*	0.8797*	0.4205*	0.808*							
0.3348*	0.8106*	0.1525	0.9201*	0.7798*	0.7919*	0.8541*	0.5436*						
0.3702*	0.95*	0.1012	0.8425*	0.8391*	0.3871*	0.8879*	0.7663*	0.7278*					
0.1427	-0.0943	0.1639	-0.1611	0.0895	0.002	-0.1608	0.0005	-0.1184	-0.1908				
0.4974*	0.9551*	0.2793	0.903*	0.9785*	0.6217*	0.9265*	0.8614*	0.8271*	0.8656*	0.0431			
0.2844*	0.917*	0.0581	0.9657*	0.8722*	0.6968*	0.9922*	0.7435*	0.8915*	0.8704*	-0.1659	0.9068*		
0.278*	0.7542*	-0.0415	0.5581*	0.6098*	-0.0039	0.6338*	0.6607*	0.3652*	0.8938*	-0.2113	0.6154*	0.5946*	
0.068	0.3255*	-0.0277	0.568*	0.3476*	0.7641*	0.4433*	0.0291	0.7737*	0.1812	-0.0627	0.3987*	0.5241*	-0.2303*

*** = Significant at 5% probability level, CL = Cob length, DTM = Days taken to maturity, EFL = Exertion above flag leaf, FTD = 50% tassel emergence, GY = Grain yield, HGW = 100-grain weight, IND = Internode diameter, INL = Internode length, LA = Leaf area, LL = Leaf length, LW = Leaf width, PH = Plant height, PL = Paduncle length, ST = Seed thickness, SW = Seed width, SL = Seed length, TSL = Tassel stalk length, CPP = Cobs per plant, SPR = Seeds per row, CD = Cob diameter, TED = Tassel emergence days, Branches = No. lateral tassel branches, Silk = 50% silk emergence days, CW = Cob weight, SRPC= Seed rows per cob**

Table 5: Analysis of variance for various morphological and grain yield traits in maize F_1 hybrids

Source	DF	Days to Maturity	Cob length	Cob diameter	Cob weight	Exertion flag leaf length	100-grain weight	Tassel Lateral branches	Cobs per plant	Peduncle length	Seed rows per cob	Seeds per row
Genotypes	15	92.9687	24.08333	7.58219	1822.83	37.2187	59.1354	9.91667	4.1249	5.3	5.0333	22.325
Error	16	16.0203	3.95237	0.52546	912.3	2.9462	23.1437	2.64373	0.1382	0.4717	0.0423	0.7017

* = Significant at 5% probability level

Table 5a: Analysis of variance for various morphological and grain yield traits in maize F_1 hybrids

Source	DF	50% Silk Emergence days	Tassel Emergence days	Yield kg/acre	Leaf area	Leaf length	Leaf width	Internode length	Internode diameter	Plant height	Tassel length	Tassel stalk length
Genotypes	15	10.3667	9.8666	111808	3930.02	5.2	1.1479	8.2333	3.1459	73.8333	9.6583	10.2583
Error	16	0.4127	0.2788	17.11	162.52	0.1767	0.017	0.7031	0.1138	0.1117	0.0707	0.4017

* = Significant at 5% probability level

Table 6: Estimations of genetic components for various morphological and grain yield traits in maize F_1 hybrids

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h^2bs%	GA%
Days to maturity	92.969*	110.170	25.649	48.251	41.670	61.501	16.020	38.133	61.554	6.330
Cob Length	24.083*	23.861	6.710	53.031	10.663	66.848	3.952	40.699	62.933	15.115
Cob Diameter	7.582*	4.904	2.352	69.255	2.878	76.600	0.525	32.732	81.740	49.619
Cob Weight	1822.83*	156.490	303.510	139.265	1215.810	278.734	912.30	241.449	24.964	9.762
Exertion Flag leaf length	37.219*	47.673	11.424	48.953	14.370	54.903	2.946	24.860	79.498	11.094
100-grain weight	59.135*	27.423	11.997	66.143	35.141	113.201	23.144	91.867	34.140	12.952
Tassel lateral Branches	9.917*	13.111	2.424	43.001	5.068	62.173	2.644	44.905	47.835	14.415
Cobs Per Plant	4.125*	2.486	1.329	73.119	1.467	76.827	0.138	23.580	90.580	77.465
Peduncle Length	5.300*	20.361	1.609	28.115	2.081	31.971	0.472	15.221	77.334	9.616
Seed Rows Per Cob	5.033*	12.611	1.664	36.321	1.706	36.780	0.042	5.792	97.520	17.726
Seeds Per Row	22.325*	42.798	7.208	41.038	7.909	42.989	0.702	12.805	91.128	10.509
50% Silk Emergence Days	10.367*	110.170	3.318	17.354	3.731	18.402	0.413	6.120	88.938	2.736
50% Tassel Emergence Days	9.867*	48.611	3.196	25.641	3.475	26.736	0.279	7.573	91.976	6.190
Grain Yield	111808.0*	3053.010	37263.63	349.364	37280.74	349.444	17.110	7.486	99.954	11.094
Leaf Area	3930.02*	419.140	1255.833	173.096	1418.353	183.955	162.52	62.269	88.542	13.962
Leaf Length	5.200*	62.736	1.674	16.337	1.851	17.178	0.177	5.307	90.454	3.443
Leaf Width	1.148*	8.892	0.377	20.590	0.394	21.049	0.017	4.372	95.685	11.854
Internode Diameter	8.233*	20.111	2.510	0.353	3.213	0.089	0.703	18.698	78.118	12.220
Internode Length	3.146*	4.594	1.011	46.904	1.125	49.474	0.114	15.739	89.880	36.410
Plant Height	73.833*	162.360	24.574	38.904	24.686	38.993	0.112	2.623	99.548	5.346
Tassel Length	9.658*	47.298	3.196	25.994	3.267	26.280	0.071	3.866	97.836	6.561
Tassel Stalk Length	10.258*	14.548	3.286	47.523	3.687	50.344	0.402	16.617	89.106	20.641

* = Significant at 5% probability level, Mean Sum of Squares (MS), Grand mean (GM), Genotypic variance (GV), Genotypic coefficient of variance (GCV %), Phenotypic variance (PV), Phenotypic coefficient of variance (PCV %), Environmental Variance (EV), Environmental coefficient of variance (ECV %), Broad sense heritability (h^2bs %), Genetic advance (GA)

Table 7: Mean significance differences among different maize F_1 hybrids for various crop yielding traits

Crosses	Days to Maturity	Cob length	Cob diameter	Cob weight	Exertion flag leaf length	100-grain weight	Tassel Lateral branches	Cobs per plant	Peduncle length	Seed rows per cob	Seeds per row	50% Silk Emergence days
77×570	120.49A	22.486DE	4.4856B	129.49L	49.486B	22.486I	12.486DE	2.0851ABC	18.486DE	11.486 E	39.486GH	49.486B
77×680	110.49C	24.486C	4.9856AB	144.49H	51.486A	24.486GH	11.486EF	2.1852AB	20.486BC	12.486DE	40.486 G	52.486A
210×82	115.49B	22.486DE	4.4856B	126.49M	46.486DE	22.486I	14.486BC	2.1856AB	19.486CD	11.486 E	38.486 H	48.486BC
210×460	110.49C	28.486A	6.1856A	240.49A	43.486G	38.486A	12.486DE	2.6856A	17.486 E	16.486 A	50.486 A	47.486CD
590×510	100.49E	24.486C	5.4856AB	178.49C	44.486FG	34.486C	15.486AB	2.1856AB	18.486DE	14.486BC	42.486DE	48.486BC
210×530	105.49D	26.486B	5.2857AB	163.49E	45.486EF	33.486C	12.486DE	2.3856BC	22.486A	12.486DE	45.486 B	49.486 B
210×600	120.49A	22.486DE	4.4856 B	138.49J	48.486BC	24.486GH	16.486 A	2.2856AB	0.486BC	11.486 E	42.486DE	51.486 A
210×640	105.49D	21.486E	4.4856 B	132.49K	46.486DE	23.486HI	15.486AB	2.1856ABC	20.486BC	11.486 E	40.486FG	49.486 B
470×135	120.49A	26.486B	5.8857AB	220.49B	44.486FG	36.486 B	12.486DE	2.5856B	19.486CD	5.486AB	49.486 A	49.486 B
470×570	105.49D	22.486DE	4.7857AB	148.49G	45.486EF	25.486FG	10.486 F	2.0856ABC	18.486DE	12.486DE	41.486EF	47.486CD
470×680	110.49C	24.486C	5.0856AB	169.49D	47.486CD	28.486 D	13.486CD	2.0856ABC	22.486 A	3.486CD	3.486CD	49.486 B
590×82	120.49A	22.486DE	4.4856B	140.49I	52.486A	26.486EF	11.486EF	2.2856AB	20.486BC	11.486 E	40.486FG	51.486 A
590×410	90.486F	23.486CD	4.5856 B	139.49IJ	49.486B	24.486GH	14.486BC	2.1856AB	22.486 A	11.486 E	42.486DE	44.486 E
210×510	105.49D	22.486DE	4.4856 B	133.49K	48.486BC	23.486HI	10.486F	2.0856ABC	22.486 A	11.486 E	40.486FG	6.486 D
590×530	105.49D	24.486C	4.7857AB	158.49F	47.486CD	27.486DE	14.486BC	2.1856AB	0.486BC	12.486DE	44.486BC	44.486 E
560×600	115.49B	22.486DE	4.4853B	139.49IJ	51.486A	22.486I	11.486EF	2.0856ABC	21.486AB	11.486 E	42.486DE	47.486CD

Table 7a: Mean significance differences among different maize F_1 hybrids for various crop yielding traits

Crosses	Tassel Emergence days	Yield kg/acre	Leaf area	Leaf length	Leaf width	Internode length	Internode diameter	Plant height	Tassel length	Tassel stalk length
77×570	51.486DE	2750.5P	444.71ABC	62.486CD	9.4856 AB	18.48EF	4.085AB	159.49 F	48.486BC	12.486FG
77×680	53.486BC	2930.5 K	385.12 BC	60.486 E	8.4856 BC	20.486CD	4.1856AB	164.49 D	46.486 DE	14.486 DE
210×82	49.486FG	2800.5 O	507.31 A	64.486 AB	11.486 A	16.486 G	4.1856AB	145.49 H	47.486 CD	13.486 EF
210×460	52.486CD	3585.5 A	374.42 C	62.486 CD	7.9856 C	21.486BC	3.9857AB	166.49 C	46.486 DE	15.486 CD
590×510	54.486 B	3230.5 C	391.49BC	65.486A	8.4856BC	23.486A	5.8856A	159.49 F	50.486 A	18.486 A
210×530	51.486DE	3170.5 D	404.21 BC	63.486 BC	8.485BC	18.486EF	4.0856AB	160.49 F	47.486 CD	11.486 GH
210×600	48.486G	2986.5 I	385.12 BC	60.486 E	8.4856BC	19.486DE	4.1857AB	162.49 E	49.486 AB	14.486 DE
210×640	51.486DE	2846.5 M	397.85BC	62.486 CD	8.4856 BC	22.486AB	4.4856AB	172.49 A	48.486 BC	16.486 BC
470×135	56.486 A	3474.5 B	515.17 A	64.486 AB	11.485AB	21.486BC	4.7857AB	168.49 B	47.486 CD	15.486 CD
470×570	52.486CD	2980.5 J	391.49 BC	61.486 DE	8.4856BC	20.486CD	4.3857AB	164.49 D	47.486 CD	13.486 EF
470×680	50.486 EF	3140.5 F	458.94 AB	64.486AB	9.4856 AB	20.486CD	4.5856AB	160.49 F	48.486 BC	16.486 BC
590×82	54.486 B	3027.5 H	397.85BC	62.486 CD	8.4856 BC	19.486DE	4.4856AB	159.49 F	50.486 A	15.486 CD
590×410	52.486CD	3082.5 G	458.94 AB	61.486DE	10.486 AB	21.486BC	4.5856AB	162.49 E	42.486 F	15.486 CD
210×510	50.486EF	2876.5L	397.85 BC	62.486 CD	8.4856BC	17.486FG	4.6856AB	157.49 G	45.486 E	17.486 AB
590×530	48.486 G	3154.5E	410.58 BC	64.486AB	8.4856 BC	22.486AB	4.4856AB	165.49CD	43.486 F	11.486 GH
560×600	53.486BC	2810.5 N	385.12 BC	60.486 E	8.4856 BC	17.486 DE	4.5856AB	168.49 B	46.486 DE	10.486 H

Table 8: Correlation among various morphological and crop yielding traits in maize F_1 hybrids

Traits	DTM	CL	CD	CW	EFL	HGW	Branches	CPP	PL	SRPC	SPR	50% SED	FTD	GY	LA	LL	LW	IND	INL	PH	TL
CL	-0.0567																				
CD	0.0092	0.8327*																			
CW	0.0359	0.8708*	0.7218*																		
EFL	0.2273	-0.4773	-0.3739	-0.6364*																	
HGW	-0.0536	0.8878*	0.7628*	0.932*	-0.663*																
Branches	-0.1353	-0.017	0.1348	-0.047	-0.1896	0.0478															
CPP	0.0592	0.2518	0.676*	0.0154	0.1818	0.0936	0.2607														
PL	-0.2626	-0.1076	-0.0785	-0.3758*	0.456	-0.2801*	0.0412	0.2945*													
SRPC	0.0208	0.8654*	0.8876*	0.9345*	-0.5896*	0.8965*	0.0526	0.3015*	-0.3523*												
SPR	0.0124	0.8806*	0.7282*	0.9199*	-0.5304*	0.8657*	0.0169	0.1485	-0.1228	0.832*											
50%SED	0.6223*	0.0003	0.1594	-0.0581	0.2632*	0.0155	0.0375	0.2152	-0.05	0.0434	-0.1342										
FTD	0.0735	0.32*	0.4744*	0.4136*	0.0347	0.4331*	-0.3078*	0.2203	-0.1586	0.4711*	0.3143*	0.2609*									
GY	-0.1126	0.8604*	0.674*	0.9492*	-0.606*	0.9397*	0.0562	0.0021	-0.2473	0.8615*	0.9105*	-0.125	0.3487*								
LA	-0.0912	0.0583	0.2571	-0.1192	0.0805	-0.0983	0.3327*	0.5194*	0.3271*	0.0385	-0.0397	-0.1274	0.0426	-0.0688							
LL	-0.2561*	0.3027*	0.2926*	0.1667	-0.2	0.2065	0.2519*	0.297*	0.3308*	0.1963	0.2632*	-0.3849*	-0.0559	0.2811	0.8018*						
LW	-0.0363	-0.006	0.2375*	-0.185	0.1477	-0.1687	0.3322*	0.552*	0.3052*	-0.0017	-0.1142	-0.0484	0.0652	-0.1558	0.9881*	0.7017*					
IND	-0.0681	0.1765	0.2358	0.2315*	0.1607	0.0619	0.0418	0.24*	0.1502	0.2018	0.4215*	-0.1707	0.221	0.2049	-0.0245	0.0618	-0.0452				
INL	0.1036	0.1682	0.5421	-0.1205	0.2228*	0.0399	0.2955*	0.9007*	0.4568*	0.133	0.0642	0.3158*	0.1774	-0.1014	0.5121*	0.264*	0.5524*	0.0715			
PH	-0.0798	0.2005	0.2433*	0.3338*	-0.0311	0.2238	-0.0169	0.0823	-0.0095	0.2762*	0.4641*	-0.0093	0.3399*	0.2902*	-0.3531	-0.2119	-0.366	0.8477*	0.0098		
TL	0.673*	-0.0906	0.1088	-0.109	0.2716*	-0.0685	-0.0669	0.2225	-0.1146	0.0026	-0.2039	0.9661*	0.2114	-0.208	-0.162	-0.4112*	-0.0816	-0.1465	0.2609*	-0.0175	
TSL	-0.1218	0.2799*	0.2616*	0.1267	-0.1189	0.2244*	-0.0725	0.2162	0.4876*	0.1148	0.2166	0.2422*	0.0161	0.21	0.1767	0.3493*	0.1223	-0.108	0.2715*	0.0042	0.2035

*** = Significant at 5% probability level, CL = Cob length, DTM = Days taken to maturity, EFL = Exertion above flag leaf, FTD = 50% tassel emergence, GY = Grain yield, HGW = 100-grain weight, IND = Internode diameter, INL = Internode length, LA = Leaf area, LL = Leaf length, LW = Leaf width, PH = Plant height, PL = Paduncle length, ST = Seed thickness, SW = Seed width, SL = Seed length, TSL = Tassel stalk length, CPP = Cobs per plant, SPR = Seeds per row, CD = Cob diameter, Branches = No. lateral tassel branches, Silk = 50% silk emergence days, CW = Cob weight, SRPC= Seed rows per cob**

DISCUSSION:

Genetic characterization of crosses was another objective of the study by exploiting RAPD analyses. RAPD analyses were carried out to ensure the crossing of the genomes. Successes obtained in crosses were judged by RAPD. We found a substantial difference between the banding pattern of parents and offspring (Figure II, III, IV, V). This is in agreement to Asif *et al*., [8] and Takatsu *et al.,* [11] who found different RAPD pattern between parents and offspring of Gladiolus hybrids. Among RAPD markers, the band patterns in the hybrids were found not to be completely additive. A similar phenomenon also appears in the interspecific hybridization in Cyrtandra [12-15], and intraspecific crosses of sugarcane varieties [16]. Sources of polymorphisms in RAPD assay may include base changes within priming site sequence, deletions of priming site, insertions that render priming sites too distant to support amplification, and deletions or insertions that change the size of a DNA fragment without preventing its amplification also proposed by Williams *et al.,* [17]. Therefore, differences in markers from parents to offspring may be the result of DNA recombination, mutation, or random segregation of chromosome in meiosis processing during hybridization [18, 19]. Chromosomal crossing-over during meiosis may result in the loss of priming sites and thus fragments are present in parents but not in offspring [11]. Non-parental bands (Type VII fragments) may be generated from the recombination and mutation in meiosis processing during hybridization [18, 19] and may be also created by heteroduplex formation [12, 13]. It is useful in identification of new cultivars [18, 23]. Male specific bands (type III fragments) in the offspring indicate that offspring is true hybrid [20-23]. On the basis of similarity coefficient of RAPD data was used to generate dendrograms, which showed the similarity and relatedness among individuals but this relatedness does not relate with phenotypic characters noted in green house. This result suggest that RAPD analysis might not be sufficient to classify accurately, but it can be effectively used as a tool for breeding[10] in case of Gladiolus, it was found RAPD good enough for classification and grouping of cultivars into proper phenotypic groups by RAPD analysis, this may be because of high number of primers and high number of cultivars used in his analysis [13]. No RAPD fragments associated to the high yielding character was observed [20] for shattering resistance in *Brassica rapa*, possibly because of relatively small sample size and low number of primers used for the purpose [21].

It was suggested from results that higher tassel branches indicated that pollen production will be higher for the genotypes heaving higher tassel branches. Higher cob length indicated that the number grains per cob will be higher and caused to increase the grain yield of maize. Higher days taken to maturity and 50% silk emergence indicated that the genotypes showed late maturing characteristics [22, 24-26]. It was found from results that the F_1 crosses 590×510, 210×460 and 470×135 performed better for most of the studied yield related traits. It was suggested that the F_1 crosses 590×510, 210×460 and 470×135 may be used for the improvement of grain yield of maize. The traits like 100-grain weight, seeds per row, seed rows per cob, cob weight, cob length, cob diameter and cobs per plant played an important role in the grain yield of maize and hence the selection on the basis of these traits may be helpful to improve grain yield and production of maize [27-31]. It was suggested from results that the inbred lines with higher leaf length, leaf width, leaf area, internode length and internode diameter may be used for the development of synthetic and hybrids for higher green fodder yield of maize. Higher 100-grain yield indicated that the seed size for the inbred lines was higher and may be used as selection criteria to improve grain yield of maize[25].

It was revealed form results that higher seed width, seed length, seed rows per cob and seeds per row of cob indicated that the seed was with good health and higher reserved organic compounds and inbred lines may be used for the development of synthetic varieties and hybrids with higher grain yield [28, 29]. The results showed that higher cob diameter, seed length and cob weight indicated that the selection of inbred lines for developing higher yield maize synthetic varieties and hybrids may be helpful to improve crop yield and production. The higher seed length suggested that the seed to stover ratio will be higher that caused to improve grain yield in order to store organic compounds in the seed. The higher seed size indicated the selection may be helpful to develop large seeded maize genotypes [32-34]. Higher genetic advance indicated that the selection of inbred lines to fix the genetic effects may be helpful. The improvement in the traits will be increased in next generations as the genetic advance showed fixable gene action like additive type of gene action. The synthetic maize varieties may be developed on selecting inbred lines with higher genetic advance traits while the higher heritability may help to develop hybrids in maize as heritability here indicating dominance type of gene action [33]. Higher heritability indicated that the selection of F_1 hybrids may be helpful to improve grain yield of maize and also the heterotic effects may also be increased as broad sense heritability indicated the dominant gene action. Higher genetic advance indicated that the increase in the traits may be fixed in next generations to improve grain yield of maize as genetic advance revealed additive type of gene action. The selection of genotypes on the basis of genetic advance helped plant breeder and plant geneticist to develop synthetic varieties while heritability to develop hybrids [3, 27, 29]. The significant and strong correlations suggested that the development of higher grain yielding maize genotypes may be achieved on selecting strongly correlated traits with grain and its contributing traits. The positive correlations indicated that the increase in the grain yield may be fixed in next generations. The synthetic and hybrids of maize may be developed to improve grain yield. The negative correlation indicated that the traits significantly and strongly correlated with grain yield may not be helpful to improve grain yield. The negative correlation may be used to fix decrease in the negatively correlated [35, 36]. It was revealed from table 8 that significant and strong correlation was found among the traits including cob length, cob diameter, cob weight, 100-grain weight, grain yield, cobs per plant, seed rows per cob, leaf length leaf area leaf width, internode diameter and plant height. The positive and significant correlation of grain yield with its contributing traits indicated that the F_1 hybrids performed well for grain yield. The selection of parents may be helpful to improve grain yield of maize through the development of F_1 hybrids [23, 28, 29, 31, 37].

CONCLUSION

It was concluded from results that the higher heritability and genetic advance was found for yield related traits for parent inbred lines and F_1 hybrids. Significant and strong correlation was also observed among these traits. The F_1 crosses 590×510, 210×460 and 470×135 performed better for most of the studied yield related traits. The Four RAPD primers (R-8, R-15, S-13 and S-19) were used for molecular assessment of true hybrids and parents for RAPD analysis. Patterns of inheritance of RAPD fragments (bands) were noted and seven types of

RAPD fragments were identified. Clustering was performed and dendrograms were generated using RAPD data. The adopted pattern of molecular analysis and multiplication is an ideal way of exploiting variability which usually arises during the hybridization of two parental lines.

CONFLICT OF INTEREST

The authors showed no conflict of interest in the manuscript publication.

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