

## COMBINING ABILITY IN MAIZE UNDER TWO NITROGEN LEVELS AND ASSESSING GENETIC DIVERSITY USING RAPD MARKER

Kamara, M. M.\* and M. R. Rehan\*\*

\*Agronomy Dep., Faculty of Agriculture 33516, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

\*\*Genetics Dep., Faculty of Agriculture 33516, Kafrelsheikh University, Kafr El-Sheikh, Egypt.



### ABSTRACT

A half diallel cross among seven white maize inbred lines was made in 2012 growing season. The resulted 21  $F_1$  crosses and the commercial check hybrid SC10 were evaluated under two different nitrogen levels, i.e. 80 and 120 kg N  $fad^{-1}$  at the Experimental Farm, Faculty of Agriculture, Kafrelsheikh University in 2013 growing season, to estimate general and specific combining ability effects (GCA and SCA) and their interactions with nitrogen levels as well as identify the superior inbred lines and crosses. Data were collected for number of days to 50% silking, ear length, ear diameter, number of rows  $ear^{-1}$ , number of kernels  $row^{-1}$  and grain yield  $plant^{-1}$  and were analyzed according to Griffing (1956) method-4 model-1 (fixed model). The results revealed that, the mean squares due to nitrogen levels (N), genotypes (G), crosses (Cr.),  $G \times N$  interaction and  $Cr. \times N$  interaction were significant for all the studied traits. General and specific combining ability (GCA and SCA) mean squares were significant for all the studied traits under the two nitrogen levels and their combined data. Both GCA and SCA effects were significantly interacted with nitrogen levels for most of the studied traits. The non-additive gene action played an important role in the inheritance of all the studied traits, except days to 50% silking and grain yield  $plant^{-1}$  under the two nitrogen levels and their combined data. The inbred lines  $P_5$ ,  $P_6$  and  $P_7$  showed the best desirable GCA effects for earliness, whereas  $P_1$ ,  $P_2$  and  $P_4$  were the best general combiners for grain yield  $plant^{-1}$ . The best crosses showed desirable SCA effects were  $P_3 \times P_5$ ,  $P_3 \times P_7$ ,  $P_5 \times P_6$  and  $P_6 \times P_7$  for earliness and  $P_1 \times P_4$ ,  $P_2 \times P_4$ ,  $P_3 \times P_6$  and  $P_5 \times P_7$  for grain yield  $plant^{-1}$  under the two nitrogen levels and the combined data. Two crosses  $P_1 \times P_4$  and  $P_2 \times P_4$  gave significantly positive superiority in grain yield over the check hybrid SC10 under the two nitrogen levels and the combined data. The genetic diversity (GD) among the seven parental inbred lines was investigated using Random Amplified Polymorphic DNA (RAPD) markers. Seven random primers were used to give a total 70 reproducible RAPD fragments, of them 56 (77.88%) being polymorphic. The GD among the inbred lines differed from 0.333 to 0.655 with an average of 0.503. The estimate value of correlation coefficient between GD and mean performance of the  $F_1$  hybrids for grain yield  $plant^{-1}$  was low ( $r = 0.335$ ) or not high enough to be of predictive value. Therefore, the RAPD marker could not be predicted about the mean performance of the grain yield  $plant^{-1}$  in this study.

**Keywords:** *Zea mays* L, Inbred lines, GCA, SCA, Nitrogen levels, RAPD, Genetic diversity.

### INTRODUCTION

Maize (*Zea mays* L.) is one of the major cereal crops used worldwide for a human food, poultry and livestock feed in addition to many industrial purposes. Recently, it has been used as a biomass for bioenergy purposes.

In Egypt, one of the main objectives is to increase maize production in order to decrease its import and respond to its high consumption. The development of superior hybrids could contribute to the improvement of maize productivity. Therefore, intense efforts are being made by maize breeders to explore the genetic material in order to develop new maize hybrids which characterized by high yielding potentiality. Knowledge of combining ability of the parents and the nature of gene action involved in the expression of the trait to be improved are important for selection of suitable parents in hybridization and identification of promising hybrids (Chawla and Gupta, 1984 and Hallauer, 1990). The diallel cross analysis is one of the most informative methodology for generating information on gene action controlling traits, and the combining ability of the parents. The two main genetic parameters of diallel analysis are general and specific combining ability (GCA and SCA). The GCA is the average performance of a line in its hybrid combinations which is proportional to favorable allelic frequencies in parents and additive effects, while SCA is related to dominance or non-additive genetic components and defined as the superiority of a certain hybrid compared to other hybrids derived from hybridization of different parents (Sprague and Tatum, 1942). Both additive and non-additive gene effects have been reported to be important in the genetic expression of many maize traits including grain yield (Rameeh *et al.*, 2000, Desai and Singh, 2001 and Estakhr and Heidari, 2012). However, the magnitude of the additive genetic effects represented the major role in the inheritance of maize grain yield and days to 50% silking date (Wu *et al.*, 2003, Yu *et al.*, 2003, Badu-Apraku and Oyekunle, 2012 and Badu-Apraku *et al.*, 2013), although the non-additive genetic effects played an effective role in the inheritance of maize grain yield and most of its contributing traits (Makumbi *et al.*, 2011 and Abdel-Moneam *et al.*, 2014).

Understanding the genetic diversity and distance of maize inbred lines is important for planning crosses, assigning inbred lines to specific groups and designing breeding strategies (Oyekunle *et al.*, 2015). Besides morphological and quantitative data based diversity analysis of the inbred lines, molecular markers that reveal polymorphism at the DNA level (Smith and Smith, 1992) have been shown to be a very powerful tool for estimation of genetic diversity as they were independent of the confounding effects of environmental factors. Random amplified polymorphic DNA (RAPD) which is relatively simple rapid, cost effective and detect high polymorphism, have been extensively used to study the genetic diversity and relationships among maize inbred lines (Lanza *et al.*, 1997, Liu *et al.*, 1998, Wu, 2000, Bruel *et al.*, 2007 and Devi and Singh, 2011). Assessment of genetic diversity among maize inbred lines using RAPD molecular markers and determining their associations with the performance of the F<sub>1</sub> hybrids for grain yield are invaluable in selecting parental inbred lines for development of productive hybrids with high yielding ability. In view of the above, the present investigation was carried out to establish the magnitude of both GCA and SCA effects and their interactions with nitrogen levels, assess the genetic diversity among the studied maize inbred lines using RAPD markers and determining the relationship between the RAPD based distances of the

parental inbred lines and the performance of their F<sub>1</sub> hybrids for grain yield plant<sup>-1</sup>.

## **MATERIALS AND METHODS**

### **Plant materials**

Seven white inbred lines of maize were used as parents in this study i.e., P<sub>1</sub> (Inb. 4), P<sub>2</sub> (Inb. 17), P<sub>3</sub> (Inb. 53), P<sub>4</sub> (Inb. 76), P<sub>5</sub> (Inb. 81), P<sub>6</sub> (Inb. 94) and P<sub>7</sub> (Inb. 120). These inbred lines were obtained from Maize Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt.

### **Field experiments**

In 2012 growing season, all possible combinations excluding reciprocals were made among the seven inbred lines at the Experimental Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt. In 2013 growing season, the resulted 21 F<sub>1</sub> hybrids and the commercial check hybrid SC10 were evaluated in two separate experiments represented two different nitrogen levels; 80 (N1) and 120 (N2) kg N fad<sup>-1</sup>. Nitrogen fertilizer was added in two equal doses before 1<sup>st</sup> and 2<sup>nd</sup> irrigations. A randomized complete block design with three replications was used for each experiment. Each plot consisted of two ridges, 6 m long and 0.70 m width. Planting was made in hills spaced at 0.25 m with three kernels per hill on one side of the ridge. The seedlings were thinned to one plant per hill after 21 days from planting. All other agricultural practices were carried out according to standard commercial recommendations for maize production. The soil analysis of the experimental site before sowing in 2013 growing season indicated that the soil was clay (49% clay, 35.2% silt and 15.8% sand), pH (8.1) and EC (0.355 dSm<sup>-1</sup>). The total organic matter was 1.6% and the available N, P and K were 33.5, 12.7 and 291.5 mg/kg soil, respectively.

Data were recorded for number of days to 50% silking (day), ear length (cm), ear diameter (cm), number of rows ear<sup>-1</sup>, number of kernels row<sup>-1</sup> and grain yield plant<sup>-1</sup> (g) which was adjusted for 15.5% moisture

### **DNA isolation**

The genomic DNA was isolated from the leaf tissues of the seven inbred lines by CTAB method with minor modification according to Tamari *et al.* (2013). Briefly, a 100 mg of plant leaves was grinded in liquid nitrogen and placed in 2 ml eppendorf tube. A 800 µl of pre-heated (65 C°) CTAB buffer was added followed by incubated for 30 min at 60 C°. Chloroform/Isoamyl alcohol mix (800 µl) was added and tubes gently mixed. The mixture was centrifuged and the DNA was precipitated by adding 550 µl of pre-cold isopropanol. DNA was collected and the pellet was washed in 200 µl washing buffer (70 % ethanol and 10 mM ammonium acetate) followed by TE + RNase A buffer for RNA removal. The DNA collected again with 100 µl (7.5 M NH<sub>4</sub>-acetate) and 750 µl absolute ethanol. After pellet drying, DNA suspended in 50 µl TE buffer and stored at -20 C° until use.

### **RAPD-PCR analysis**

Seven decamer RAPD primers (G1, G2, G3, G4, G5, G6 and G7) (Cat. No.: A069653-A531559-to-65, Bio Basic Inc, Canada) were used (Table

1) to screen the genomics DNA in a single primed PCR reaction using i-Taq master mix (iNtRON Biotechnology, Korea). Each reaction was performed in a 20 µl reaction volume 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 at 94°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 20 sec, 20 sec of annealing at 30°C and 3 min of elongation at 72°C. The program ended with a final elongation step at 72°C for 3 min. Amplification products were separated on 1 % agarose gel, stained with ethidium bromide and visualized under UV-Gel documentation system.

**Table (1): List of random amplified polymorphic DNA (RAPD) primers and their nucleotide sequence.**

No.	Primer name	Sequence (5'→3')	Catalog Numbers
1	OP-G1	CCCAAGGTCC	A069653-A531559
2	OP-G2	CATACCGTGG	A069653-A531560
3	OP-G3	AGCATGGCTC	A069653-A531561
4	OP-G4	GACCAATGCC	A069653-A531562
5	OP-G5	TGAGGGTCCC	A069653-A531563
6	OP-G6	GGGTCTCGGT	A069653-A531564
7	OP-G7	AGAGCCGTCA	A069653-A531565

#### Data analysis

The experimental obtained data were statistically analyzed for analysis of variance according Steel and Torrie (1980). The combined analysis of the two experiments was done whenever homogeneity of variance was detected. General and specific combining ability were estimated according to Griffing (1956), method-4, model-1(fixed model). Superiority percentage (Sup. %) for grain yield plant<sup>-1</sup> was calculated for individual crosses as the percentage deviation of F<sub>1</sub> mean performance from check hybrid SC10 mean value.

#### Genetic relationships

The data generated from the band patterns of the seven RAPD primers were introduced to software (<http://genomes.urv.cat/UPGMA/index.php>) (Garcia-Vallve *et al.*, 2000) according to binary values of (1) and (0) for the presence and absence of bands, respectively. The genetic distance and phylogenetic relationship between the seven inbred lines was conducted based on RAPD analysis on the basis of Jaccard's (Tanimoto) coefficient.

## RESULTS AND DISCUSSION

The analysis of variance for all the studied traits in each nitrogen level and their combined data are presented in Table (2). Mean squares due to nitrogen levels (N) were significant for all studied traits, indicating overall differences between the two nitrogen levels. Genotypes (G) and crosses (Cr.) mean squares were found to be highly significant for all the studied traits under the two nitrogen levels and their combined data, indicating a wide diversity among the genetic materials used in the present study. Mean

squares due to genotypes × nitrogen levels (G × N) and crosses × nitrogen levels (Cr. × N) interactions were significant for all the studied traits, revealing that the tested genotypes behaved differently from nitrogen level to another. Mean squares due to crosses vs. check were significant for days to 50% silking under N2 level and the combined data, ear diameter under N1 level and the combined data and grain yield plant<sup>-1</sup> under both nitrogen levels and the combined data. Insignificant interaction mean squares between crosses vs. check and nitrogen levels were observed for all the studied traits.

**Table (2): Mean squares from ordinary analysis of variance and combining ability analysis for all the studied traits under the two nitrogen level and their combined data.**

S.O.V	df	Mean squares					
		Days to 50% silking	Ear diameter	Ear length	No. of Rows ear <sup>-1</sup>	No. of kernels row <sup>-1</sup>	Grain yield plant <sup>-1</sup>
N1 (80 kg N fad <sup>-1</sup> )							
Genotypes (G)	21	26.79**	0.535**	10.05**	3.42**	48.142**	1532.57**
Crosses (Cr.)	20	27.93**	0.548**	10.41**	3.59**	50.50**	1543.79**
GCA	6	61.71**	0.907**	10.69**	7.02**	78.27**	3576.69**
SCA	14	13.45**	0.394**	10.29**	2.12**	38.59**	672.55**
Cr. vs. Check	1	3.99	0.275*	2.85	0.020	1.02	1308.17**
Error	42	1.40	0.05	1.05	0.78	2.77	127.73
GCA/SCA		1.0\	0.50	0.21	0.94	0.42	1.27
N2 (120 kg N fad <sup>-1</sup> )							
Genotypes (G)	21	32.70**	0.587**	9.56**	2.58**	35.74**	1145.23**
Crosses (Cr.)	20	33.53**	0.603**	9.94**	2.66**	37.47**	1153.39**
GCA	6	81.15**	0.935**	17.78**	4.06**	72.82**	2966.97**
SCA	14	13.12**	0.461**	6.58**	2.06*	22.32**	376.14**
Cr. vs. Check	1	16.10**	0.267	1.96	0.980	0.98	982.03*
Error	42	1.68	0.07	1.32	0.90	3.69	144.98
GCA/SCA		1.39	0.44	0.63	0.55	0.74	2.44
Combined over the two nitrogen levels							
Nitrogen (N)	1	205.40**	9.688**	197.01**	97.83 **	230.00*	11025.22*
Rep/N	4	2.32	0.097	2.447	1.28	28.38	1390.54
Genotypes (G)	21	56.63**	1.007**	16.88**	4.36**	76.85**	2437.67**
Crosses (Cr.)	20	58.56**	1.031**	17.49**	4.57**	80.58**	2445.62**
GCA	6	137.75**	1.691**	24.44**	8.51**	144.85**	6303.78**
SCA	14	24.62**	0.748**	14.52**	2.88**	53.04**	792.13**
Cr. vs. Check	1	18.03**	0.527**	4.68	0.160	2.16	2278.67**
G × N	21	2.86*	0.115*	2.73**	1.64*	7.03**	240.13*
Cr. × N	20	2.90*	0.120*	2.86 **	1.68*	7.38**	251.56*
GCA × N	6	5.11**	0.151*	4.03**	2.57**	6.24	239.87
SCA × N	14	1.95	0.107*	2.36*	1.30	7.87**	256.56*
Cr. vs. Check × N	1	2.06	0.02	0.13	0.84	0.03	11.53
Error	84	1.54	0.06	1.19	0.84	3.23	136.35
GCA/SCA		1.18	0.47	0.35	0.75	0.57	1.88
GCA × N /GCA		0.04	0.09	0.165	0.30	0.04	0.04
SCA × N /SCA		0.08	0.14	0.163	0.45	0.15	0.32

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

### Mean performance

Mean performance of all the tested crosses for all the studied traits under the two nitrogen levels and their combined data as well as superiority percentage (Sup. %) relative to check hybrid SC10 for grain yield plant<sup>-1</sup> are presented in Table 3. In general the mean values of the crosses were higher under the high nitrogen level (120 kg N fad<sup>-1</sup>) as compared to those under low level of nitrogen (80 kg N fad<sup>-1</sup>) for all the studied traits, except for days to 50% silking. The increase in mean performance of these traits at high nitrogen level might be due to the simulating effect of nitrogen on metabolic process in maize plants. These results are in general agreement with those obtained by Medici *et al.* (2004), Ngaboyisonga *et al.* (2009), El-Badawy (2013) and Kamara *et al.* (2014).

Four crosses P<sub>3</sub>×P<sub>5</sub>, P<sub>3</sub>×P<sub>7</sub>, P<sub>5</sub>×P<sub>6</sub> and P<sub>6</sub>×P<sub>7</sub> under the two nitrogen levels and their combined data were found significantly earlier than the check hybrid SC10. Earliness in maize is favorable for saving water irrigation and escaping destructive injuries caused by the stem corn borers. Two single crosses P<sub>1</sub>×P<sub>5</sub> and P<sub>2</sub>×P<sub>4</sub> under the two nitrogen levels and their combined data significantly surpassed the check hybrid SC10 for ear diameter. Concerning ear length, the crosses P<sub>1</sub>×P<sub>6</sub> and P<sub>2</sub>×P<sub>4</sub> under N1 and the combined data and P<sub>1</sub>×P<sub>2</sub> and P<sub>1</sub>×P<sub>4</sub> under both nitrogen levels and the combined data exhibited significantly increased values as compared to the check hybrid SC10. The cross P<sub>4</sub>×P<sub>6</sub> under N2 level and the crosses P<sub>1</sub>×P<sub>5</sub> and P<sub>2</sub>×P<sub>4</sub> under the two nitrogen levels and the combined data gave the highest mean value for number of rows ear<sup>-1</sup> and significantly surpassed the check hybrid SC10. Five crosses P<sub>1</sub>×P<sub>2</sub>, P<sub>1</sub>×P<sub>4</sub>, P<sub>1</sub>×P<sub>6</sub>, P<sub>3</sub>×P<sub>6</sub> and P<sub>3</sub>×P<sub>7</sub> under the two nitrogen levels and the combined data significantly possessed higher number of kernels row<sup>-1</sup> than the check hybrid SC10. The mean values of the grain yield plant<sup>-1</sup> ranged from 119.58 g for P<sub>5</sub>×P<sub>6</sub> to 204.17g for P<sub>1</sub>×P<sub>4</sub> under N1 level and from 144.03 g for P<sub>6</sub>×P<sub>7</sub> to 218.20 g for P<sub>1</sub>×P<sub>4</sub> under N2 level, whereas it ranged from 137.14 g for P<sub>5</sub>×P<sub>6</sub> to 211.19 g for P<sub>1</sub>×P<sub>4</sub> under the combined data. Superiority percentage (Sup. %) for grain yield plant<sup>-1</sup> relative to the check hybrid SC10 (Table 3) revealed that two crosses P<sub>1</sub>×P<sub>4</sub> and P<sub>2</sub>×P<sub>4</sub> under the two nitrogen levels and the combined data had positive and significant superiority percentage over the check hybrid SC10. The cross P<sub>1</sub>×P<sub>2</sub> gave positive superiority percentage over the check hybrid SC10, but it was not significant. Hence it could be concluded that these crosses offer possibility for improving grain yield of maize. These results are in harmony with those obtained by EL-Hosary *et al.* (2006), El-Ghonemy and Ibrahim (2010) and El-Badawy (2013). They reported positive and significant superiority percentages compared to the check hybrids for maize grain yield. The fluctuation of hybrids performance from nitrogen level to another was detected for most traits. These results could be due to significant interaction between crosses and nitrogen levels.

**Table (3): Mean performance of all the tested crosses for all the studied traits under the two nitrogen levels and their combined data as well as superiority percentage (Sup. %) relative to the check hybrid SC10 for grain yield plant<sup>-1</sup>.**

Cross	Days to 50% silking			Ear diameter (cm)			Ear length (cm)			No. of rows ear <sup>-1</sup>		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub> xP <sub>2</sub>	63.00	60.67	61.83	4.80	5.37	5.08	22.00	24.70	23.35	14.33	16.34	15.34
P <sub>1</sub> xP <sub>3</sub>	61.33	59.67	60.50	3.77	4.60	4.18	16.50	23.00	19.75	13.47	14.33	13.90
P <sub>1</sub> xP <sub>4</sub>	62.67	61.67	62.17	4.37	4.87	4.62	23.00	25.80	24.40	14.10	14.89	14.49
P <sub>1</sub> xP <sub>5</sub>	61.55	57.33	59.44	5.13	5.73	5.43	18.90	21.60	20.25	16.25	17.00	16.63
P <sub>1</sub> xP <sub>6</sub>	58.67	58.00	58.33	4.30	4.80	4.55	22.00	23.50	22.75	12.77	13.80	13.28
P <sub>1</sub> xP <sub>7</sub>	59.00	57.00	58.00	4.70	5.37	5.03	17.00	21.67	19.33	13.77	14.33	14.05
P <sub>2</sub> xP <sub>3</sub>	61.53	59.80	60.67	4.60	4.79	4.69	19.33	21.60	20.47	13.00	15.05	14.02
P <sub>2</sub> xP <sub>4</sub>	63.67	61.33	62.50	5.15	5.70	5.43	22.40	23.45	22.92	15.74	17.05	16.40
P <sub>2</sub> xP <sub>5</sub>	60.33	56.33	58.33	4.03	4.50	4.27	19.20	23.45	21.33	14.77	15.42	15.09
P <sub>2</sub> xP <sub>6</sub>	61.33	57.00	59.17	4.67	5.10	4.88	19.80	20.33	20.07	13.90	15.80	14.85
P <sub>2</sub> xP <sub>7</sub>	62.00	57.67	59.83	4.17	4.62	4.39	18.55	20.78	19.67	14.33	16.20	15.27
P <sub>3</sub> xP <sub>4</sub>	63.33	61.67	62.50	4.73	5.34	5.04	18.44	19.33	18.89	13.34	16.26	14.80
P <sub>3</sub> xP <sub>5</sub>	55.17	50.33	52.75	3.70	5.29	4.50	18.00	21.27	19.63	11.23	16.41	13.82
P <sub>3</sub> xP <sub>6</sub>	60.67	57.67	59.17	3.67	4.20	3.93	20.80	23.00	21.90	12.10	14.66	13.38
P <sub>3</sub> xP <sub>7</sub>	56.78	52.22	54.50	4.07	4.43	4.25	20.00	22.00	21.00	13.13	15.10	14.12
P <sub>4</sub> xP <sub>5</sub>	59.00	58.00	58.50	4.57	5.08	4.82	18.00	20.07	19.03	13.80	16.33	15.07
P <sub>4</sub> xP <sub>6</sub>	59.50	59.00	59.25	4.40	4.92	4.66	17.50	19.03	18.27	14.10	16.65	15.38
P <sub>4</sub> xP <sub>7</sub>	60.67	57.67	59.17	4.47	4.93	4.70	17.90	20.27	19.08	14.28	16.15	15.21
P <sub>5</sub> xP <sub>6</sub>	53.00	51.33	52.17	4.10	4.55	4.33	17.20	20.00	18.60	13.53	14.53	14.03
P <sub>5</sub> xP <sub>7</sub>	60.26	57.00	58.63	3.97	4.47	4.22	19.00	20.00	19.50	14.10	15.26	14.68
P <sub>6</sub> xP <sub>7</sub>	53.22	51.78	52.50	4.12	4.22	4.17	18.33	20.47	19.40	14.10	15.47	14.78
CheckSC10	60.50	58.00	59.25	4.67	5.20	4.93	20.21	22.50	21.36	14.00	15.00	14.50
LSD 5%	1.95	2.14	1.43	0.36	0.43	0.28	1.69	1.90	1.25	1.46	1.57	1.05
LSD 1%	2.61	2.86	1.89	0.49	0.58	0.37	2.26	2.54	1.66	1.95	2.10	1.40

**Table (3): Cont.**

Cross	No. of kernels row <sup>-1</sup>			Grain yield plant <sup>-1</sup> (g)			Sup. % relative to SC10 for grain yield plant <sup>-1</sup>		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub> xP <sub>2</sub>	44.00	47.00	45.50	182.78	190.75	186.76	3.75	-0.51	1.53
P <sub>1</sub> xP <sub>3</sub>	40.43	45.00	42.72	172.08	182.47	177.27	-2.32	-4.83	-3.63
P <sub>1</sub> xP <sub>4</sub>	46.67	47.00	46.83	204.17	218.20	211.19	15.89**	13.81*	14.81**
P <sub>1</sub> xP <sub>5</sub>	40.79	41.00	40.90	166.25	190.17	178.21	-5.63	-0.81	-3.12
P <sub>1</sub> xP <sub>6</sub>	44.00	45.89	44.94	166.83	192.38	179.61	-5.30	0.34	-2.36
P <sub>1</sub> xP <sub>7</sub>	42.11	43.00	42.56	161.41	167.24	164.33	-8.38	-12.77*	-10.67**
P <sub>2</sub> xP <sub>3</sub>	37.07	45.00	41.03	163.33	166.31	164.82	-7.29	-13.26*	-10.40**
P <sub>2</sub> xP <sub>4</sub>	42.00	43.88	42.94	196.39	212.92	204.65	11.48*	11.05*	11.26**
P <sub>2</sub> xP <sub>5</sub>	40.33	43.00	41.67	157.50	164.33	160.91	-10.60*	-14.29**	-12.52**
P <sub>2</sub> xP <sub>6</sub>	36.11	39.58	37.85	121.33	181.81	151.57	-31.13**	-5.17	-17.60**
P <sub>2</sub> xP <sub>7</sub>	35.55	37.55	36.55	143.15	168.64	155.90	-18.74**	-12.04*	-15.25**
P <sub>3</sub> xP <sub>4</sub>	37.33	40.00	38.67	154.58	175.00	164.79	-12.26*	-8.72	-10.41**
P <sub>3</sub> xP <sub>5</sub>	39.00	41.40	40.20	123.32	154.58	138.95	-30.00**	-19.37**	-24.46**
P <sub>3</sub> xP <sub>6</sub>	45.00	46.70	45.85	157.50	173.66	165.58	-10.60*	-9.42	-9.98**
P <sub>3</sub> xP <sub>7</sub>	44.00	46.00	45.00	140.47	151.08	145.78	-20.26**	-21.20**	-20.75**
P <sub>4</sub> xP <sub>5</sub>	30.00	39.00	34.50	149.51	173.08	161.29	-15.13**	-9.73	-12.32**
P <sub>4</sub> xP <sub>6</sub>	35.00	36.33	35.67	154.58	166.66	160.62	-12.26*	-13.07*	-12.68**
P <sub>4</sub> xP <sub>7</sub>	36.70	36.90	36.80	134.28	151.78	143.03	-23.78**	-20.83**	-22.24**
P <sub>5</sub> xP <sub>6</sub>	35.90	38.13	37.02	119.58	154.70	137.14	-32.12**	-19.31**	-25.45**
P <sub>5</sub> xP <sub>7</sub>	36.89	39.00	37.95	148.11	157.50	152.81	-15.93**	-17.85**	-16.93**
P <sub>6</sub> xP <sub>7</sub>	38.00	41.00	39.50	133.58	144.03	138.81	-24.18**	-24.88**	-24.54**
Check SC10	40.00	42.60	41.30	176.17	191.72	183.95	-	-	-
LSD 5%	2.74	3.17	2.06	18.65	19.87	13.41	-	-	-
LSD 1%	3.67	4.24	2.74	24.95	26.58	17.78	-	-	-

### Combining ability

The analysis of variance for combining ability for all the studied traits under the two nitrogen level and their combined data are presented in Table (2). Mean squares due to general combining ability (GCA) and specific combining ability (SCA) were highly significant for all the studied traits under the two nitrogen levels and the combined data, indicating that both additive and non-additive gene effects were important in the inheritance of these traits. These results are in general agreement with those previously reported by Lima *et al.* (1995), Rameeh *et al.* (2000), Desai and Singh (2001), Katta *et al.* (2007) and Estakhr and Heidari (2012). The GCA/SCA ratio was more than unity for days to 50% silking and grain yield plant<sup>-1</sup> under the two nitrogen levels and their combined data, indicating that these traits were predominantly controlled by the additive gene action. These findings are in agreement with those of Ogunbodede *et al.* (2000), Wu *et al.* (2003), Yu *et al.* (2003), Abuali *et al.* (2012) and Badu-Apraku and Oyekunle (2012). On the contrary, GCA/SCA ratio was less than unity for ear diameter, ear length, number of rows ear<sup>-1</sup> and number of kernels row<sup>-1</sup> under the two nitrogen levels and their combined data, indicating the preponderance of the non-additive gene action in controlling these traits. These results are in accordance with those obtained by Abdel-Moneam *et al.* (2009), EI-Badawy (2013), Katta *et al.* (2013) and Abdel-Moneam *et al.* (2014).

Mean squares due to the interactions of both GCA and SCA with nitrogen levels were significant for all the studied traits, except GCA × N for number of kernels row<sup>-1</sup> and grain yield plant<sup>-1</sup> and SCA × N for days to 50% silking and number of rows ear<sup>-1</sup> since these traits were not significant. These results suggested that the behavior of the two types of gene action varied from nitrogen level to another. It is fairly evident that the ratio of SCA × N/ SCA was higher than the ratio of GCA × N/ GCA for all the studied traits, except ear length. This result indicated that the non-additive effects were more influenced by nitrogen levels than the additive genetic effects for these traits. Mosa *et al.* (2010) reported that the non-additive genetic effects were more affected by nitrogen levels than additive gene actions for grain yield and most of its components.

### General combining ability (GCA) effects

Estimates of general combining ability (GCA) effects of the seven inbred lines under the two nitrogen levels and their combined data are shown in Table 4. High positive values of GCA effects would be of interest for all studied traits in question, except days to 50% silking where high negative values would be useful from the breeder point of view. The inbred lines P<sub>5</sub>, P<sub>6</sub> and P<sub>7</sub> exhibited highly significant and negative GCA effects for days to 50% silking under the two nitrogen levels and their combined data, indicating that these inbred lines could be considered as good combiners for earliness. On the contrary, significant and positive GCA effects were obtained by the inbred lines P<sub>1</sub>, P<sub>2</sub> and P<sub>4</sub> for ear diameter; P<sub>1</sub> and P<sub>2</sub> for ear length; P<sub>2</sub> and P<sub>4</sub> for number of rows ear<sup>-1</sup>; P<sub>1</sub> and P<sub>3</sub> for number of kernels row<sup>-1</sup> and P<sub>1</sub>, P<sub>2</sub> and P<sub>4</sub> for grain yield plant<sup>-1</sup> under the two nitrogen levels and their combined data. These results indicated that these parental inbred lines possess favorable genes and that improvement in respective traits may be



attained if they are incorporated in maize hybridization program. It is worth noting that the inbred line which possessed high GCA effects for grain yield plant<sup>-1</sup> showed desirable GCA effect for one or more of the traits contributing to grain yield. El-Badawy (2013) and Katta *et al.* (2013) reported that GCA effects were desirable and significant for earliness, grain yield and its components.

**Table (4): Estimates of general combining ability (GCA) effects of the seven inbred lines for all the studied traits under the two nitrogen levels as well as the combined data.**

Inbred line	Days to 50% silking			Ear diameter			Ear length		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub>	1.43**	2.12**	1.77**	0.19**	0.27**	0.23**	0.80**	2.04**	1.42**
P <sub>2</sub>	2.56**	1.81**	2.19**	0.26**	0.14*	0.20**	1.18**	0.84**	1.01**
P <sub>3</sub>	-0.05	-0.48	-0.26	-0.32**	-0.15*	-0.23**	-0.46	0.02	-0.22
P <sub>4</sub>	1.96**	3.12**	2.54**	0.31**	0.29**	0.30**	0.37	-0.43	-0.03
P <sub>5</sub>	-1.95**	-2.69**	-2.32**	-0.13*	0.05	-0.04	-1.02**	-0.74**	-0.88**
P <sub>6</sub>	-2.53**	-1.80**	-2.16**	-0.18**	-0.32**	-0.25**	0.05	-0.75**	-0.35
P <sub>7</sub>	-1.42**	-2.08**	-1.75**	-0.13*	-0.27**	-0.20**	-0.92**	-0.98**	-0.95**
LSD 5% (gi)	0.57	0.63	0.42	0.11	0.13	0.08	0.49	0.56	0.37
LSD 1% (gi)	0.76	0.84	0.55	0.14	0.17	0.11	0.66	0.74	0.49
LSD 5%(gi-gj)	0.87	0.96	0.64	0.16	0.19	0.12	0.76	0.85	0.56
LSD 1%(gi-gj)	1.17	1.28	0.85	0.22	0.26	0.16	1.01	1.14	0.74

**Table (4): Cont.**

Inbred line	No. of rows ear <sup>-1</sup>			No. of kernels row <sup>-1</sup>			Grain yield plant <sup>-1</sup>		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub>	0.36	-0.55*	-0.10	4.35**	3.36**	3.85**	24.95**	20.40**	22.67**
P <sub>2</sub>	0.64**	0.48*	0.56**	-0.24	0.78	0.27	7.14*	9.11**	8.12**
P <sub>3</sub>	-1.32**	-0.32	-0.82**	1.32**	2.40**	1.86**	-3.50	-7.22*	-5.36**
P <sub>4</sub>	0.49*	0.78**	0.64**	-1.71**	-1.80**	-1.75**	12.95**	11.68**	12.31**
P <sub>5</sub>	0.16	0.30	0.23	-2.67**	-2.11**	-2.39**	-12.90**	-8.97**	-10.94**
P <sub>6</sub>	-0.48*	-0.50*	-0.49**	-0.45	-0.89	-0.67*	-15.08**	-5.20	-10.14**
P <sub>7</sub>	0.16	-0.19	-0.01	-0.60	-1.73**	-1.17**	-13.56**	-19.79**	-16.67**
LSD 5% (gi)	0.43	0.46	0.31	0.80	0.93	0.60	5.46	5.82	3.93
LSD 1% (gi)	0.57	0.61	0.41	1.08	1.24	0.80	7.31	7.78	5.21
LSD 5%(gi-gj)	0.65	0.70	0.47	1.23	1.42	0.92	8.34	8.89	6.00
LSD 1%(gi-gj)	0.87	0.94	0.63	1.64	1.90	1.22	11.16	11.89	7.95

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

### Specific combining ability (SCA) effects

Estimates of specific combining ability (SCA) effects of the 21 single crosses for all the studied traits under the two nitrogen levels and their combined data are presented in Table 5. Four crosses P<sub>3</sub>×P<sub>5</sub>, P<sub>3</sub>×P<sub>7</sub>, P<sub>5</sub>×P<sub>6</sub> and P<sub>6</sub>×P<sub>7</sub> showed significant and negative SCA effects for days to 50% silking towards earliness under the two nitrogen levels and their combined data. The crosses P<sub>2</sub>×P<sub>3</sub> under N1 level, P<sub>3</sub>×P<sub>5</sub> under N2 level and P<sub>1</sub>×P<sub>5</sub>, P<sub>1</sub>×P<sub>7</sub>, P<sub>2</sub>×P<sub>4</sub>, P<sub>2</sub>×P<sub>6</sub> and P<sub>3</sub>×P<sub>4</sub> under the two nitrogen levels and the combined data exhibited significant and positive SCA effects for ear diameter. Regarding to ear length, the crosses P<sub>1</sub>×P<sub>6</sub> and P<sub>5</sub>×P<sub>7</sub> under N1 and the combined data, P<sub>2</sub>×P<sub>5</sub> under N2 and the combined data and P<sub>1</sub>×P<sub>4</sub>, P<sub>2</sub>×P<sub>4</sub>,

P<sub>3</sub>×P<sub>6</sub> and P<sub>3</sub>×P<sub>7</sub> under the two nitrogen levels and their combined data showed significant and positive SCA effects for this trait.

**Table (5): Estimates of specific combining ability (SCA) effects of the 21 F<sub>1</sub> crosses for all the studied traits under the two nitrogen levels and their combined data.**

Cross	Days to 50% silking			Ear diameter			Ear length		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub> ×P <sub>2</sub>	-0.84	-0.55	-0.69	0.001	0.06	0.03	0.79	0.14	0.46
P <sub>1</sub> ×P <sub>3</sub>	0.11	0.74	0.42	-0.46**	-0.42**	-0.44**	-3.07**	-0.74	-1.91**
P <sub>1</sub> ×P <sub>4</sub>	-0.56	-0.86	-0.71	-0.49**	-0.59**	-0.54**	2.60**	2.51**	2.55**
P <sub>1</sub> ×P <sub>5</sub>	2.22**	0.61	1.41**	0.72**	0.52**	0.62**	-0.12	-1.38	-0.75
P <sub>1</sub> ×P <sub>6</sub>	-0.08	0.39	0.16	-0.07	-0.05	-0.06	1.92**	0.53	1.23**
P <sub>1</sub> ×P <sub>7</sub>	-0.85	-0.33	-0.59	0.29**	0.47**	0.38**	-2.11**	-1.07	-1.59**
P <sub>2</sub> ×P <sub>3</sub>	-0.82	1.18	0.18	0.31**	-0.10	0.10	-0.61	-0.95	-0.78
P <sub>2</sub> ×P <sub>4</sub>	-0.69	-0.89	-0.79	0.23*	0.38**	0.30**	1.62**	1.35*	1.48**
P <sub>2</sub> ×P <sub>5</sub>	-0.13	-0.08	-0.10	-0.45**	-0.58**	-0.52**	-0.19	1.67**	0.74*
P <sub>2</sub> ×P <sub>6</sub>	1.46*	-0.31	0.58	0.23*	0.39**	0.31**	-0.66	-1.44*	-1.05**
P <sub>2</sub> ×P <sub>7</sub>	1.02	0.65	0.83*	-0.32**	-0.14	-0.23**	-0.94	-0.77	-0.85**
P <sub>3</sub> ×P <sub>4</sub>	1.58**	1.74**	1.66**	0.39**	0.30*	0.34**	-0.70	-1.94**	-1.32**
P <sub>3</sub> ×P <sub>5</sub>	-2.68**	-3.79**	-3.24**	-0.21*	0.49**	0.14	0.25	0.30	0.28
P <sub>3</sub> ×P <sub>6</sub>	3.41**	2.65**	3.03**	-0.19	-0.23	-0.21**	1.98**	2.05**	2.02**
P <sub>3</sub> ×P <sub>7</sub>	-1.59*	-2.51**	-2.05**	0.16	-0.05	0.06	2.15**	1.28*	1.72**
P <sub>4</sub> ×P <sub>5</sub>	-0.85	0.28	-0.29	0.03	-0.15	-0.06	-0.58	-0.45	-0.51
P <sub>4</sub> ×P <sub>6</sub>	0.23	0.39	0.31	-0.09	0.05	-0.02	-2.15**	-1.47**	-1.81**
P <sub>4</sub> ×P <sub>7</sub>	0.30	-0.66	-0.18	-0.07	0.01	-0.03	-0.78	0.00	-0.39
P <sub>5</sub> ×P <sub>6</sub>	-2.36**	-1.48*	-1.92**	0.05	-0.07	-0.01	-1.06*	-0.19	-0.63
P <sub>5</sub> ×P <sub>7</sub>	3.79**	4.48**	4.13**	-0.13	-0.20	-0.17*	1.71**	0.04	0.87*
P <sub>6</sub> ×P <sub>7</sub>	-2.67**	-1.63*	-2.15**	0.07	-0.09	-0.01	-0.03	0.52	0.24
LSD 5% (sij)	1.13	1.23	0.82	0.21	0.25	0.16	0.98	1.10	0.72
LSD 1% (sij)	1.51	1.65	1.09	0.28	0.33	0.21	1.30	1.47	0.96
LSD 5% (sij-sik)	1.75	1.91	1.27	0.32	0.39	0.25	1.51	1.70	1.12
LSD 1% (sij-sik)	2.34	2.56	1.69	0.43	0.52	0.33	2.02	2.27	1.48
LSD 5% (sij-ski)	1.51	1.66	1.10	0.28	0.33	0.22	1.31	1.47	0.97
LSD 1% (sij-ski)	2.02	2.22	1.46	0.001	0.06	0.03	1.75	1.97	1.28

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

**Table (5): Cont.**

Cross	No. of rows ear <sup>-1</sup>			No. of kernels row <sup>-1</sup>			Grain yield plant <sup>-1</sup>		
	N1	N2	Comb.	N1	N2	Comb.	N1	N2	Comb.
P <sub>1</sub> ×P <sub>2</sub>	-0.48	0.83	0.18	0.51	0.84	0.68	-4.11	-11.96*	-8.03*
P <sub>1</sub> ×P <sub>3</sub>	0.62	-0.37	0.12	-4.61**	-2.77**	-3.69**	-4.16	-3.91	-4.04
P <sub>1</sub> ×P <sub>4</sub>	-0.57	-0.91*	-0.74*	4.65**	3.42**	4.04**	11.48*	12.92*	12.20**
P <sub>1</sub> ×P <sub>5</sub>	1.92**	1.67**	1.80**	-0.27	-2.26*	-1.26*	-0.59	5.54	2.47
P <sub>1</sub> ×P <sub>6</sub>	-0.93*	-0.72	-0.82**	0.72	1.41	1.07	2.16	3.98	3.07
P <sub>1</sub> ×P <sub>7</sub>	-0.57	-0.51	-0.54	-1.01	-0.64	-0.83	-4.78	-6.57	-5.67
P <sub>2</sub> ×P <sub>3</sub>	-0.13	-0.68	-0.41	-3.39**	-0.20	-1.79**	4.89	-8.78	-1.94
P <sub>2</sub> ×P <sub>4</sub>	0.80	0.21	0.51	4.57**	2.88**	3.73**	21.51**	18.92**	20.21**
P <sub>2</sub> ×P <sub>5</sub>	0.16	-0.94*	-0.39	3.86**	2.32*	3.09**	8.47	-9.01	-0.27
P <sub>2</sub> ×P <sub>6</sub>	-0.07	0.25	0.09	-2.58**	-2.32*	-2.45**	-25.53**	4.69	-10.42**
P <sub>2</sub> ×P <sub>7</sub>	-0.28	0.33	0.02	-2.99**	-3.52**	-3.25**	-5.23	6.12	0.45
P <sub>3</sub> ×P <sub>4</sub>	0.36	0.24	0.30	-1.65*	-2.62**	-2.13**	-9.66	-2.66	-6.16
P <sub>3</sub> ×P <sub>5</sub>	-1.42**	0.86	-0.28	0.98	-0.90	0.04	-15.08**	-2.42	-8.75*
P <sub>3</sub> ×P <sub>6</sub>	0.09	-0.08	0.00	4.76**	3.18**	3.97**	21.28**	12.88*	17.08**
P <sub>3</sub> ×P <sub>7</sub>	0.48	0.04	0.26	3.91**	3.31**	3.61**	2.73	4.89	3.81
P <sub>4</sub> ×P <sub>5</sub>	-0.67	-0.32	-0.50	-5.00**	0.89	-2.05**	-5.33	-2.84	-4.08
P <sub>4</sub> ×P <sub>6</sub>	0.27	0.81	0.54	-2.22**	-2.99**	-2.60**	1.91	-13.03*	-5.56
P <sub>4</sub> ×P <sub>7</sub>	-0.19	-0.02	-0.11	-0.36	-1.59	-0.98	-19.91**	-13.32*	-16.61**
P <sub>5</sub> ×P <sub>6</sub>	0.04	-0.84	-0.40	-0.36	-0.88	-0.62	-7.24	-4.33	-5.79
P <sub>5</sub> ×P <sub>7</sub>	-0.04	-0.43	-0.23	0.78	0.83	0.81	19.77**	13.06*	16.41**
P <sub>6</sub> ×P <sub>7</sub>	0.60	0.58	0.59	-0.33	1.61	0.64	7.41	-4.19	1.61
LSD 5% (sij)	0.84	0.91	0.61	1.58	1.83	1.19	10.77	11.47	7.74
LSD 1% (sij)	1.13	1.21	0.81	2.12	2.45	1.58	14.41	15.35	10.27
LSD 5% (sij-sik)	1.30	1.40	0.94	2.45	2.83	1.85	16.68	17.77	11.99
LSD 1% (sij-sik)	1.75	1.88	1.25	3.28	3.79	2.45	22.32	23.78	15.91
LSD 5% (sij-ski)	1.13	1.21	0.82	2.13	2.45	1.60	14.45	15.39	10.39
LSD 1% (sij-ski)	1.51	1.63	1.08	2.84	3.28	2.12	19.30	20.59	13.78

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

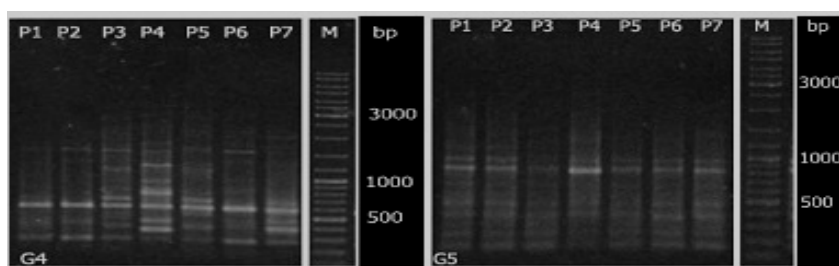
The cross  $P_1 \times P_5$  for number of rows ear<sup>-1</sup> and the crosses  $P_1 \times P_4$ ,  $P_2 \times P_4$ ,  $P_2 \times P_5$ ,  $P_3 \times P_6$  and  $P_3 \times P_7$  for number of kernels row<sup>-1</sup> under the two nitrogen levels and the combined data had significant and positive SCA effects. Four crosses  $P_1 \times P_4$ ,  $P_2 \times P_4$ ,  $P_3 \times P_6$  and  $P_5 \times P_7$  under the two nitrogen levels and their combined data had significant and positive SCA effects for grain yield plant<sup>-1</sup>. 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 plant<sup>-1</sup> also showed high SCA effects for one or more traits of yield components. For example, the cross  $P_2 \times P_4$  which showed high SCA effects for grain yield plant<sup>-1</sup> also showed high SCA effects for ear diameter, ear length and number of kernels row<sup>-1</sup>. In most traits, the values of SCA effects were mostly different from nitrogen level to another. These findings coincided with that discussed elsewhere in this study where significant SCA by nitrogen levels mean squares were detected (Table 2).

#### **Polymorphism of RAPD markers**

Seven random primers used to assess genetic diversity among the seven inbred lines generated a total of 70 reproducible RAPD bands with an average of 10 bands per primer. Of which 14 bands (22.12 %) were monomorphic, while 56 bands (77.88 %) were polymorphic (Table 6). Primer OP-G7 gave 100 % polymorphism while, primer OP-G5 (Fig. 1) produced the most monomorphic bands. The level of polymorphism (77.88 %) found in this study was higher than that reported (73.02 %) in other selected group of maize inbred lines (Mukharib *et al.*, 2010). Molin *et al.* (2013) reported (81.9%) polymorphism in RAPD based screening of 48 varieties of maize landraces and clustered them based on their genetic diversity. The genetic polymorphism detected among the inbred lines in this study can be used to expand the genetic resources in breeding programs.

**Table (6): Maize RAPD primers, their amplified fragments, monomorphic, polymorphic and the polymorphism percentage.**

No.	Primer	Total amplified fragment	Monomorphic bands	Polymorphic bands	Polymorphism %
1	OP-G1	5	1	4	80
2	OP-G2	10	1	9	90
3	OP-G3	9	1	8	88.89
4	OP-G4	16	3	13	81.25
5	OP-G5	8	6	2	25
6	OP-G6	10	2	8	80
7	OP-G7	12	0	12	100
Total		70	14	56	77.88



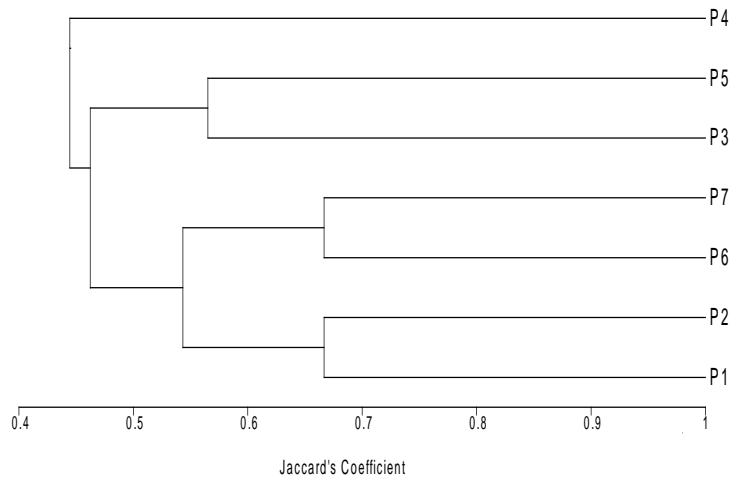
**Fig. (1): DNA-RAPD patterns generated by OP-G4 and OP-G5 primers with the seven inbred lines (P1 - P7). (M) refers to the DNA ladder.**

**Genetic diversity for RAPD marker**

Based on the RAPD profiles, a genetic distance (GD) matrix was constructed using the shared bands (monomorphic) and the variable bands (polymorphic) among the seven inbred lines. The Lowest genetic distance (0.333) was obtained between the inbred lines (P<sub>1</sub> and P<sub>2</sub>) and (P<sub>6</sub> and P<sub>7</sub>), whereas the highest genetic distance was (0.655) scored between the inbred lines P<sub>2</sub> and P<sub>4</sub> (Table 7). The average of genetic distance among all parents was (0.501). Cluster analysis classified the seven inbred lines into two main clusters (Fig. 2) in addition to the out group consists of the inbred line P<sub>4</sub>. The first main cluster included four inbred lines P<sub>1</sub>, P<sub>2</sub>, P<sub>6</sub> and P<sub>7</sub> and this cluster separated into two sub-clusters; the first sub-cluster grouped the inbred lines P<sub>1</sub> and P<sub>2</sub>. While, the second sub cluster contained the inbred lines P<sub>6</sub> and P<sub>7</sub>. The inbred lines P<sub>3</sub> and P<sub>5</sub> were grouped in the second main cluster. RAPD technique can be used as a tool for determining the extent of genetic diversity among maize inbred lines, for allocating genotypes into different groups and is successful in confirming hypothesized relationship (Parentoni *et al.*, 2001 and Devi and Singh, 2011).

**Table (7): Genetic distance based on Jaccard's coefficient for the seven inbred lines of maize revealed by RAPD.**

Inbred lines	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>4</sub>	P <sub>5</sub>	P <sub>6</sub>	P <sub>7</sub>
P <sub>1</sub>	-						
P <sub>2</sub>	0.333	-					
P <sub>3</sub>	0.545	0.619	-				
P <sub>4</sub>	0.576	0.655	0.610	-			
P <sub>5</sub>	0.490	0.640	0.435	0.467	-		
P <sub>6</sub>	0.395	0.463	0.543	0.550	0.460	-	
P <sub>7</sub>	0.468	0.500	0.542	0.475	0.462	0.333	-



**Fig. (2): Dendrogram generated based on UPGMA clustering method and Jaccard's coefficient using RAPD data among the parental inbred lines.**

**Correlation between GD and mean performance of grain yield plant<sup>-1</sup>**

The estimate value of correlation coefficient between GD of the parental inbred lines and mean performance of the F<sub>1</sub> hybrids for grain yield plant<sup>-1</sup> was low and positive ( $r = 0.335$ ). This specific tendency could be predicted about the relationship of GD for grain yield plant<sup>-1</sup> in this study. A similar finding was reported in earlier studies of Shieh and Thseng (2002) and EL-Hosary *et al.* (2006) wherein the correlation between RAPD-based genetic distance of the parental inbred lines and F<sub>1</sub> hybrids for grain yield in general was low or not high enough to be of predictive value. The results of the present study were different from that of Lanza *et al.* (1997) who reported positive correlation between RAPD-based genetic distances and F<sub>1</sub> hybrids grain yield. RAPD marker can be used as a tool for determining the extent of genetic diversity among maize inbred lines into different groups but when used a large number of primers to detect the variation over all DNA or used a new marker like SSR.

**CONCLUSION**

Both additive and non-additive gene effects were important in the inheritance of all the studied traits with preponderance of non-additive gene action in the inheritance of all the studied traits except days of 50% silking and grain yield plant<sup>-1</sup> under the two nitrogen levels and their combined data.

Two crosses  $P_1 \times P_4$  and  $P_2 \times P_4$  had positive and significant superiority percentage relative to the check hybrid SC10 for grain yield  $\text{plant}^{-1}$  under the two nitrogen levels and their combined data. These crosses offer possibility for improving grain yield in maize and may be useful for testing under different locations and environments. The polymorphism percentage based on overall RAPD primers was 77.88 %. The correlation between RAPD-based genetic distance of the parental inbred lines and hybrids grain yield  $\text{plant}^{-1}$  was low and can't be used to precisely predict the  $F_1$  hybrids grain yield performance. RAPD marker can be used as a tool for determining the extent of genetic diversity among maize inbred lines into different groups but when used a large number of primers to detect the variation over all DNA or used a new marker like SSR.

## REFERENCES

- Abdel-Moneam, M.; A. Attia; M. El-Emery and E.A. Fayed (2009). Combining ability and heterosis for some agronomic traits in crosses of maize. Pak. J. Biol. Sci., 12: 433-438.
- Abdel-Moneam, M.; M. Sultan; S. Salama and A. El Oraby (2014). Evaluation of combining ability and heterosis for yield and its components traits of five maize inbreds under normal and stress nitrogen fertilization. Asian J. Crop Sci., 6: 142-149.
- Abuali, A.I.; A.A. Abdelmulla; M.M. Khalafalla, A.E. Idris and A.M. Osman (2012). Combining ability and heterosis for yield and yield components in maize (*Zea mays* L.). Aust. J. Basic Appl. Sci., 6: 36-41.
- Badu-Apraku, B. and M. Oyekunle (2012). Genetic analysis of grain yield and other traits of extra-early yellow maize inbreds and hybrid performance under contrasting environments. Field Crops Res., 129: 99-110.
- Badu-Apraku, B.; M. Oyekunle; M.A.B. Fakorede; I. Vroh; R. O. Akinwale and M. Aderounmu (2013). Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. Euphytica, 192: 413-433.
- Bruel, D.C.; V. Carpentieri-Pipolo; C. de Fátima Ruas; A.C. Gerage and S.G.H. de Souza (2007). Assessment of genetic diversity in maize inbred lines using RAPD markers. Crop Breed. Appl. Biot., 7 (2): 173-178.
- Chawla, H. and V. Gupta (1984). Index India-Agric. Calcutta Agric. Soc. Indian, 28 (4): 261-265.
- Desai, S.A. and R. Singh (2001). Combining ability studies for some morphophysiological and biochemical traits related to drought tolerance in maize (*Zea mays* L.). Indian J. Genet. Plant Breed., 61(1): 34-36.
- Devi, P. and N.K. Singh (2011). Heterosis, molecular diversity, combining ability and their interrelationships in short duration maize (*Zea mays* L.) across the environments. Euphytica, 178 (1): 71-81.

- El-Badawy, M.E.M. (2013). Heterosis and combining ability in maize using diallel crosses among seven new inbred lines. *Asian J. Crop Sci.*, 5(1): 1-13.
- El-Ghonemy, M. and M. Ibrahim (2010). Diallel analysis of yellow maize for combining ability and heterosis. *J. Plant Production, Mansoura Univ.*, 1 (6): 779-792.
- El-Hosary, A.; M.E.M. EL-Badawy and Y.M. Abdel-Tawab (2006). Genetic distance of inbred lines and prediction of maize single cross performance using RAPD and SSR markers. *Egypt. J. Genet. Cytol.*, 35: 209-224.
- Estakhr, A. and B. Heidari (2012). Combining ability and gene action for maturity and agronomic traits in different heterotic groups of maize inbred lines and their diallel crosses. *J. Crop Sci. Biotech.*, 15(3): 219-229.
- Garcia-Vallve, S.; A. Romeu and J. Palau (2000). Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. *Mol. Biol. Evol.*, 17 (3): 352-361.
- Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.*, 9(4): 463-493.
- Hallauer, A.R. (1990). Improvements in yield of maize hybrids. UDC 63: 193-198p Zagreb.
- Kamara, M.M.; I.S. El-Degwy and H. Koyama (2014). Estimation combining ability of some maize inbred lines using line  $\times$  tester mating design under two nitrogen levels. *Aust. J. of Crop Sci.*, 8(9):1336-1342.
- Katta, Y.S.; M.S.M. Abd El-Aty; M.A. El-Hity and M.M. Kamara (2007). Estimate of heterosis and combining ability of some white inbred lines of maize (*Zea mays* L.). *J. Agric. Sci. Mansoura Univ.*, 32(9): 7077-7088.
- Katta, Y.S.; M.M. Kamara; M.A. El-Hity and H. Koyama (2013). Combining ability for some growing and yielding traits in maize under two nitrogen levels. *Egypt J. of Plant Breed.*, 17(2): 331-345.
- Lanza, L.L.B.; J.r. de Souza; L.M.M. Ottoboni; M.L.C. Vieira and A.P. de Souza (1997) Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. *Theor. Appl. Genet.*, 94(8): 1023-1030.
- Lima, M.; J. deMiranda and P. Furlani (1995). Diallel cross among inbred lines of maize differing in aluminum tolerance. *Braz. J. Genet.*, 18(4): 579-584.
- Liu, X.; Z. Peng; J. Fu; C. Huang; X. Liu; Z. Peng; J. Fu and C. Huang (1998). Maize inbred line grouping by using cluster analysis of RAPD molecular marker, phenotype and heterosis. *Acta Agriculturae Boreali Sinica*, 13: 36-41.
- Makumbi, D.; J.F. Betrán; M. Bänziger and J.M. Ribaut (2011). Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica*, 180: 143-162.

- Medici, L.; M. Pereira; P.J. Lea and R.A. Azevedo (2004). Diallel analysis of maize lines with contrasting responses to applied nitrogen. *J. Agric. Sci.*, 142(5): 535-541.
- Molin, D.; C. Coelho; D. Máximo; F. Ferreira; J. Gardingo and R. Matiello (2013). Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. *Genet. Mol. Res.*, 12 (1): 99-114.
- Mosa, H.E.; A.A. Motawei and A.M.M. Abd El-Aal (2010). Nitrogen fertilization influence on combining ability for grain yield and resistance to late wilt disease in maize. *J. Agric. Res. Kafrelsheikh Univ.*, 36: 278-291.
- Mukharib, D.; V. Patil; D. Biradar; P. Salimath and V. Chimmad (2010). Assessment of molecular diversity in selected maize inbreds. *Karnataka J. Agric. Sci.*, 23: 409-412.
- Ngaboyisonga, C; K. Njoroge; D. Kirubi and S.M. Githiri (2009). Effects of low nitrogen and drought on genetic parameters of grain yield and endosperm hardness of quality protein maize. *Asian J. Agric. Res.*, 3: 1-10.
- Ogunbodede, B.; S. Ajibade and S. Olakojo (2000). Heterosis and combining ability for yield and yield related characters in some Nigerian local varieties of maize (*Zea mays*). *Moor J. Agric. Res.* 1 (1): 37-43.
- Oyekunle, M.; B. Badu-Apraku; S. Hearne and J. Franco (2015). Genetic diversity of tropical early maturing maize inbreds and their performance in hybrid combinations under drought and optimum growing conditions. *Field Crops Res.*, 170: 55-65.
- Parentoni, S.N.; J.V. Magalhães; C.A.P. M.X. Pacheco Santos; T. Abadie; E.E.G. Gama; P.E.O. Guimarães; W.F. Meirelles; M.A. Lopes; M.J.V. Vasconcelos and E. Paiva (2001). Heterotic groups based on yield-specific combining ability data and phylogenetic relationship determined by RAPD markers for 28 tropical maize open pollinated varieties. *Euphytica*, 121:197-208.
- Rameeh, V.; A. Rezai and A. Arzani (2000). Estimates of genetic parameters for yield and yield components in corn inbred lines using diallel crosses. *J. Sci. Tech. Agri. Natural Resources*, 4(2): 95-104.
- Shieh, G. and F. Thseng (2002). Genetic diversity of Tainan-white maize inbred lines and prediction of single cross hybrid performance using RAPD markers. *Euphytica*, 124 (3): 307-313.
- Smith, J. and O. Smith (1992). Fingerprinting crop varieties. *Adv. Agron.*, 47: 85-140.
- Sprague, G.F. and L.A. Tatum (1942). General vs. specific combining ability in single crosses of corn. *J. Amer. Soc. Agron.*, 34: 923-932.
- Steel, R.G.D. and J.H. Torrie (1980). *Principles and Procedures of Statistics. A Biometrical Approach*. 2<sup>nd</sup> Ed. Mc Graw Hill, N.Y., USA.
- Tamari, F.; C.S. Hinkley and N. Ramprashad (2013). A comparison of DNA extraction methods using *Petunia hybrida* tissues. *J. Biomol. Tech.*, 24(3):113.
- Wu, G.; Y. Xue and D. He (2003). Combining ability analysis of maize inbred lines. *J. Maize Sci.*, 11: 32-36.



- Wu, M. (2000). Genetic diversity and its relationship to hybrid performance and heterosis in maize as revealed by AFLPs and RAPDs. Maize Genetics Cooperation Newsletter, (74): 62-63.
- Yu, H.; K. Xu; X. Chen; Z. Wu; Z. Jin and X. Shen (2003). Analysis of combining ability and hereditary parameters of a major drought resistant in maize for yield traits. J. Maize Sci., 11: 12-18.

**القدرة على التآلف فى الذرة الشامية تحت مستويين من التسميد النيتروجينى و تقدير التباعد الوراثى باستخدام المعلمات الجزيئية RAPD**  
**\*محمد محمد وجيد قمره و\*\*مدحت رمضان ربحان**  
**\*قسم المحاصيل- كلية الزراعة- جامعة كفر الشيخ- كفر الشيخ- مصر**  
**\*\*قسم الوراثة- كلية الزراعة- جامعة كفر الشيخ- كفر الشيخ- مصر**

تم عمل التهجين النصف دائرى بين سبعة سلالات من الذرة الشامية البيضاء فى موسم ٢٠١٢. تم تقييم ال ٢١ هجين فردى الناتجة بالإضافة الى هجين المقارنة (هجين فردى ١٠) فى تجربتين منفصلتين تحت مستويين من التسميد النيتروجينى ٨٠ و ١٢٠ كجم نيتروجين/ فدان بمزرعة كلية الزراعة - جامعة كفر الشيخ فى موسم ٢٠١٣. وذلك لتقدير تأثيرات القدرة العامة والخاصة على التآلف وتفاعلها مع مستويات التسميد ولتحديد السلالات والهجن المتوقعة وتم دراسة الصفات التالية: عدد الأيام حتى ظهور ٥٠ % من الحراير ، طول الكوز، قطر الكوز، عدد الصفوف/ كوز، وعدد الحبوب/ صف ومحصول الحبوب/ نبات وتم تحليلها وراثياً وفقاً للطريقة الرابعة-الموديل الأول لجريفيج ١٩٥٦. أظهرت النتائج أن التباين الراجع لكل من مستويى التسميد، التراكيب الوراثية، الهجن ، التراكيب الوراثية x النيتروجين و الهجن x النيتروجين كان معنوياً لجميع الصفات تحت الدراسة. كانت التباينات للقدرة العامة والخاصة على التآلف معنوية لكل الصفات تحت الدراسة فى كلا المستويين من التسميد و التحليل المشترك. تفاعلت كل من القدرة العامة والخاصة على التآلف معنوياً مع التسميد النيتروجينى لمعظم الصفات تحت الدراسة. كان الفعل الجينى غير المضيف هو الاكثر اهمية فى وراثة جميع الصفات ما عدا صفتى عدد الأيام حتى ظهور ٥٠ % من الحراير ومحصول الحبوب / نبات. أظهرت السلالات الأبوية رقم ٥ ، ٦ ، ٧ أفضل القيم لتأثيرات القدرة العامة على الائتلاف للتبكير بينما أظهرت السلالات رقم ١ ، ٢ ، ٤ قدرة عامة جيدة على التآلف لصفة محصول الحبوب/ نبات. أظهرت النتائج أن أفضل الهجن للقدرة الخاصة على التآلف (المرغوب) هى الهجن  $P_6 \times P_7$   $P_5 \times P_6$  ،  $P_3 \times P_5$  ،  $P_3 \times P_7$  ،  $P_3 \times P_5$  لصفة التزهير المبكر والهجن  $P_1 \times P_4$  ،  $P_2 \times P_4$  ،  $P_3 \times P_6$  ،  $P_5 \times P_7$  لصفة محصول الحبوب/ نبات فى كلا المستويين من التسميد و التحليل المشترك. تفوق محصول الهجينان  $P_1 \times P_4$  و  $P_2 \times P_4$  تفوقاً معنوياً على محصول هجين المقارنة (هجين فردى ١٠) تحت كلا المستويين من التسميد و التحليل المشترك. تم تقدير التباعد الوراثى باستخدام تكنيك ال RAPD بين السبعة سلالات الأبوية. كان عدد شظايا ال DNA الناتجة من سبعة بادئات من RAPD هى ٧٠ شظية حققت ٥٦ منهم عدد متباين من الاختلافات بنسبة ٧٧.٨ % . تراوحت قيمة التباعد الوراثى بين ٠.٣٣٣ الى ٠.٦٥٥ . بمتوسط ٠.٥٣٣ . كانت قيمة الارتباط بين التباعد الوراثى و متوسط أداء الهجن لمحصول الحبوب/ نبات كانت موجبة و لكن منخفضة (٠.٣٣٥) . لذلك لا يمكن الاعتماد على طريقة المعلمات الجزيئية (RAPD) فى التنبؤ بمحصول الحبوب للنبات الفردى للهجن المتكونة من الاباء فى هذه الدراسة.