25 *Egypt. J. Agron*. **Vol. 38,** No. 3, pp.471– 488 (2016)

Diallel Analysis and Genetic Diversity of Some Yellow Maize Inbred Lines (*Zea mays* L.) Using RAPD and SSR Markers

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INFORMATION about combining ability and genetic diversity among maize inbred lines is fundamental in designing future breeding strategies for improving grain yield. Twenty one F_1 hybrids were generated by crossing seven yellow maize inbred lines in a half diallel mating scheme in 2013 season. The 21 F_1 hybrids plus the two check hybrids (SC166 and SC173) were evaluated in a randomized complete block design with three replications during 2014 season, to estimate combining ability effects and identify type of gene action governing the inheritance of grain yield and other important traits. Results showed that both general (GCA) and specific (SCA) combining ability mean squares were highly significant for all the studied characters. The GCA/SCA ratio was more than unity for all the studied traits, except days to 50% silking, plant height and grain yield, indicating the preponderance of the additive gene effects in the inheritance of these traits. The inbred line P_1 appeared to be the best general combiner for earliness, grain yield and its components. The crosses $P_1 \times P_2$, $P_3 \times P_7$, $P_4 \times P_7$, and $P_5 \times P_6$ had the best SCA effects for grain yield and most of its component traits. The cross $P_1 \times P_2$ significantly out-yielded the two check hybrids (SC166 and SC173). The genetic diversity among the seven yellow parental inbred lines was assessed using two types of molecular markers; Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR). Seven random primers were used in the RAPD reactions, resulting in the amplification of 82 bands with 95.12% polymorphism. While, eight pairs of SSR primers were used and resulted in 49 fragments with 100% polymorphism. Genetic similarity among all possible pairs of inbred lines varied from 0.12 to 0.69, with an average of 0.34, for RAPD markers, and from 0.01 to 0.56, with an average of 0.24, for SSR markers. The similarity matrices for RAPD and SSR data were not significantly correlated ($r=0.34$, $p > 0.05$). Genetic distances based on RAPD and SSR markers were not significantly correlated with F_1 hybrids grain yield ($r=0.02$ and $r=0.429$, $P>0.05$, respectively). Therefore, the parental genetic distance could not be used to predict the grain yield of the F_1 hybrids in this study.

Keywords: Maize, Combining ability, Genetic distance, RAPD, SSR.

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Maize (*Zea mays* L.) is the third important cereal crop worldwide. It is used in human food, fodder and fuel (Ranum *et al.,* 2014). In Egypt, substantial amount of maize is imported for high domestic consumption and one of the major objectives is to increase maize production in order to decrease its import. The identification of parental inbred lines that perform superior hybrids is the most costly and time consuming phase in maize hybrid development. Combining ability analysis is useful in identifying the mode of gene action and valuable to assess the potentiality of inbred lines.

Assessment of genetic diversity and relatedness among breeding materials has a preponderant role in a breeding program. Molecular markers in many crop species including maize have proven valuable in genetic diversity analysis. Their expression, unlike morphological markers, is not affected by environmental factors; hence they reflect the actual level of the genetic difference existed among the genotypes (Legesse *et al.,* 2007). Several DNA marker technologies are available to study genetic diversity in maize. Among them, Random Amplified Polymorphic DNA (RAPD) markers have been widely used in the maize diversity studies probably because they detected a high level of polymorphism in a rapid and simple manner in addition to its low cost (Bruel *et al.,* 2007 and Devi & Singh, 2011). Simple Sequence Repeats (SSR) or Microsatellites are useful markers as they have a multi-allelic nature, co-dominant inheritance and reproducibility which make it ideal for genetic diversity studies in maize (Akaogu *et al.,* 2012; Sserumaga *et al.* 2014 and Nyaligwa *et al.,* 2015).

The association between DNA marker-based genetic distance (GD) and F_1 hybrids performance for grain yield has been studied in maize. Phumichai *et al.* (2008), Makumbi *et al.* (2011) and Kamara (2016) showed that the GD was significantly correlated with F_1 hybrids grain yield. However, Menkir *et al.* (2010) and Akaogu *et al.* (2012) and Kamara & Rehan (2015) reported insignificant correlation between GD and F_1 hybrids grain yield. The objectives of this study were to: (1) Estimate combining ability effects and identify type of gene action governing the inheritance of the studied traits, (2) Assess the genetic diversity among the studied maize inbred lines using RAPD and SSR markers and (3) Estimate the correlations of RAPD and SSR genetic distances with F_1 hybrids grain yield.

Materials and Methods

Field experiments

Seven yellow maize inbred lines, derived from different sources by maize breeding program (Table 1) were crossed during 2013 season in all possible combinations excluding reciprocals. The resultant 21 F_1 crosses along with the two check hybrids SC166 and SC173 were evaluated in a randomized complete block design (RCBD) with three replications at the Experimental Farm of the Faculty of Agriculture, Kafrelsheikh University, during 2014 season. Each plot consisted of two ridges of five meters length, 70cm width and 25cm between the hills. Two kernels were planted per hill and later thinned to one plant per hill.

The other cultural practices were followed as usual for ordinary maize field in the area. Data were recorded for days to 50% silking (day), plant height (cm), ear length (cm), ear diameter (cm), ear height (cm), number of rows/ear, number of kernels/row and grain yield (ardab/faddan) adjusted to 15.5% moisture content (one ardab = 140 kg, one faddan = 4200 m^2). The ordinary analysis of variance was done according to Steel & Torrie (1980). Combining ability analysis was done following the procedure of Griffing (1956), method-4, model-1.

TABLE 1. The code, name and pedigree of the parental maize inbred lines.

Parent code	Name	Pedigree
P_1	Inb. 174	BS-10-1
P_2	Inb. 202	H-111
P_3	Inb. 203	CP X 888
P_4	Inb. 236	Turk 13
P_5	Inb. 239	Turk-24
P_6	Inb. 247	Young-6
P_7	Inb. 207	Sd.7 x GZ.614 BC1 S7Imported MSA 1967

Molecular analysis

DNA isolation

The young leaves were harvested from 15 to 20 seedlings of each inbred line after twenty days from planting and stored at -80 °C. Genomic DNA was isolated using CTAB method (Doyle & Doyle, 1990). DNA quantity as well as quality was assessed using NanoDrop spectrophotometer (ND-1000, USA).

RAPD analysis

Seven decamer RAPD primers were used for RAPD analysis in this study (Table 6). Polymerase chain reaction (PCR) was carried out in 20 µl reaction mixture containing 1 X Taq buffer, X mM dNTPs, 0.5 µM primer, 1 U of Taq polymerase and 1.0 µl of template DNA. The PCR reaction consisted of an initial denaturation for 2 min at 94°C, followed by 35 cycles consisting of denaturation at 94°C for 20 sec, 20 sec of annealing at 30°C, extension of 3 min at 72°C and a final extension of 3 min at 72 °C.

SSR analysis

Eight specific maize microsatellite markers (SSR) were used to carry out the SSR analysis (Table 6). PCR was performed in a volume of 10 µl reaction mixture containing 1 µL of 20 ng/µL genomic DNA template, 1 unit Taq DNA polymerase (Promega, USA), 2mM MgCl2, 0.2mM dNTPs and 0·5 µM of reverse and forward primer. The PCR reaction was initially started by denaturation at 94°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, 30 sec of annealing at 55°C, 30 sec of extension at 72°C and a final extension of 3 min at 72 °C. Amplified products of both RAPD and SSR analysis were electrophoresed on 1.5 % agarose gel. The gels were stained with ethidium bromide and then distained with tap water and photographed using gel documentation system (UVITEC, UK).

Data analysis

RAPD and SSR gels were scored as presence (1) and absence (0) of each band for each inbred line for all tested primers. Similarity coefficient matrices were calculated using the Jaccard similarity algorithm (Jaccard, 1908) using PAST program. Cluster analysis was performed to produce a denderogram using method of unweighted pair-group with arithmetic averages (UPGMA).

Results and Discussion

Analysis of variance

The mean squares due to genotypes (G) were partitioned into crosses (C), checks (Ch) and C *vs*. Ch. Genotypes (G) and crosses (C) mean squares were found to be highly significant for all the studied traits, indicating a wide diversity among the genetic materials used in the present study. The differences between the two check hybrids (Ch) were not significant for all the studied traits. Mean squares for crosses *vs.* checks (C *vs*. Ch.) were significant for all the measured traits, except ear height and ear diameter. Results in Table 2 showed that both general (GCA) and specific (SCA) combining ability mean squares were highly significant for all the studied characters. These results would suggest the importance of both additive and non-additive gene effects in determining the performance of these characteristics. However, the magnitude of GCA/SCA ratio exceeded the unity for ear height, ear length, ear diameter, No. of rows/ear and No. of kernels/row, indicating the greatest role of the additive type of gene action in the inheritance of these traits. These results are in accordance with those obtained by Badu-Apraku & Oyekunle (2012), Abd El-Mottalb *et al.* (2013) and Mousa (2014) for ear height; Hefny (2010) for ear length; El-Badawy (2013) and Ibrahim (2014) for No. of rows/ear and Abo El-Haress (2015) and Kamara (2015) for ear diameter and No. of kernels/row. On the other hand, GCA/SCA ratio was less than unity for days to 50% silking, plant height and grain yield, indicating that these traits were predominantly controlled by the non-additive type of gene action. These findings are in agreement with those of Ahmed (2013), El-Ghonemy (2015) and Hassan *et al.* (2016) for plant height and Dodiya & Joshi (2002), Attia *et al.* (2015) and Hassan (2015) for days to 50% silking and grain yield.

TABLE 2. Mean squares from ordinary and combining ability analysis for all the studied traits.

	df	Days to 50% silking	Plant height (cm)	Ear height (cm)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of kernels/ row	Grain yield (ard/ fad)
Genotypes (G)	22	22.71**	521.01**	184.14**	14.51**	0.13**	2.67**	62.89**	29.99**
F_1 Crosses (C)	20	24.53**	507.77**	191.03**	12.42**	0.13**	2.70**	64.39**	31.14**
GCA	6	17.47**	481.21**	287.32**	19.63**	0.25**	3.81**	74.71**	14.69**
SCA	14	27.56**	519.16**	149.76**	9.33**	0.09**	2.22**	59.96**	38.19**
Checks (Ch)	1	2.53	253.50	13.50	0.38	0.03	0.26	1.50	1.50
C *vs.* Ch	1	6.54*	1053.14**	217.05	70.34**	0.09	4.59**	94.31**	35.47**
Error	44	1.02	63.56	57.91	1.09	0.03	0.54	4.96	4.12
GCA/SCA		0.63	0.93	1.92	2.10	2.80	1.72	1.25	0.38

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

Mean performance

Mean performance of the 21 F_1 crosses and the two check hybrids SC166 and SC173 for all the studied traits are shown in Table 3. The results showed that the mean values of number of days to 50% silking ranged from 60.5 days ($P_5 \times P_7$) to 70.67 days ($P_1 \times P_2$) with an average of 65.25 days. The seven crosses ($P_1 \times P_4$), ($P_1 \times P_5$), ($P_1 \times P_6$), ($P_2 \times P_3$), ($P_2 \times P_4$), ($P_4 \times P_5$) and ($P_5 \times P_7$) were significantly earlier than the earliest check hybrid SC166. Earliness in maize is favorable for saving water irrigation and escaping destructive injuries caused by the stem corn borers. Plant height means ranged from 226.25cm ($P_4 \times P_5$) to 270cm ($P_1 \times P_6$) with an average of 244.63cm. Seven crosses ($P_1 \times P_3$), ($P_2 \times P_6$), ($P_3 \times P_4$), ($P_4 \times P_5$), ($P_4 \times P_6$), ($P_5 \times P_6$) and ($P_5 \times P_7$) were significantly shorter than the shortest check hybrid SC166. Meanwhile, sixteen crosses were significantly shorter than the check hybrid SC173. As for ear height, two crosses ($P_1 \times P_5$) and ($P_4 \times P_5$) had significantly lower ear placement compared with the lower check hybrid SC166, and the crosses means ranged from 121cm ($P_4 \times P_5$) to 153cm ($P_1 \times P_7$) with an average of 129.21cm. Concerning ear length, the crosses mean values ranged from 15.2 ($P_2 \times P_4$) to 22cm ($P_1 \times P_2$ and $P_1 \times P_6$) with an average of 18.17cm. None of the crosses significantly surpassed the two check hybrids. Meanwhile, two crosses ($P_1 \times P_2$) and ($P_1 \times P_6$) were similar in ear length to the best check hybrid SC173. Regarding ear diameter, the crosses ranged from 4.6cm ($P_3 \times P_4$) to 5.4cm ($P_1 \times P_5$) with an average of 4.9cm. Only one cross ($P_1 \times P_5$) gave significantly increased value compared to the best check hybrid SC166. Concerning, number of rows per ear, the crosses ranged from 12.17 ($P_4 \times P_6$) to 15.5cm ($P_4 \times P_7$) with an average of 13.79. Four crosses ($P_1 \times P_6$), ($P_2 \times P_3$), ($P_3 \times P_7$) and ($P_4 \times P_7$) were not significantly different from the best check hybrid SC173. Regarding number of kernels per row, the crosses ranged from 29.0 ($P_5 \times P_7$) to 45.9 ($P_4 \times P_7$) with an average of 38.35. None of the studied crosses significantly surpassed the highest check hybrid SC173. While, three crosses ($P_1 \times P_2$), ($P_1 \times P_5$) and ($P_4 \times P_7$) did not differ significantly from the check hybrid SC173. Concerning grain yield (ard/fad), the crosses mean values ranged from 20.21 ard/fad ($P_5 \times P_7$) to 32.46 ard/fad ($P_1 \times P_2$) with an average of 25.96 ard/fad. Only the cross ($P_1 \times P_2$) significantly outyielded the highest yielding check hybrid SC173. Moreover, five and two crosses insignificantly out-yielded the check hybrids SC166 and SC173, respectively.

General combining ability (GCA) effects

Estimates of GCA effects assigned to each inbred line for all the traits are shown in Table 4. High positive values of ($\hat{g}_i$) effects would be of importance for all studied traits in question, except days to 50% silking, plant and ear heights where high negative values would be of interest from the breeder point of view. The results showed that the inbred lines P_1 and P_5 were the best combiners for earliness, as they had highly significant negative ($\hat{g}_i$) effects for days to 50% silking. Meanwhile, the inbred lines P_4 and P_5 showed significant or highly significant negative ($\hat{g}_i$) effects for plant height and ear height, indicating that these inbred lines could be considered as a good combiners for developing short

and lower ear placement hybrids. On the other hand, significant or highly significant positive ($\hat{g}_i$) effects were obtained by the inbred lines P_1, P_2 and P_6 for ear length; P_1 and P_2 for ear diameter; P_1 and P_3 for No. of rows/ear; P_1 and P_6 for No. of kernels/row and P_1 for grain yield. From the previous results, it could be concluded that the inbred line P_1 appeared to be the best general combiner for earliness, grain yield and its components. Attia *et al.* (2015) and El-Shamarka *et al.* (2015), reported desirable and significant ($\hat{g}_i$) effects for earliness, grain yield and its components in their respective studies.

TABLE 3. Mean performance of the 21 F_1 crosses and the two check hybrids SC166 and SC173 for all the studied traits.

Cross	Days to 50% silking	Plant height (cm)	Ear height (cm)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of kernels/ row	Grain yield (ard/fad)
$P_1 \times P_2$	70.67	245.00	129.57	22.00	5.33	14.31	45.00	32.46
$P_1 \times P_3$	64.50	231.00	125.00	19.50	4.70	14.00	41.17	28.33
$P_1 \times P_4$	61.50	253.75	128.75	17.30	4.80	13.33	33.80	23.25
$P_1 \times P_5$	63.50	251.25	121.25	18.20	5.40	14.33	44.17	25.00
$P_1 \times P_6$	61.50	270.00	140.00	22.00	5.10	15.00	40.50	27.70
$P_1 \times P_7$	64.30	268.75	153.00	18.80	4.90	14.00	40.83	24.00
$P_2 \times P_3$	60.83	266.25	137.50	18.50	5.10	15.00	36.83	23.67
$P_2 \times P_4$	63.33	248.75	121.92	15.20	4.70	13.33	38.17	24.83
$P_2 \times P_5$	65.00	253.75	126.25	17.50	4.90	12.67	32.00	23.33
$P_2 \times P_6$	67.00	227.50	122.50	19.30	5.10	13.83	40.83	25.73
$P_2 \times P_7$	66.00	240.33	132.00	20.10	5.00	13.33	37.17	28.67
$P_3 \times P_4$	65.50	236.25	126.50	15.60	4.60	14.50	34.67	24.17
$P_3 \times P_5$	66.00	240.00	125.00	15.50	4.70	13.00	36.50	25.00
$P_3 \times P_6$	67.00	242.83	126.25	15.40	4.85	14.00	37.83	27.66
$P_3 \times P_7$	66.00	247.50	141.25	16.20	4.90	15.33	30.33	28.93
$P_4 \times P_5$	63.50	226.25	121.00	18.30	4.79	13.00	36.67	25.00
$P_4 \times P_6$	68.50	231.25	125.00	19.70	4.93	12.17	42.33	20.40
$P_4 \times P_7$	66.80	243.75	122.00	20.00	4.70	15.50	45.90	31.00
$P_5 \times P_6$	69.47	238.00	130.33	19.00	4.80	13.00	39.33	30.15
$P_5 \times P_7$	60.50	227.50	131.25	16.40	4.83	12.33	29.00	20.21
$P_6 \times P_7$	69.00	247.67	127.00	17.00	4.70	13.67	42.33	25.58
SC166	65.70	252.00	134.00	21.50	5.10	14.50	42.00	28.00
SC173	67.00	265.00	137.00	22.00	4.95	14.92	43.00	29.00
LSD 0.05	1.67	13.16	12.56	1.72	0.29	1.21	3.68	3.35
LSD 0.01	2.23	17.60	16.80	2.30	0.39	1.62	4.92	4.48

TABLE 4. Estimates of general combining ability ($\hat{g}_i$) effects of the seven yellow maize inbred lines for all the studied traits.

Inbred line	Days to 50% silking	Plant height (cm)	Ear height (cm)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of kernels/ row	Grain yield (ard/fad)
P_1	-1.12**	10.39**	4.47*	1.76**	0.17**	0.44*	3.07**	1.00*
P_2	0.26	2.75	-1.10	0.72**	0.15**	-0.06	-0.02	0.59
P_3	-0.34	-0.80	1.25	-1.66**	-0.11*	0.62**	-2.55**	0.41
P_4	-0.48	-5.56**	-6.01**	-0.58*	-0.17**	-0.18	0.29	-1.42**
P_5	-0.72**	-6.21**	-4.03*	-0.82**	0.01	-0.88**	-2.49**	-1.41**
P_6	2.18**	-2.11	-0.83	0.68**	0.02	-0.22	2.61**	0.30
P_7	0.21	1.54	6.25**	-0.10**	-0.07	0.28	-0.91	0.53
LSD (0.05) gi	0.49	3.85	3.68	0.50	0.09	0.35	1.08	0.98
LSD (0.01) gi	0.65	5.15	4.92	0.67	0.11	0.47	1.44	1.31
LSD (0.05) gi-gj	0.74	5.88	5.62	0.77	0.13	0.54	1.64	1.50
LSD (0.01) gi-gj	1.00	7.87	7.51	1.03	0.18	0.72	2.20	2.00

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

Specific combining ability (SCA) effects

As shown in Table 5, the most desirable significant or highly significant ($\hat{S}_{ij}$) effects were obtained by the crosses ($P_1\times P_4$), ($P_1\times P_6$), ($P_2\times P_3$), ($P_2\times P_4$) and ($P_5\times P_7$) for days to 50% silking (towards earliness), ($P_1\times P_2$), ($P_1\times P_3$), ($P_2\times P_6$), ($P_2\times P_7$) and ($P_5\times P_7$) for plant height (towards shorter plants), ($P_1\times P_3$), ($P_1\times P_5$), ($P_4\times P_7$) and ($P_6\times P_7$) for ear height (towards lower ear placement), ($P_1\times P_2$), ($P_1\times P_3$), ($P_1\times P_6$), ($P_2\times P_3$), ($P_2\times P_7$), ($P_4\times P_5$), ($P_4\times P_6$) and ($P_4\times P_7$) for ear length, ($P_1\times P_5$), ($P_3\times P_7$) and ($P_4\times P_6$) for ear diameter, ($P_1\times P_5$), ($P_1\times P_6$) and ($P_4\times P_7$) for No. of rows/ear, ($P_1\times P_2$), ($P_1\times P_3$), ($P_1\times P_5$), ($P_3\times P_5$), ($P_4\times P_7$) and ($P_6\times P_7$) for No. of kernels/row and ($P_1\times P_2$), ($P_3\times P_7$), ($P_4\times P_7$) and ($P_5\times P_6$) for grain yield. These crosses may find prime importance in breeding programs for the traditional breeding procedures. It is notable that the crosses that showed high SCA effects for grain yield also showed high SCA effects for one or more traits of yield components. For example, the cross ($P_4\times P_7$) which showed high SCA effects for grain yield also showed high SCA effects for ear length, No. of rows/ear and No. of kernels/row.

Levels of polymorphism

The genetic diversity within the seven yellow maize parental lines was assessed using two types of molecular markers (RAPD and SSR). The obtained results are presented in Table 6. Seven RAPD primers were used and generated a total of 82 reproducible DNA bands/alleles, of which 78 bands (95.12%) were polymorphic (Fig. 1). The number of polymorphic bands detected with each primer ranged from 8 (primer OP-1) to 14 (primer OP-7) with an average of 9.75 bands per primer. The level of RAPD polymorphism observed in the study was higher than those reported in previous studies of maize by Lanza *et al.* (1997) (80.6%), Mukharib *et al.* (2010) (73.02 %) and Molin *et al.* (2013) (81.9%).

TABLE 5. Estimates of specific combining ability ($\hat{S}_{ij}$) effects of the 21 F_1 crosses for all the studied traits.

Cross	Days to 50% silking	Plant height (cm)	Ear height (cm)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of kernels/ row	Grain yield (ard/fad)
$P_1 \times P_2$	6.27**	-12.78**	-3.01	1.35**	0.12	0.13	3.60**	4.91**
$P_1 \times P_3$	0.70	-23.23**	-9.93**	1.23*	-0.26**	-0.85*	2.30*	0.97
$P_1 \times P_4$	-2.16**	4.29	1.09	-2.05**	-0.10	-0.72*	-7.91**	-2.29*
$P_1 \times P_5$	0.07	2.44	-8.39*	-0.91	0.32**	0.98**	5.23**	-0.55
$P_1 \times P_6$	-4.83**	17.09**	7.16	1.39**	0.01	0.98**	-3.54**	0.44
$P_1 \times P_7$	-0.05	12.19**	13.07**	-1.03*	-0.10	-0.52	0.32	-3.49**
$P_2 \times P_3$	-4.34**	19.66**	8.14*	1.27*	0.16	0.65	1.06	-3.28**
$P_2 \times P_4$	-1.70**	6.92	-0.18	-3.11**	-0.18*	-0.22	-0.45	-0.30
$P_2 \times P_5$	0.20	12.57**	2.17	-0.57	-0.16	-0.19	-3.84**	-1.80
$P_2 \times P_6$	-0.70	-17.78**	-4.78	-0.27	0.03	0.31	-0.11	-1.12
$P_2 \times P_7$	0.27	-8.59*	-2.36	1.31*	0.02	-0.69	-0.25	1.59
$P_3 \times P_4$	1.07*	-2.03	2.05	-0.33	-0.02	0.28	-1.42	-0.78
$P_3 \times P_5$	1.80**	2.37	-1.43	-0.19	-0.10	-0.52	3.19**	0.05
$P_3 \times P_6$	-0.10	1.11	-3.38	-1.79**	0.04	-0.19	-0.57	1.00
$P_3 \times P_7$	0.87	2.12	4.54	-0.21	0.18*	0.64	-4.56**	2.04*
$P_4 \times P_5$	-0.56	-6.61	1.84	1.53**	0.06	0.28	0.52	1.87
$P_4 \times P_6$	1.54**	-5.71	2.64	1.43**	0.19*	-1.22**	1.09	-4.43**
$P_4 \times P_7$	1.81**	3.14	-7.45*	2.51**	0.05	1.61**	8.17**	5.93**
$P_5 \times P_6$	2.74**	1.69	5.99	0.97	-0.12	0.31	0.86	5.30**
$P_5 \times P_7$	-4.25**	-12.46**	-0.18	-0.85	0.00	-0.86*	-5.96**	-4.87**
$P_6 \times P_7$	1.35**	3.61	-7.63*	-1.75**	-0.15	-0.19	2.28*	-1.20
LSD 5% (s_{ij})	0.96	7.60	7.25	0.99	0.17	0.70	2.12	1.93
LSD 1% (s_{ij})	1.29	10.16	9.70	1.33	0.23	0.94	2.84	2.59
LSD 5% (s_{ij}-s_{ik})	1.49	11.77	11.23	1.54	0.26	1.08	3.29	2.99
LSD 1% (s_{ij}-s_{ik})	1.99	15.74	15.03	2.06	0.35	1.45	4.40	4.01
LSD 5% (s_{ij}-s_{kl})	1.29	10.19	9.73	1.33	0.23	0.94	2.85	2.59
LSD 1% (s_{ij}-s_{kl})	1.72	13.61	12.99	1.78	0.30	1.25	3.80	3.46

TABLE 6. Details of the selected primers used in RAPD and SSR profiling analysis.

Marker type	Primer name	Sequence	TNB	NMB	NPB	PPB
RAPD	OP-1	CCCAAGGTCC	8	-	8	100
	OP-2	CATACCGTGG	12	-	12	100
	OP-3	AGCATGGCTC	10	-	10	100
	OP-4	GACCAATGCC	15	-	15	100
	OP-5	TGAGGGTCCC	9	-	9	100
	OP-6	GGGTCTCGGT	13	3	10	76.92
	OP-7	AGAGCCGTCA	15	1	14	93.33
	Total		82	4	78	95.12
SSR	phi072	F: ACCGTGCATGATTAATTTCTCCAGCCTT R: GACAGCGCGCAAATGGATTGAACT	2	-	2	100
	phi024	F: ACTGTTCCACCAAACCAAGCCGAGA R: AGTAGGGGTTGGGGATCTCCTCC	1	-	1	100
	Umc1014	F: GAAAGTCGATCGAGAGACCCTG R: CCCTCTCTTCACCCCTTCCTT	5	-	5	100
	phi299852	F: GATGTGGGTGCTACGAGCC R: AGATCTCGGAGCTCGGCTA	1	-	1	100
	phi112	F: TGCCCTGCAGGTTCACATTGAGT R: AGGAGTACGCTTGGATGCTCTTC	4	-	4	100
	phi015	F: GCAACGTACCGTACCTTTCCGA R: ACGCTGCATTCAATTACCGGGAAG	3	-	3	100
	umc1033	F: CTTCTTCGTAAAGGCATTTTGTGC R: GTGCGGGATTCCTTAGTTTGC	3	-	3	100
	phi301654	F: GAATGCATGCTTTTCAAGGAC R: CGCACAGAGAGCAGAACG	2	-	2	100
	Total		21		21	100

TNB: Total number of bands; NMB: number of monomorphic bands; NPB: number of polymorphic bands; PPB: percent of polymorphic bands.

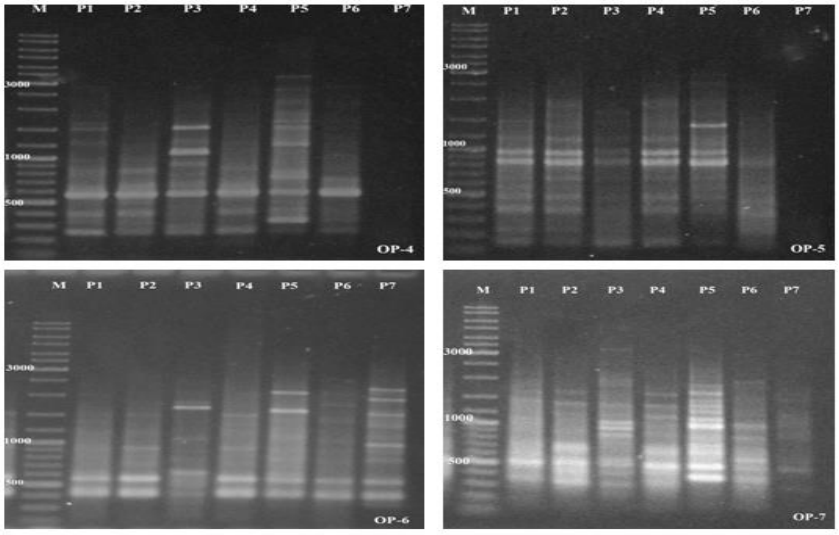

Fig.1. DNA-RAPD patterns generated by four arbitrary primers (OP-4, OP-5, OP-6 and OP-7) with the seven inbred lines (P1 - P7). (M) refers to the DNA ladder.

For SSR analysis, eight primer pairs used and all were polymorphic. A total of 21 polymorphic bands/ alleles were amplified with 100% polymorphism (Table 6 and Fig. 2). This result was in agreement with Sun *et al.* (2001) and Souza *et al.* (2008). They also detected 100% polymorphism within maize inbreds using SSR markers. The number of polymorphic bands detected with each primer ranged from 1 (primers phi024 and phi299852) to 5 (primer Umc1014) with an average of 2.63 bands per primer. The mean number of SSR bands per primer detected in this study was similar to those obtained by Menkir *et al.* (2004) and Akinwale *et al.* (2014). However, it was lower than those reported by Nyaligwa *et al.* (2015) and Pandit *et al.* (2016).

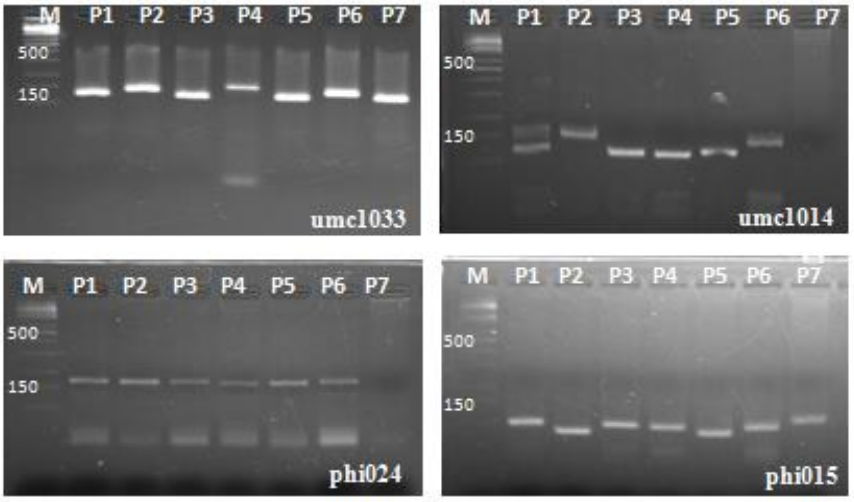

Fig. 2. DNA-SSR patterns generated by four specific primers (umc1033, umc1014, phi112 and phi015) with the seven inbred lines (P1 - P7). (M) refers to the DNA ladder.

The high level of polymorphism observed in this study for both marker types (RAPD and SSR) is consistent with the previous studied of Senior *et al.* (1998), Souza *et al.* (2008) and Abdellatif & Khidr (2010) which reported that the polymorphism levels for different types of molecular markers were high in maize. Souza *et al.* (2008) found that SSR markers obtained higher levels of polymorphism than RAPD markers in maize. The high level of polymorphism associated with SSR markers is caused by replication slippage and the co-dominant nature of this marker, which responsible for generating SSR allelic diversity (Pejic *et al.*, 1998 and Souza *et al*,. 2008).

Genetic distance and cluster analysis

Two independent genetic similarity matrices were produced for the RAPD and SSR data according to Jaccard's coefficient (Jaccard, 1908). The similarity coefficient for RAPD markers ranged from 0.12 to 0.69 with an average of 0.34

(Table 7, above diagonal). The lowest genetic similarity (0.12) was detected between P_3 and P_7 also, obtained between P6 and P7. Meanwhile, the highest genetic similarity was (0.69) observed between the inbred lines P_2 and P_4. Based on SSR data, the similarity coefficient ranged from 0.01 to 0.56 with an average of 0.24 (Table 7, below diagonal). The lowest genetic similarity (0.01) was detected between P_2 and P_7, whereas the highest genetic similarity was (0.56) observed between the inbred lines P3 and P5. In general, the SSR markers data showed less similarity than those obtained from the RAPD markers. These results corresponded well with the findings of Sun *et al.* (2001) and Laborda *et al.* (2005) which showed that the SSR markers had a better ability to investigate diversity, showing higher polymorphism and consequently higher variability.

TABLE 7. Similarity matrices based on RAPD (above diagonal) and SSR (below diagonal) markers among maize inbred lines (P_1- P_7).

	P_1	**P_2**	**P_3**	**P_4**	**P_5**	**P_6**	**P_7**
P_1		0.56	0.39	0.57	0.47	0.29	0.15
P_2	0.15		0.39	0.69	0.38	0.37	0.16
P_3	0.27	0.30		0.36	0.40	0.40	0.12
P_4	0.21	0.23	0.36		0.44	0.29	0.14
P_5	0.23	0.36	0.56	0.42		0.34	0.13
P_6	0.33	0.15	0.17	0.31	0.23		0.12
P_7	0.22	0.01	0.13	0.09	0.22	0.10	

The dendrogram generated from RAPD data separated the studied inbred lines into two main clusters as represented in Fig. 3A. The inbred line P_7 was placed alone in the first main cluster. The second main cluster contained the rest of the inbred lines and this cluster separated into two sub-clusters; the first one grouped the inbred lines P_3 and P_6, while, the second sub-cluster divided into two sub-sub clusters; the first contained P_5 and the second included P_1, P_2 and P_4.

The dendrogram obtained with SSR markers (Fig. 3B), also divided the inbred lines into two main clusters. The inbred line P_7 placed alone as in RAPD markers in the first main cluster. The second main cluster was divided into two sub-clusters; the first had the inbred lines P_1 and P_6, whereas, the second sub-cluster divided into two sub-sub clusters; the first contained P_2 and the second included P_3, P_4 and P_5. Both RAPD and SSR markers showed kind of similarity in the topology of their respective dendrograms. Even though, notable differences in the position of the inbred lines were observed, except the inbred line P_7 which separated into out group in both methods (RAPD and SSR) that indicate this inbred is highly diverse than other maize inbreeds.

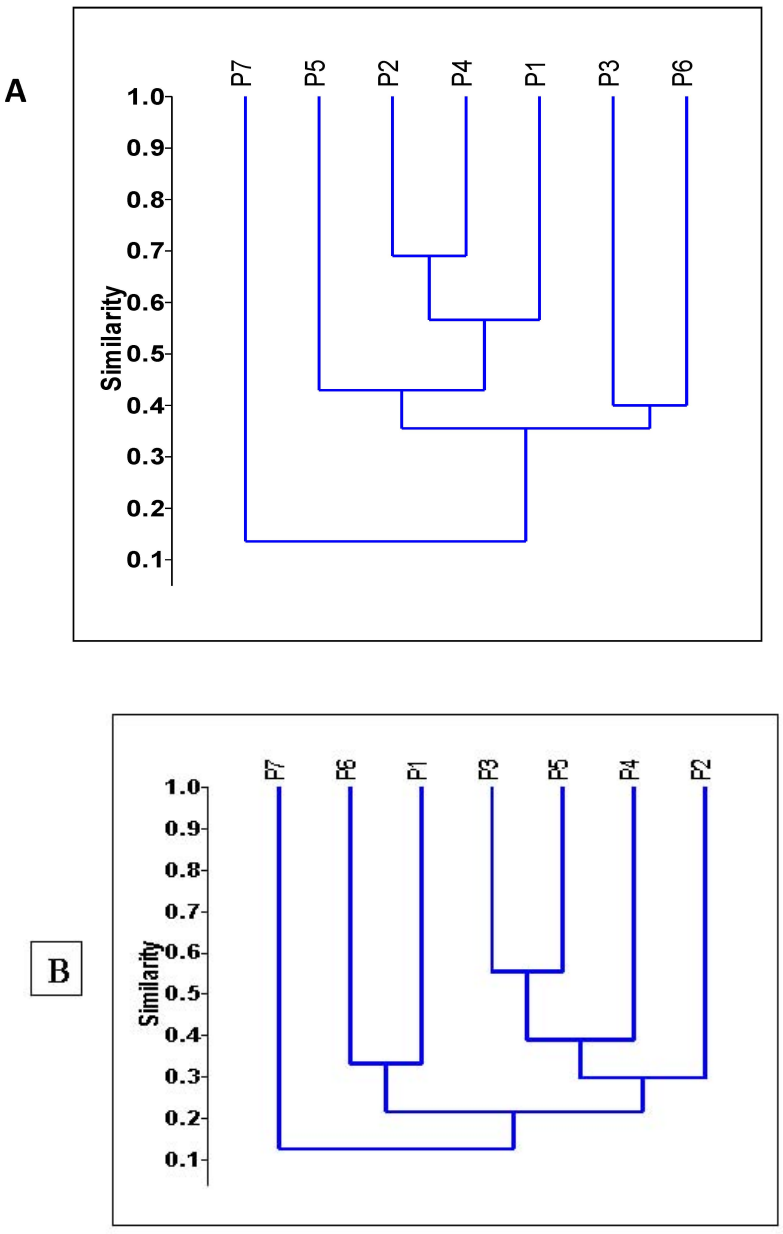

Fig. 3. Dendrogram generated based on UPGM clustering method using PAST program and Jacquard's coefficient using RAPD (A) and SSR (B) data among the seven inbred lines.

Correlation between RAPD and SSR markers

The correlation coefficient (r) between the similarity matrices obtained with RAPD and SSR markers was not significant (r=0.34, p >0.05). These results are similar with those reported by Garcia *et al.* (2004), who observed a correlation of (0.33) between RAPD and SSR markers. Also, Cholastova *et al.* (2011) found that RAPD and SSR markers were poorly correlated (0.11) when studying genetic diversity in maize. However, Souza *et al.* (2008) and Leal *et al.* (2010) found high and significant correlations (0.57 and 0.55) between RAPD and SSR markers in maize, respectively. The main reason for the limited correlation between RAPD and SSR markers might be the fact the RAPD markers are dominant, whereas, SSR are co-dominant. Moreover, the number of primers used and nature of genetic polymorphism detected by the two markers may affect the correlation between them (Pejic *et al.* 1998 and Sun *et al.*, 2001).

Correlations of genetic distances with F_1 hybrids grain yield

Genetic distances (GD) based on each of RAPD and SSR markers were insignificantly correlated with F_1 hybrids grain yield, the correlation values being (r =0.02 and 0.429 P>0.05), respectively. These results indicate that it is difficult to predict the F_1 hybrids grain yield from the genetic distance of the inbred lines used in this study. These results agree well with the findings of Menkir *et al.* (2010), Akaogu *et al.* (2012) and Oyekunle *et al.* (2015). They reported insignificant correlation between molecular marker-based GD and F_1 hybrids grain yield. However, these results are contrary to the findings of Phumichai et al (2008) and Makumbi *et al.* (2011) who reported significant correlation between GD and F_1 hybrids grain yield. The lack of correlation between GD and F_1 hybrids performance could be due to the absence of linkage between genes controlling the trait and markers used to estimate GD, inadequate genome coverage, random marker distribution and diversified effect of dominance (Bernardo, 1992).

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(*Received* 12/10/2016;
accepted 7/12/2016)

تحليل الهجن التبادلية والتباعد الوراثى لبعض سلالات من الذرة الشامية الصفراء بإستخدام الدلائل الجزيئية RAPD وSSR

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تعتبر المعلومات عن القدرة على التآلف والتباعد الوراثى بين سلالات الذرة الشامية أمر هام و أساسى في تصميم استراتيجيات التربية المستقبلية لتحسين محصول الحبوب. تم تكوين ٢١ هجين فردى عن طريق إجراء التهجين النصف دائرى بين سبعة سلالات مرباه داخلياً من الذرة الشامية الصفراء فى موسم٢٠١٣. تم تقيم ال٢١ هجين فردى الناتجة مع إثنين من هجن المقارنة الصفراء (هجين فردى١٦٦ وهجين فردى ١٧٣) في تجربة ذات تصميم قطاعات كاملة العشوائية بثلاث مكررات فى موسم ٢٠١٤ وذلك لتقدير تأثيرات القدرة العامة والخاصة على التآلف ولتحديد الفعل الجينى المتحكم فى وراثة صفة محصول الحبوب وغيرها من الصفات الهامة .أظهرت النتائج أن التباين الراجع للقدرة العامة والخاصة على التآلف كان عالى المعنوية لجميع الصفات تحت الدراسة. كانت النسبة بين تباين القدرة العامة على التآلف و تباين القدره الخاصة على التآلف أكبر من الوحده لجميع الصفات تحت الدراسة ما عدا صفات عدد الأيام حتي ظهور ٥٠٪ من الحرايز، إرتفاع النبات ومحصول الحبوب مما يدل على ان الفعل الجينى المضيف هو المتحكم فى وراثة تلك الصفات . أظهرت السلالة الأبوية P_1 افضل قدرة عامة على التآلف لصفة التبكير و صفة المحصول ومكوناتة . كانت أفضل الهجن فى تأثيرات القدرة الخاصة على التآلف هى $P_1\times P_2$, $P_3\times P_7$ $P_4\times P_7$, $P_5\times P_6$, لصفة المحصول وواحد أو أكثر من مكوناتة. تفوق محصول الهجين $P_1\times P_2$ تفوقاً معنوياً على محصول هجنى المقارنة (هجين فردى١٦٦ وهجين فردى ١٧٣). تم تقدير التباعد الوراثى بين السبعة سلالات الأبوية بإستخدام نوعين من الدلائل الجزيئية وهما RAPD وال SSR. تم إستخدام سبعة بادئات فى تفاعل الRAPD

والتى أعطت ٨٢ حزمة بنسبة تعدد شكل مظهرى ٩٥,١٢٪. بينما تم إستخدام ٨ أزاوج من بادئات الSSR والتى أعطت ٤٩ شظية بنسبة ١٠٠٪ تعد شكل مظهرى. تراوحت قيم التشابة الوراثى بين كل زوج من السلالات الأبوية بناءاً على دلائل الRPAD من ٠,١٢ إلى ٠,٦٩ بمتوسط ٠,٣٤ بينما تراوحت من ٠,٠١ إلى ٠,٥٦ بمتوسط ٠,٢٤ بناءاً على دلائل ال SSR. كان الارتباط بين مصفوفتى التشابة الوراثى لكلا من RAPD وال SSR غير معنوى. أظهرت النتائج أن قيم الإرتباط بين التباعد الوراثى المقدر بإستخدام كلًا من الRAPD والSSR مع متوسط أداء الهجن لصفة محصول الحبوب كانت غير معنوية، لذلك لا يمكن الاعتماد على التباعد الوراثى بين السلات الأبوية المستخدمة فى هذه الدراسة فى التنبؤ بمحصول حبوب الهجن الناتجة من هذه السلالات .