MOLECULAR AND GENETIC ANALYSES OF THE CROSS BETWEEN MAIZE AND TEOSINTE

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ABSTRACT

The aim of this investigation was to obtain a hybrid between Zea mays and Zea mexicana of high yield to estimate genetic parameters for yield and its components in this hybrid and to measure variation between genotypes on the molecular level. The F_1 and its reciprocal between maize single cross and line of teosinte (136A) which was derived from crossing the local race of teosinte and an introduced race from CIMMYT called Central Plateau were obtained 2005 season, at Serw Agric. Res. Station of ARC. Grains of F_2 were obtained in 2006. In the summer season of 2007 and 2008, 5 entries (2 parents, F_1 and F_2) were field evaluated at the same station. Young and fresh seedling samples of the 4 genotypes were used to isolate DNA and RAPD analysis was performed. The results showed that the teosinte line (136A) had the highest number of tillers $plant^{-1}$, number of leaves $plant^{-1}$ and number of ears $plant^{-1}$ with the means of 12.58, 85.58 and 107.18, respectively. While, the single cross 30K8 was the highest for 100-grain weight and grain yield $plot^{-1}$ with the means of 36.33 g and 6.01 kg $plot^{-1}$, respectively. The differences between G.C.V and P.C.V were narrow, suggesting little effect of environment on these traits. Heritability in broad sense expressed high values for the studied traits, ranging from 80.56 to 99.5%. This indicated that these characters were less affected by environment and largely influenced by different components of genetic variance. RAPD was found to be a reliable for the analysis of genetic diversity in this study. Cluster analysis divided the samples into two distinct groups (I and II). The group I included teosinte line (136A) and the maize single cross 30K8. On the other hand, the group two included F_1 Zea mexicana x Zea mays and reciprocal F_1 Zea mays x Zea mexicana. The percentage of polymorphism in this investigation was 7.3%. Genetic distances were on average greater (0.93) for genotypes 1 and 2 than for genotypes 3 and 2 (0.84) and genotypes 3 and 4 (0.90).

Key words : *Zea mays, Zea mexicana, Genetic parameters, DNA isolated, RAPD analysis.*

INTRODUCTION

The successful introgression of this germplasm (maize and teosinte) would help to expand both the limited germplasm base from which modern crop cultivars evolved (Frankel 1974) and the narrow genetic base which continues to be the primary source of elite lines and highly adapted breeding populations. When germplasm from a wild species or an exotic race contributes to a breeding program, usually the transfer of qualitative rather than quantitative genes is involved (Stalker 1980). During the seventies of last century the study about the possibility of hybridization

between maize and teosinte and its impact on the chromsome segregation has been started (De Wet *et al* 1970).

Aulicino and Magoja(1991), crossed a maize inbred to Balsas teosinte and to Guatemala teosinte. They evaluated parents and F_1's and found that heterosis was high for most traits and ranged from 3.4% for productive nodes/ tiller to 104.1% for number of ears in the uppermost node in Balsas teosinte cross. Sreekumer and Bai-Dis (1995) evaluated 9 fodder maize types and found high estimates of genotypic coefficient of variation, heritability and genetic advance, for plant height. The use of the polymerase chain reaction (PCR) in generating random amplified polymorphic DNA (RAPD) by Williams *et al* (1990) has already proven valuable in genetic analysis. The amplified DNA fragments represent anonymous regions distributed randomly throughout the genome of the organism and provide a fingerprint of the plants being investigated (Rossetto *et al* 1997 and Terzi 1997). The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for the development of populations or to facilitate the identification of diverse parents to cross in hybrid combination in order to maximize the expression of heterosis (Smith *et al* 1990 and Santos *et al* 1994). Many investigators employed RAPD-PCR to assess genetic fingerprint in wide range of plants (Aimin *et al* 1998, Rashed *et al* 1998, Barkat, *et al* 1999, Aly *et al* 2000, Abdel-Tawab *et al* 2001, Hassan 2001 a & b, cheng *et al* 2002 and Zhuravlev *et al* 2003). They reported that polymorphisms detected by arbitrarily primed PCR are useful molecular markers in population studies.

De Wet and Harlan (1976) reported in cytogenetic evidence that teosinte (*Zea mays ssp. mexicana* (Schrad.) Iltes) and maize (*Zea mays* L.) are conspecific. In addition the compartive morphological and genetical studies indicated that it is more probable that maize originated from a teosinte-like ancestor under domestication, than that a maize-like plant gave rise to teosinte through a series of mutations. On the other hand, (Lubberstedt *et al* 1998) studied application from maize to teosinte and their hybrids. Also, they made a cross between the maize inbred A188 and an individual of *Zea mays* ssp.*mexicana*, and obtained amplification products for maize and teosinte originated from the same genomic location for each of nine microsatellites investigated. The results from this work indicates the application of maize microsatellites to teosinte for fingerprinting or marker-assisted introgression of genomic regions from teosinte into cultivated maize appears promising. In addition Matsuoka *et al* (2002) have successfully used maize SSRs in maize and teosinte for measuring intraspecific variation and studying phylogeographic relationships. Recent studies in maize demonstrated that MAS could be more economical and yield greater gains than phenotypic selection,

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especially for traits that were difficult or costly to measure (Dreher *et al* 2000 and Yousef and Juvik 2001). Lanaza *et al*(1997) evaluated the genetic diversity of 18 maize inbred lines, and determine the correlation between genetic distance and single cross hybrid performance. They used the RAPD techniques, genetic divergence was determined using jaccard's similarity coefficient, and a final dindrogram was constructed using a weighted pair group with arthimetical averages (UPGMA). Cluster analysis divided the samples into three distanict groups that were confirmed by principal coordinate analysis. The genetic distances were correlated with important agronomic traits for single cross hybrids and heterosis.

The aim of this investigation was to obtain a hybrid between *Zea mays* and *Zea mexicana* of high yield, estimate the genetic parameters for yield and its components and measure variation in molecular level.

MATERIALS AND METHODS

Field trials

Genetic materials used in this study involved one teosinte line (136A) derived from segregating generations of hybrid between local race (Rayana) and Central Plateau, and one commercial corn single cross hybrid Pioneer 30K8. F_1 and its reciprocal cross were obtained at Serw Agricultural Research Station in 2005. The seeds production from these entries which included 2 parental genotypes, F_1, F_{1r} and F_2 were divided to evaluate at Serw Agricultural Research Station in the two years; 2007 and 2008.

A randomized complete blocks design was used in both years with three replications, Plot size was one row, 4m length and 80 cm apart, 30 cm between hills. Seeds were planted along the row at the rate of three kernels per hill. Seedlings were thinned to one plant per hill after 21 days from planting. All agronomic field practices were applied as recommended for maize cultivation.

Observations were recorded on 10 plants chosen at random from each plot. The measurements included the following traits: number of tillers per $plant^{-1}$, number of leaves $plant^{-1}$, 100 grain weight (g) and number of ears $plant^{-1}$ and grain yield $plot^{-1}$. Several analyses of variances were made in order to test the significant of the differences among the studied genotypes. In addition a combined analysis of variance across two years was computed for the genotypes according to Cochran and Cox (1980). The differences between any two means were tested for significance using the least significant difference values (LSD) test at both 5% and 1% levels of probability. Percentage of heterosis (H) was determined as the increase in the mean of the F_1 hybrids over mid-parents (M.P) or better parent (B.P) as follows: Heterosis over mid parent: H MP% = and heterosis over the better parent, H BP% = . The percenuage of inbreeding depression was measured

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as the deviation of F_2 generation than from the corresponding F_1 hybrid. Potence ratio was calculated to determine the degree of dominance and its direction according to Mather and Jinks (1982) as follows:

$$\text{Potence ratio (P)} = \frac{\dot{F}_1 - \frac{1}{2}(\dot{P}_1 + \dot{P}_2)}{\frac{1}{2}(\dot{P}_1 - \dot{P}_2)}$$

Absence of dominance is considered when (p) = zero, then genes are called additive genes (Allard1960) complete dominance is assumed when (P) = ±1, Partial dominance when (P): -1 > P>+1. Over dominance - 1>P<+1. Heritability in broad (H_b) sense is referred to as the ratio of genetic variance to total variance as follows according to Johanson et al (1955). Heritability in broad sense ($H_b\%$) = , where genotypic σ^2 g and phenotypic σ^2 p variances were calculated according to (Al - Jibouri et al 1958).

Genotypic and phenotypic coefficient of variability (GCV and PCV) were measured according to Burton (1952). Genetic advance under selection (Gs) was estimated using a selection intensity of 10% according the formula, Gs%=

DNA was isolated from 50 mg of young leaves using Qiagen Kit (Hilden, Germany) for DNA extraction. The concentration and purity were determined. The purity of DNA for all samples was between 90-97% and the ratio between 1.7-1.8. The RAPD analysis was performed according to Williams et al (1990).The primers used were 10-mer oligonucleotide. Ten primers were selected as potentially useful. The sequences of the used primers are shown in Table (1). Randomly amplified polymorphic DNA (RAPD) was done according to the following procedure.

Table 1. List of the primer names and their nucleotide sequences used in the study for RAPD procedure.

No	Name	Sequence	No	Name	Sequence
1	OP-A09	5′ GGG TAA CGC C 3′	6	OP-D11	5′ AGC GCC ATT G 3′
2	OP-A10	5′ GTG ATC GCA G 3′	7	OP-C08	5′ TGG ACC GGT G 3′
3	OP-A12	5′ TCG GCG ATA G 3′	8	OP-B04	5′ GGA CTG GAG T 3′
4	OP-B13	5′ TTC CCC CGC T 3′	9	OP-O 18	5′ GGG AGC GCT T 3′
5	OP-A19	5′ CAA ACG TCG G 3′	10	OP-B05	5′ TGC GCC CTT C 3′

DNA isolation procedure

Young and fresh seedling samples were used for DNA isolation according to Dellaporta *et al* 1983). For RAPD analysis, PCR amplifications were carried out in a total volume 25 µl. Electrophoresis sample preparation were 15.0 µl PCR product, 5.0µl Loading dye (Sambrook and Russell 2001). Gel preparation agarose was cooked and specific amplification products were scored as present (1) or absent (0) for each of the four genotypes with ten primers (Table 1). Genetic similarity between all genotypes were estimated by simple matching co-efficient (Sokal and Michener 1958). Finally the gel was visualized and photographed using gel documentation system. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C, 1 min at 36° C, and 2 min at 72° C. The reaction was finally stored at 72° C for 10 min. PCR products for all samples were then electrophorased (15 ul) at 75 constant volt for 2h through 1% agarose containing ethedium bromide (0.5 ug/ml). The RAPD patterns were visualized with UV transilluminator. Gel was analyzed using gel documentation system. The different molecular size of bands was determined against DNA marker (Promega G317A). Construction of the dendrogram tree was performed using the un-weighted pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS program version 10.

RESULTS AND DISCUSSION

An analysis of variance was made separately each year (Table 2). In addition to analysis of variance across two years (Table 3). Highly significant differences were found between studied genotypes for all studied traits. The significance of mean squares of genotypes is an indicator to the presence of genetic variation among these genotypes.

However, years and genotypes by year's interaction mean squares were highly significant in most of studied traits except for grain yield plot[1] weight. This indicated that these genotypes gave different performances under conditions of different years with respect to these traits.

Table 2. Mean squares for all studied traits from the data in year1 (Y1) and year2 (Y2).

	df	Tillers plant[1]		Leavesplant[1]		100G.W (g)		Ears plant[1]		Grain yieldKgplot[1]	
		Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Reps	2	0.003	0.06	2.06	7.21	0.006	124.3	3.30	22.75	0.139	0.092
Geno.	4	66.92**	67.7**	2171.3**	2703.6**	335.3**	211.9**	4363.5**	6613.0**	15.92**	13.6**
Error	8	0.07	0.05	9.05	9.2	0.4	0.7	21.7	35.1	0.16	0.09

*,** Significant at 0.05 an⁻¹ 0.01 levels of probability , respectively.

Table 3. Combined analysis of variance cross years for all studied traits.

S.O.V	df	Tillersplant[-1]	Leavesplant[-1]	100G.W(g)	Earsplant[-1]	Grain yield Kg plot[-1]
Year	1	0.427*	181.0**	1.4	1713.2**	0.03
R/ year.	4	0.021	4.6	0.35	38.1	0.2
Genotype	4	.134.2**	4789.1**	537.8**	10623.0**	29.23**
Geno*year	4	0.304**	85.8**	9.4**	353.54**	0.3
Error	16	0.06	9.11	0.54	28.4	0.12

The mean performance of the studied genotypes was determined separately for each year and obtained results are presented in Table (4). However data combined across two years for all studied traits are presented in Table (5). The means showed that, the teosinte line (136.A) was the highest for number of tillers per plant, number of leaves per plant and number of ears per plant with the means of 12.58, 85.58, and 107.18, respectively, while the maize hybrid (30 k8) was the highest for 100 grain weight and grain yield per plot with the mean of 36.33 g and 6.01 Kg plot[-1], respectively. The F_1 hybrid was higher than F_2 generation due to inbreeding depression.

Table 4. Mean performance of the studied genotypes at first (Y_1) and second (Y_2) for the studied traits.

Genotype	Tillersplant[-1]		Leavesplant[-1]		100G.W(g)		Earsplant[-1]		Grain yield Kg plot[-1]	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
136-A	12.5	12.67	82.26	88.9	6.5	5.70	102.1	112.3	1.23	1.22
30 k8	1.00	1.00	15.0	14.73	31.96	27.4	2.0	1.94	5.94	6.08
F_1	2.55	3.56	39.13	56.4	10.07	10.53	79.44	119.56	5.64	4.86
F_{1r}	2.65	2.53	40.9	39.4	8.7	10.32	81.2	88.85	5.25	5.18
F_2	1.83	1.94	17.86	20.36	8.71	9.87	70.08	87.83	1.64	2.01
LSD 0.05	0.51	0.42	13.9	5.7	0.6	1.61	33.4	11.16	0.73	0.55

Table 5. Mean performance of the studied traits across two years.

Genotype	Tillers plant[-1]	Leavesplant[-1]	100G.W(g)	Ears plant[-1]	Grain yiel Kgplot[-1]
136.A	12.58	85.58	6.09	107.18	1.22
30 k8	1.55	14.9	36.33	1.99	6.01
F_1	3.05	47.75	10.30	99.50	5.25
F_{1r}	2.59	40.16	9.51	85.02	5.21
F_2	1.88	19.11	9.3	78.96	1.83
LSD 0.05	0.42	5.22	1.27	9.22	0.61

The Variances in terms of genotypic (V_G) and phenotypic (V_P) as well as, genotypic (G.C.V) and phenotypic (P.C.V) coefficient of variability, heritability in broad sense (h^2_b), potence ratio (P), heterosis relative to mid parent (HMP%) and better parent (HBP%) values; in addition to studied, inbreeding depression (I.D%) and genetic advance under selection using 10% selection intensity are presented in Table (6). Similarly, these parameters were determined from the combined data across two years for all studied traits (Table 7). The results revealed that the genotypic variance (V_G) relative to environmental variation (V_E) was large in magnitude for all traits. The differences between G.C.V and P.C.V were narrow, suggesting little effects of environments on these traits. Heritability in broad sense expressed high percentage for all studied traits ranging from 92.6 to 99.4%. This indicated that these characters were less affected by environment and largely influenced by components of genetic variance which may include additive, dominance and epistasis variances. This result is in agreement with the results obtained by Geiger et al (1986). The results exhibited relative both F_1 and F_{1r} positive and highly significant heterotic value over mid-parents (HMP) for number of ears per plant and grain yield plot^{-1} but number of tillers per plant and number of leaves per plant showed negative and highly significant (HMP) values. The heterosis values over mid parent ranged from − 61.71 % to 82.3 % for number of tillers per plant in F_{1r} and number of ears per plant for F_1 , respectively. This result is in agreement with the results obtained by Aulicino and Magoja (1991) in maize Balsas teosinte and maize Guatemala teosinte hybrids for number of tillers per plants and number of ears per plant.

The heterosis over better parent (HBP)in both F_1 and F_{1r} was negatively significant for grain yield per plant and highly significant for other traits except for number of ears per plant in F_1 which was insignificant. Concerning inbreeding depression (ID), positive and highly significant value for all studied traits except for 100 grain weight which was insignificant. Also, the results showed that potence ratio (P) was positive or negative and less than unity for all studied traits for F_1 and F_{1r} , indicating partial dominance and may explain the absence of heterosis in most of studied traits especially heterosis over better parent. The estimates of expected genetic advance (G.S) recommended that selection of 10% of plants would improve studied traits. The average of expected advance value for number of tillers per plant, number of leaves per plant, 100-grain weight, number of ears per plant and grain yield per plant are 9.4 , 54.6 , 18.5 , 80.02 , and 4.34 respectively. Thus, from the previous results, it could be concluded that selection in advanced segregating generations of this combination is good to improve these traits.

Table 6 .Genotypic (V_G) and phenotypic (V_P) variance, genotypic (GCV) and phenotypic (PCV) coefficient of variation, potence ratio (P), heterosis over mid parent (HMP) and better parent (HBP), inbreeding dipression (ID) and genetic advance (G_S) for all studied traits at the first (Y_1) and second (Y_2) year.

Parameter	Season	Tillersplant⁻¹	Leavesplant⁻¹	100G.W(g)	Earsplant⁻¹	Grain yield Kg plot⁻¹
VE	Y_1	0.07	9.05	0.39	21.68	0.159
	Y_2	0.05	7.17	0.68	35.09	0.09
VG	Y_1	22.3	720.74	111.62	1447.26	15.76
	Y_2	22.51	898.15	70.4	2192.65	4.5
VP	Y_1	22.4	729.8	112.02	1468.94	15.92
	Y_2	22.55	907.32	71.1	2227.74	4.59
H^2_b	Y_1	99.5	98.75	99.60	98.5	98.9
	Y_2	99.8	98.9	99.0	98.42	98.03
G.C.V	Y_1	100.0	68.76	80.07	56.79	100.0
	Y_2	100.0	68.17	65.77	57.03	54.8
P.C.V	Y_1	100.0	69.16	80.21	57.22	100.0
	Y_2	100.0	68.5	66.1	57.5	55.3
$P(F_1)$	Y_1	1.12	-0.28	-0.66	0.55	0.87
	Y_2	-0.57	0.12	-0.55	1.13	0.49
$P(F_{1r})$	Y_1	1.26	0.23	-0.82	0.58	0.71
	Y_2	-0.75	0.33	0.57	0.57	0.62
HMP(F_1)%	Y_1	-62.23**	-19.60**	-47.6**	52.6**	57.54**
	Y_2	-84.71**	8.76ᴺˢ	-36.2**	109.4**	33.34**
HBP (F_1)%	Y_1	-79.53**	-52.43**	-68.5**	-22.2**	-5.05ᴺˢ
	Y_2	-71.87**	-36.6**	-51.51**	6.5ᴺˢ	-20.2**
HMP(F_{1r})%	Y_1	-60.6**	-15.96**	-54.75**	55.9**	46.65**
	Y_2	-62.95**	-23.9**	-37.5**	55.62**	41.92**
HBP(F_{1r})%	Y_1	-78.7**	-50.3**	72.8**	-20.5**	-11.62**
	Y_2	-80.01**	-55.65**	-62.2**	-20.9**	-14.8**
I.D %	Y_1	28.23**	54.35**	15.61ᴺˢ	10.89ᴺˢ	70.92**
	Y_2	45.5**	63.87ᴺˢ	6.4ᴺˢ	26.53**	58.64**
G.S.%	Y_1	9.42	53.6	21.18	75.88	7.89
	Y_2	9.52	59.87	16.78	93.35	4.21

Table 7. Genotypic (VG) and phenotypic (VP) variance, genotypic (GCV) and phenotypic (PCV) coefficient of variation, potence ratio (P), heterosis over mid parent (HMP%) and better parent (HBP%), Inbreeding depression (ID %) and genetic advance (GS%) for all studied traits (Data are combined across two years).

Parameter	Tillersplant[-1]	Leavesplant[-1]	100G.W(g)	Earsplant[-1]	Grain yield Kg plot[-1]
VE	0.047	9.11	0.54	28.40	0.12
VG	22.32	783.90	88.10	1711.60	4.82
VP	22.44	818.60	91.53	1848.40	4.99
H^2_b	99.40	95.00	96.20	92.60	96.60
G.C.V	100.00	67.50	72.30	55.50	54.34
P.C.V	100.0	68.90	73.80	57.70	55.34
$P(F_1)$	-0.64	-0.07	-0.64	0.85	0.68
$P(F_{1r})$	-0.72	-0.28	-0.71	0.57	0.66
HMP(F_1)%	-55.00**	-4.95[NS]	-42.4**	82.3**	45.43**
HBP(F_1) %	-75.6**	-44.2**	-65.32**	-7.16[NS]	-12.64*
HMP(F_{1r})%	-61.71**	-20.1**	-64.9**	55.71**	44.32**
HBP(F_{1r})%	-79.4**	-53.1**	-67.9**	-20.7**	-13.31*
I.D %	38.56**	59.97**	9.7[NS]	20.66**	65.14**
G.S. %	9.40	54.60	18.50	80.02	4.34

Random Amplified Polymorphic DNA (RAPD)

RAPD-PCR was used to analyze the genetic diversity of the four studied maize, teosinte, and their hybrid to assess their genetic relationships using similarity indices and dendrogram tree. Ten arbitrary random primers (Table 1) was used to determine RAPD polymorphism of the four maize genotypes were Primer OP- A09, Primer OP- A10, Primer OP- A12, Primer OP- A19, Primer OP- B04, Primer OP- B05, Primer OP- B13, Primer OP- C08, Primer OP- D11 and Primer OP- O 18. The resulted amplified fragments are shown in figure (1) and (2). Banding patterns were scored as present (1) or absent (0). All the 10 primers successfully amplified DNA fragments for all varieties. A total number of 490 fragments were visualized across the four investigated genotypes. The results of RAPD-PCR technique are shown as strong and weak bands produced in the RAPD reactions. Weak bands result from low homology between the primer and the pairing site on the DNA strand Thormann *et al* (1994). According to the data in the Table (9), the genotypes 1 and 2 did not give any positive or molecular marker with primers, except with primer OP-B05, OP-A18 and OP-D11. On the other hand, the genotypes 3 and 4 gave markers with OP-A09, OP-A19, OP-D11, OP-C08, OP-A18 and OP-B05. The fragment of DNA which weighted 635 pb was advanted to

genotypes 3 and 4 in the primer OP-A09. Also, the fragment with OP- B05 was 955 pb appeared in the genotypes F_1 and F_1 reciprocal.

The percentage of polymorphism in this investigation was 7.3 resulted from 28 positive and negative markers in all genotypes divided by 348 (total number of fragments produced from 10 primers RAPD-PCR with four genotypes). While, the monographic implication in all genotypes using 10 primers were 92.7 %. This ratio very high for many reasons such as the low number of genotypes, low suitable number of primers of RAPD-PCR (Huxley 1955 and Clark 1967). A heterozygote advantage describes the case in which the heterozygote genotype has a higher relative fitness than either the homozygote dominant or homozygote recessive genotype. Selection favoring the heterozygote is one of the mechanisms that maintain polymorphism and help to explain some kinds of genetic variability (Ford 1940 and 1964).

Much interest has recently arisen in the PCR-based RAPD method of DNA fingerprinting (Williams et al 1990). To evaluate the genetic diversity of 2 maize genotypes, and to determine the correlation between genetic distance and cross hybrid performance, we have used random amplified polymorphic DNA (RAPD), a PCR-based technique. Genetic divergence was determinated using Jaccard's similarity coefficient, and a final dendrogram was constructed using an unweighted pair-group method with arithmetical averages (UPGMA). Cluster analysis divided the samples into two distinct groups (I and II). The group I include genotypes 1 and 2 (teosinte line 136A and single cross 30 k8), respectively. On the other hand, the group two included genotypes 3 and 4 (F_1 Zea mexicana x Zea mays and reciprocal F_1 Zea mays x Zea mexicana), respectively. The two groups were appeared the production of crosses and the parents which founded in the same group, to investigate genetic distances among maize material and hybrid performance. Genetic distances (GDs) were on average greater (0.93) for genotypes 1 and 2 than for genotypes 3 and 2 (0.84) and genotypes 3 and 4 (0.90) as shown in Table (8).In this investigation the RAPD- PCR had ability and simplicity as a molecular tool to distinguish between genotypes. The simplicity of laboratory assays for RAPD is an attractive method for the analysis of genetic diversity among maize landraces. The polymorphism detected among the accessions can be used in breeding programs to maximize the use of genetic resources (Valdemar et al 2004). The RAPD-PCR can detect the GCA and measure the genetic and breeding traits (Aleksandar et al 2008). These authors found that genetic distance revealed by RAPD markers can be used to establish consistent heterotic patterns between maize inbred lines.

Table 8. Similarity indexes among the four genotypes based on seedling RAPD-PCR using 10 primers.

	F_1	F_{1r}	136A	30 k8
F_1	100			
F_{1r}	88.9	100		
136A	86.3	87.2	100	
30 k8	83.7	86.6	92.5	100

F_1 *Zea mexicana x Zea mays*   reciprocal F_1 *Zea mays x Zea mexicana*
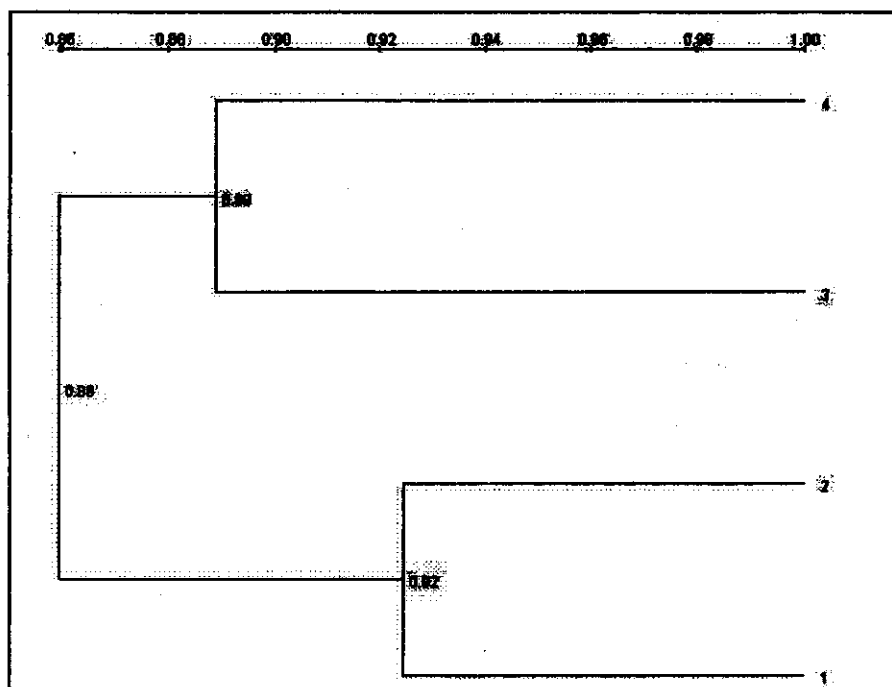
Fig. 1. Dendrogram of genetic relationships the four genotypes based on similarity indexes data using RAPD-PCR. (1) teosinte line (136A), (2) single cross (30 k8), (3) F_1 *Zea mexicana x Zea mays and (4) reciprocal F_1 Zea mays x Zea mexicana.*

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Table 9. Total positive (P) and negative (N) markers produced from four Zea mays, Zea mexicana and their hybrid using ten primers.

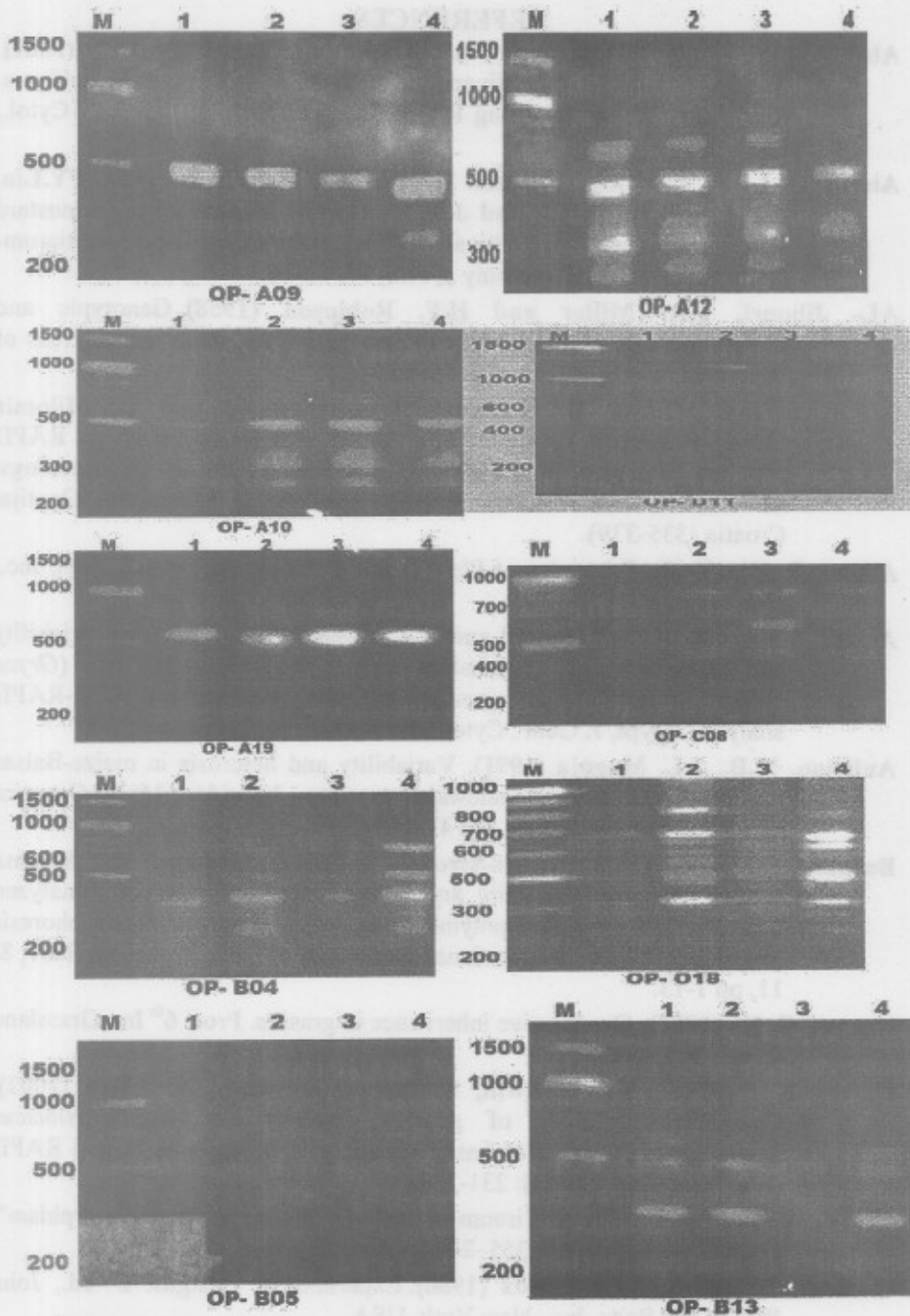| No.	Primer	%Polymorphic	Total No.of frag.	Teosinte line (136A)		Single cross (30 k8)		F₁		Reciprocal F₁		Total	
				P	N	P	N	P	N	P	N	P	N
1	OP-A09	5.6	36	-	-	-	-	1	-	1	-	2	-
2	OP-A10	0	40	-	-	-	-	-	-	-	-	-	-
3	OP-A12	0	32	-	-	-	-	-	-	-	-	-	-
4	OP-B13	0	32	-	-	-	-	-	-	-	-	-	-
5	OP-A19	5.6	36	-	-	-	1	1	-	-	-	1	1
6	OP-D11	11.1	36	1	-	1	-	-	1	-	1	2	2
7	OP-C08	3.1	32	-	-	-	-	1	-	-	-	1	-
8	OP-A18	20	80	1	2	4	1	4	-	4	-	13	3
9	OP-B04	0	28	-	-	-	-	-	-	-	-	-	-
10	OP-B05	9.4	32	1	-	1	-	-	-	1	-	3	-
	Total		384	3	2	6	2	7	1	6	1	22	6
	Grand total			5		8		8		7		28	

Fig. 2. DNA polymorphism of the 4 genotypes using randomly amplified polymorphic DNA with ten primers.

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القياسات الجزيئية والوراثية للهجين بين الذرة الشامية والذرة الريانة

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تم الحصول على الجيل الأول وكذلك الجيل الأول العكسى من التهجين بين الذرة الشامية متمثلة فى الهجين بيونير ٣٠ك٨ والذرة الريانة السلالة teosinte 136A والتى تم الحصول عليها نتيجة التهجين بـين الطـراز المحلى من الذرة الريانة وأحد الطرز المستوردة من المكسيك المسماة Central Plateau وذلك خلال الموسم الصيفى ٢٠٠٥ بمحطة البحوث الزراعية بالسرو. وتم الحصول على الجيل الثانى للجيل الأول فى الإتجاة الواحـد دون العكسى فى الموسم الزراعى ٢٠٠٦. تم تقييم الخمسة تراكيب الوراثية وهم الآبـاء والجيـل الأول والجيـل الأول الرجعى والجيل الثانى فى محطة البحوث الزراعية بالسرو خلال الموسمين الـزراعيين ٢٠٠٧ و ٢٠٠٨ . وتم إجراء تحليل التباين المشترك عبر سنتى الدراسة وذلك بغرض تقدير بعض القياسـات الوراثية مثل معامل الإختلاف الورثى GCVوكذلك معامل الإختلاف المظهرى PCVوكـذلك التحليـل الجزيـئ بإستخدام RAPD وتحديد المسافات الوراثية بين هذه التراكيب الوراثية المختلفة. وتتلخص أهم النتائج المتحصل عليها فيما يلى:

١ـ أظهرت النتائج إلى ان الفروق بين معامل الإختلاف الورثى والمظهرى كانت ضيقة مشيراً إلى التأثير القليـل للظروف البيئية على الصفات تحت الدراسة.

٢ـ كانت قيمة درجة التوريث بمفهومها الواسع مرتفعة للصفات المدروسة بقيم تتراوح بين ٩٢,٦ إلى٩٩,٤ % مشيراً إلى أن هذه الصفات أقل تأثراً لحد كبير بالبيئة وتتأثر أكثر بمكونات التباين الورثى.

٣ـ وجد أن إستخدام التحليل بواسطة الـ RAPD تعتبر دقيقة لتحليل التنوع الجزيئى للتراكيب الوراثية فى هـذه الدراسة.

٤ـ قسمت الشجرة التطورية للتراكيب الوراثية الأربعة المستخدمة فى الدراسـة إلـى مجمـوعتين رئيـسيتين : الأولى تضم التراكيب الوراثية الأول والثانى أى السلالة teosinte 136A الذرة الريانـة وهجـين الـذرة الشامية ٣٠ك٨ على الترتيب بينما المجموعة الثانية كانت تضم الجيل الأول والجيل الأول العكـسى. وكانـت نسبة التعدد فى الإختلافات الجزيئية ٧,٣% والمسافة الوراثية بين الذرة الريانة والذرة الشامية كانت ٠,٩٣ وبين الهجين والذرة الشامية كانت ٠,٨٤ بينما كانت المسافة الوراثية بين الهجين والهجين العكسى ٠,٩٠ وبذلك يمكن الأستفادة من التهجين بين الذرة الشامية والريانة فى الحصول على هجين بينهم عالى الإنتاجية فـى المحصول وبعض الصفات الأخرى المرغوبة وكذلك قياس المزيد من التباينات على المستوى الجزيئى بينهما .