Genetic diversity of maize inbred lines using ISSR markers and its implication on quantitative traits inheritance

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ABSTRACT

*Genetic distance (GD) among six maize inbred lines was determined based on the ISSR markers. A total of 96 amplification bands were produced by ten ISSR primers; 35 out of them were polymorphic (36.46 % polymorphism). Highest similarity level (94.3%) was between P_2 and P_3 that are closely related. Highest genetic diversity was between P_1 and P_4. Inheritance of earliness, grain yield and its components in three specific crosses (P_2 x P_3 low diversity, P_5 x P_6 moderate diversity and P_1 x P_4 high diversity) were studied in a trial consisting of 6 populations (P_1, P_2, F_1, F_2, Bc_1 and Bc_2) during 2013 and 2014 years. Inbred line differences occurred regarding genetic background, genetic variance within F_2 population, desirable heterosis and inbreeding depression were detected for all studied traits in the three crosses. Potence ratios were higher than unity indicating over-dominance towards the desirable parent. Additive gene effects (a) and dominance gene effects (d) were significant for most studied traits. High heritability in broad-sense was detected, except for grain yield plant^{-1} in cross No.1. Heritability in narrow-sense was low. Variance in F_2, mean performance in F_1 and GCV% increased with increasing GD in the cross P_1xP_4 followed by cross P_5xP_6 and then by cross P_2xP_3. Values of r were 0.98** between GD and variance of F_2, 0.97**between GD and mean performance of F_1 and 0.79** between GD and GCV% for grain yield plant^{-1}. Hence,ISSR markers method proved powerful, reliable, fast and inexpensive for screening genetic diversity between maize inbred lines.*

Key words: *Maize, ISSR markers, Genetic diversity, and Heritability.*

INTRODUCTION

Maize (*Zea mays* L.) is one of vital cereal crops in Egypt and the world. It is widely used in bread making in rural Egypt and in industries such as glucose, oil starch and is a main component in animal feeds. It is a general policy in Egypt to mix wheat flour with maize flour (1:4) for bread making in order to decrease wheat consumption and import. Discriminating various genotypes using morphologic markers is difficult and time-consuming. Isoenzymes and proteins that are the product of gene expression cannot demonstrate polymorphism among genotypes, and cannot identify hybrids that have close relationships. However, modern molecular markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Inter-Simple Sequence Repeats (ISSR) have been used to identify different genotypes with reliable results.

Inter-simple sequence repeat polymorphism (ISSR) markers are derived from polymorphic genomic segments that are flanked by inversely oriented, closely spaced identical micro satellite sequences (Lenka *et al.* 2015). These markers produce various polymorphisms more than RAPD markers. They have been used in assessing genetic diversity in maize genotype. Therefore, such markers are highly useful for identifying genotype, exclusion of false seeds and purification of genotypes. The ISSR amplification was described by Zietkiewicz *et al*. (1994) as, a quick stable technique and highly reliable in detection of rich polymorphisms in inter-microsatellite loci giving a good number of bands per primer, besides being relatively inexpensive.

Improving maize productivity depends on the knowledge of the heritability, gene action and interactions. The quantitative traits contain several minor genes, each of which gives a small effect and is influenced by environmental changes. For studying the genetic parameters which control the quantitative expression of gene action, commonly, there were methods depend either on genotypic mean performance or variances controlling the character. Among the other important information needed for the successful breeding program is hybrid vigor, inbreeding deteriorations, potence ratio, predicted genetic advance under selection which were explained by Johnson *et al.* (1955), El-Shouny *et al*. (2005) and El-Hosary *et al.*(2011). Generation mean analysis is a simple and useful technique for estimating gene effects for polygenic traits, where its highest advantage is to estimate epistatic gene effects, such as additive x additive (aa), dominance x dominance (dd) and additive x dominance (ad), Singh and Singh (1992) conversely Nawar *et al.* (2011) and El-Badawy (2012) reported that the type of epistatic effects additive x additive and additive x dominance as well as additive were less important than dominance and dominance x dominance effects for corn grain yield. However, the magnitude of dominance and epistatic gene effects contributed to greater extent than the additive gene effects.

The aim of the present study was to: 1) investigate possible advantages of using ISSR molecular markers, for identification and estimation of genetic diversity among six parental inbred lines of maize, and 2) investigation of the inheritance and predicted genetic gain of earliness, grain yield and its components in three specific corn crosses.

MATERIALS AND METHODS

Plant materials

Six elite white maize inbred lines were used in this study *i.e.* M27-1 (P_1), M83-A (P_2), M24-C (P_3), M43-A (P_4), M9-1 (P_5) and M122-A (P_6) which were released more than 15 year ago from SC 10, Pioneer 514, Co. 108, D.C. 183, Giza 2 and Sabaeny, respectively by Prof. Dr. Ali A. El-Hosary, Prof. of Agron., Fac. of Agric., Benha Univ. Egypt.

Molecular Analysis

Genomic DNA extraction

Genomic DNA was isolated from leaf tissues of each plant using the CTAB method (Rogers and Bendich, 1994).

Inter-simple sequence repeats (ISSR)

After estimating the concentration of the DNA samples, concentrated aliquots from each stock of DNA samples were diluted to a uniform concentration of 10 ng μL^{-1} to be used with PCR marker. Oligonucleotide sequences of the primers used in this study were selected from a set of Operon kits (Operon Technologies Inc., Alameda California, USA).

A total of ten primers (Table 1) were used in the detection of polymorphism among the six maize inbred lines.

Table (1): Name and sequence of the primers used in ISSR detection.

Primer	Sequence
ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR- 6	5'-CGCGATAGATAGATAGATA-3'
ISSR- 7	5'-GACGATAGATAGATAGATA-3'
ISSR- 8	5'-AGACAGACAGACAGACGC-3'
ISSR- 9	5'-GATAGATAGATAGATAGC-3'
ISSR- 10	5'-GACAGACAGACAGACAAT-3'

Reactions were carried out in a total volume of 25 µl containing 30 ng of genomic DNA (as a template) along with 30 pmoles of random primer, 2mM of dNTP's mix (dATP, dCTP, dTTP and dGTP, ABgene, Surrey, UK), 10 X PCR buffer, 25 mM MgCl2, and 2 units Taq DNA polymerase (Promega, USA). Amplifications were carried out in a thermo cycler (UNO II Biometra) programmed at 94ºC for 4 min (one cycle); followed by 94ºC for 45 sec, 38ºC for 1 min and 72ºC for 1 min (35 cycle) then by 72ºC for 10(one cycle) ,then 4ºC(infinitive). The amplification products were resolved by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 µg ml-1), and visualized with ultraviolet light and photographed. Size of DNA fragments was determined by comparison with the 100bp and 1Kb DNA ladder marker (Promega USA). Similarity coefficients between a pair of inbred lines were produced for the ISSR data using Nei and Li's formula (1979). A dendrogram tree was constructed by the UPGMA clustering algorithm from the SAHN option of NTSYS-PC version 2.1(Rohlf, 2000).

Field experiment

The field experiment was conducted at the Research and Experimental Station, Faculty of Agriculture, Moshtohor in two successive years (2013 and 2014). In the first year of 2013, grains of six parents were sown on 1st and 10th March. Three crosses were chosen based on genetic diversity determined by ISSR, i.e. P_2xP_3 (low diversity), P_5xP_6 (moderate diversity) and P_1xP_4 (high diversity) were made to obtain F_1 seeds. In the late season of 2013, each of F_1 and their parents were sown on 18th and 25th July. Part of the F_1 plants was backcrossed to their respective parents to produce the first and second backcrosses (Bc_1 and Bc_2), while, the other part of F_1 plants were selfed to produce F_2 seeds. Fresh seeds of F_1, from each cross, were obtained by crossing their two parents. In the summer season of 2014, the three adjacent experiments involved parents, F_1, F_2, Bc_1 and Bc_2 populations of each the three crosses. Seeds of the six populations of the three crosses were evaluated in RCBD with three replications. The dry method of planting was used. The date of planting was 4th May. Each plot contained, two ridges of each inbred lines and F_1; ten ridges of each of the two backcrosses and 20 ridges of F_2 population. Seeds were grown in ridges 6 m long and 70 cm wide. The space between hills was 25 cm with 3 kernels $hill^{-1}$ on one side of the ridge. Seedlings were thinned to one plant $hill^{-1}$. Plots were irrigated after sowing. The cultural practices were followed as done by farmers in the area. Random guarded individual 30 plants

from each parent and F_1, 250 plants for each Bc_1 and Bc_2 and 400 plants for each F_2 were taken to study the following traits: days to maturity, number of rows ear^{-1}, number of kernels row^{-1},100-kernel weight, ear weight plant^{-1} and grain yield plant^{-1}.

Various biometrical parameters were calculated, only, if the F_2 genetic variance was significant. Heterosis was expressed as "the increase of F_1 above the better parent value". Inbreeding depression was calculated as "the difference between means of the F_1 and F_2 expressed as percentage of the F_1 mean". Genetic analysis of generation means for main effect parameter (m), additive (a), dominance (d), additive x additive(aa), additive x dominance (ad) and dominance x dominance (dd) effects were all calculated according to Gamble (1962). In addition, F_2 deviation (E1) and backcross deviation (E2) were determined following the method, suggested by Mather and Jinks (1971). Heritability was calculated, in both broad and narrow senses, according to the procedure of Mather (1949). The predicted genetic advance from selection was estimated using the formula presented by Johanson *et al.*(1955) , and the potence ratio was calculated according to Peter and Frey (1966).

RESULTS AND DISCUSSION

Identification of ISSR primers

The ten primers which generated reproducible and scorable polymorphic markers were selected for further analysis. They produced multiple band profiles with a number of amplified DNA fragments ranging from 6 to 14, while the number of polymorphic fragments ranged from 2 to 10 (Table 2 and Fig. 1).

A maximum number of 14 fragments was amplified with the primers ISSR 2 and 3 and a minimum number of 6 fragments was amplified with the primer ISSR8. The total number of reproducible fragments amplified by the ten primers reached 96 bands, of which 35 were polymorphic fragments. This represented a level of polymorphism of 36.46% and an average of 3.6 markers primer^{-1}, which indicates a very high level of polymorphism among the genotypes studied. The size of the amplified fragments also varied with different primers and ranged from 100 to 1500 bp (Fig. 1).

The ISSR analysis revealed a high level of polymorphism among genotypes, which enabled accurate analysis of the genetic distance. These results agree with those of Buckler *et al.* (2006) who reported 6.8 markers primer^{-1} using 27 ISSR primer pairs .The results also agree with those of Wang and Goldman (1999), Zitoun *et al.* (2008), Dursun *et al.* (2010) and Lenka *et al.* (2015) who demonstrated that primers produced reliable and reproducible banding pattern and that the number, size of amplified DNA fragments and the percentage of generated polymorphic bands varied among primers.

Genetic similarity

The genetic similarity among the six parental inbred lines of maize was estimated based on the scored ISSR data matrix. This similarity matrix was used to generate a dendrogram using the UPGMA method. The ISSR data analysis (Table 3) show that the genetic similarity ranged from 70.7 to 94.3% with an average of 82.5%, which reflects a high level of polymorphism at their DNA level as expected. In addition to ISSR analysis the highest similarity level (94.3%) was detected between P_2 and p_3 which are closely related. However, lowest genetic similarity (70.7%) was between P_1 and P_4.

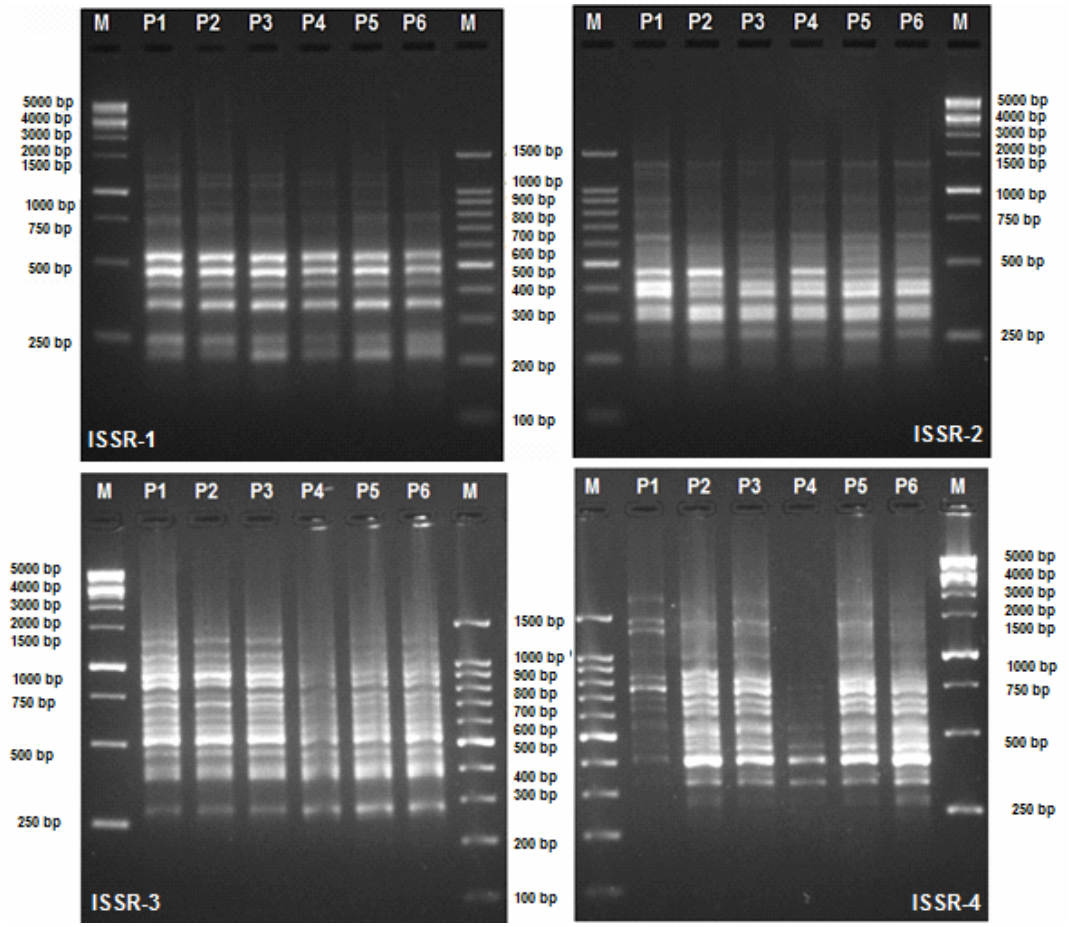

Fig. (1): Amplification of the six maize inbred lines with ISSR primers No. 1, 2, 3 and 4.

Table (2): Details of ten primers and corresponding number of ISSR DNA markers (polymorphic and monomorphic bands).

Primers Name	Total amplified bands	Monomorphic bands		Polymorphic bands	
		No.	%	No.	%
ISSR- 1	10	10	100.00	0	0.00
ISSR- 2	14	10	71.43	4	28.57
ISSR- 3	14	4	28.57	10	71.43
ISSR- 4	13	3	23.08	10	76.92
ISSR- 5	7	5	71.43	2	28.57
ISSR- 6	9	6	66.67	3	33.33
ISSR- 7	7	7	100.00	0	0.00
ISSR- 8	6	4	66.67	2	33.33
ISSR- 9	7	5	71.43	2	28.57
ISSR- 10	9	7	77.78	2	22.22
Total	96	61	63.54	35	36.46

Table (3): Genetic similarity (%) calculated from the total DNA fragments amplified from the six inbred lines using ten primers.

	P_1	P_2	P_3	P_4	P_5	P_6
P_1	100					
P_2	86.3	100				
P_3	85.1	94.3	100			
P_4	70.7	71.11	73.6	100		
P_5	86.8	83.5	86.4	79.3	100	
P_6	84.8	83.5	84.3	77.1	92.8	100

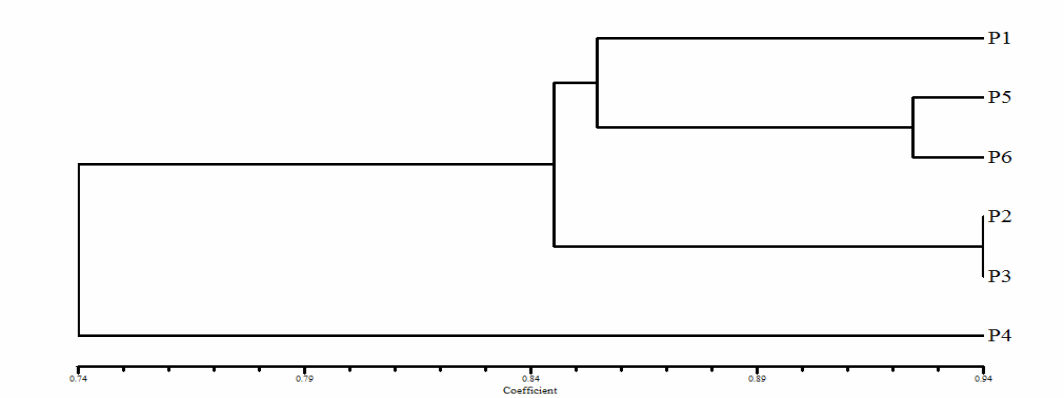

Fig. (2): Dendrogram generated based on UPGM clustering method and Jacquard's coefficient using ISSR analysis among the parental inbred lines.

A dendrogram separated the six maize inbred lines into two major clusters, the first contained P_4, while, the second contained other inbred lines, which could be divided into two subclusters. The inbred lines of P_1, P_5 and P_6 grouped together in the first sub-cluster, while the inbred lines P_2 and P_3 grouped in the second sub-cluster (Fig. 2).

Means, variances and the coefficients of variation of the six populations, i.e., parents, F_1, F_2, Bc_1 and Bc_2 for the studied traits, in the three crosses were chosen based on genetic diversity determined by ISSR markers. The crosses are P_2 x P_3 (low diversity), P_5 x P_6 (moderate diversity) and P_1 x P_4 (high diversity) (Table 4). The test of significance of parental mean performance and the genetic variance among F_2 populations in each cross for each trait are presented in Table (5). Significant inbred line differences in response to their genetic background were detected for all traits in the three crosses. Also, significant genetic variance within F_2 population was found for all traits, in the three crosses. Therefore the different biometrical parameters can be estimated according to the proposed equations of Mather and Jinks (1971) and Gamble (1962).

Heterosis, inbreeding depression, potence ratio, F_2-deviation (E1) and backcross deviation (E2) in the three crosses for maturity, yield and its components are given in Tables (5 and 6). Highly significant desirable heterotic effects compared to better parent were found for all traits in the three crosses. The significant negative heterosis for maturity is vital for escaping destructive injuries caused by *Sesamia cretica*-led, *Chilio simplex*-But and *Pyrausta nubilalis*-Hb. On the other hand,

high, significant positive heterotic effects were detected for yield and its components. The main components of yield in maize are the number of rows ear^{-1}, number of kernels row^{-1}, and 100-kernel weight. Hence if heterotic effect is found in one or more component attributes, it may lead to favorable grain yield increase in hybrids. It is worth noting that heterotic effect for grain yield was larger in magnitude than for any of its components, which is logically expected. Also, heterosis associated with number of rows ear^{-1} was smaller in magnitude than those associated with the other two components, revealing its minor effect on the expression of heterosis for grain yield. The pronounced heterotic effects, detected in the third cross, would be a value in breeding programs for high yielding ability. The significance of heterotic effects shows that non-additive genetic type of gene action affects in such traits. The current results agree with those previously reported by El-Shouny *et al.* (2005), Abou-Deif (2007) and El-Badawy (2012).

The potence ratio (P) values indicate an over-dominance (P<1) towards the desirable parent for all studied traits. Over dominance towards the lower parent was detected for days to maturity, suggesting that earliness dominated lateness. However, over-dominance towards the higher parent was detected for the remaining traits. Generally, potence values followed the same trend as those of the heterotic effects for all traits. These results are in agreement with those obtained by El-Hosary and Abd El Satar (1998) and El-Shouny *et al.* (2005). Inbreeding depression measured the reduction in performance of F_2 generations from their F_1's in the three crosses due to inbreeding. Inbreeding depression was significant and negative for days to maturity in the three crosses. Meanwhile, significant and positive inbreeding depression was detected for other cases. Both heterosis and inbreeding depression effects as it is well known are two coincides to a same particular phenomenon. Therefore, it is logically to expect that heterosis in F_1 will be accompanied by appreciable reduction in the F_2 performance and *vice versa*. Similar results were obtained by El Badawy (2012).

Significant F_2 deviation (E1) occurred since it deviated significantly from the average of F_1 and mid-parent value for all studied traits, thus indicating that epistasis was present in the inheritance of all traits. Significant backcrosses deviation (E2) was detected for all the studied traits, except for the 100-kernel weight in the second cross. It is worth noting that F_2 deviation was mostly accompanied by backcross deviation of significance in most cases under study, indicating a presence of epistasis in such large magnitude and an existence of over-dominance detected herein in most cases. This may reveal the vital role of inter-allelic gene effects on the performance of these cases.

Nature of gene action (Table 7) was investigated, according to the relationships illustrated by Gamble (1962). The estimated mean effect parameter (m), which reflects the contribution due to the overall mean plus the locus effects and interaction of the fixed loci, were highly significant for all studied characters in all crosses. The additive gene effects (a) were significant for all studied traits except number of rows ear^{-1}. These results are in agreement with those obtained by El-Shouny *et al.* (2005) and El Badawy (2012). The dominance gene effects (d) were highly significant for all traits in the three crosses, except for the number of rows ear^{-1} in the second cross and the number of kernels row^{-1} in the first cross. Dominance effects were higher in magnitude than additive gene effects. The negative value of dominance demonstrates that the smaller mean value parent had the dominant genes responsible for these cases.

Table (4): Means and variance for the six generations for all studied traits in the three studied crosses.

Trait	Cross	statistic	Population					
			P_1	P_2	F_1	F_2	Bc_1	Bc_2
Days to maturity	I(P_2xP_3)	Mean	108.700	115.300	104.967	111.670	105.344	108.122
		Variance	1.045	1.045	1.068	9.780	7.747	6.387
	II(P_5xP_6)	Mean	106.733	115.567	104.600	109.893	107.267	105.667
		Variance	1.168	2.047	1.145	9.012	8.442	6.369
	III(P_1xP_4)	Mean	114.267	106.300	100.207	107.790	108.867	107.200
		Variance	1.995	2.148	2.081	33.243	27.714	11.815
No. of rows ear^{-1}	I(P_2xP_3)	Mean	9.867	8.533	13.067	12.620	12.522	12.478
		Variance	0.533	0.809	1.030	4.424	1.581	4.575
	II(P_5xP_6)	Mean	9.733	13.520	15.467	13.290	12.467	12.200
		Variance	0.478	0.828	0.436	3.000	2.595	2.540
	III(P_1xP_4)	Mean	8.600	11.667	15.933	14.003	14.572	14.361
		Variance	0.869	0.575	0.133	3.518	2.800	2.700
No. of kernels row^{-1}	I(P_2xP_3)	Mean	23.733	17.867	33.568	29.327	28.839	23.217
		Variance	1.030	1.085	3.875	5.858	4.700	4.569
	II(P_5xP_6)	Mean	25.467	20.133	38.633	28.373	26.328	33.122
		Variance	3.223	0.533	1.551	11.272	10.121	8.577
	III(P_1xP_4)	Mean	19.067	14.300	44.300	26.000	29.210	27.145
		Variance	2.478	2.769	2.562	25.389	16.291	19.013
100-kernel weight	I(P_2xP_3)	Mean	30.688	31.303	35.400	29.913	27.744	26.828
		Variance	2.624	2.118	2.166	10.688	9.711	7.305
	II(P_5xP_6)	Mean	23.100	27.767	33.900	30.287	32.533	27.183
		Variance	3.266	2.185	2.437	18.051	15.491	15.748
	III(P_1xP_4)	Mean	26.033	29.367	42.000	36.613	32.900	30.344
		Variance	0.861	1.620	1.666	8.191	3.867	8.998
Ear weight plant^{-1}	I(P_2xP_3)	Mean	111.067	71.627	219.167	134.610	118.583	134.140
		Variance	10.616	17.790	10.489	172.867	162.088	152.031
	II(P_5xP_6)	Mean	140.267	89.767	234.500	119.447	120.978	99.283
		Variance	5.375	7.426	8.121	64.268	43.664	59.690
	III(P_1xP_4)	Mean	90.160	142.433	249.497	147.700	177.683	144.083
		Variance	4.434	3.151	4.378	320.164	243.033	229.429
Grain yield plant^{-1}	I(P_2xP_3)	Mean	72.833	49.200	159.833	99.440	89.694	119.333
		Variance	6.351	22.263	6.006	28.562	23.509	27.486
	II(P_5xP_6)	Mean	92.817	59.799	174.150	95.983	101.561	75.572
		Variance	11.219	8.611	9.995	68.070	52.572	49.755
	III(P_1xP_4)	Mean	62.033	98.700	204.560	148.440	149.439	116.278
		Variance	4.240	4.631	4.051	218.254	186.907	211.621

Table (5): Mean performance of parents, t-test of difference between parents and F-test of genetic variance among F_2 plants of the three crosses for the studied traits.

Trait	Cross	P_1	P_2	t-test	F-test
Days to maturity	I(P_2xP_3)	108.70	115.30	-25.01**	9.29**
	II(P_5xP_6)	106.73	115.57	-26.98**	6.20**
	III(P_1xP_4)	114.27	106.30	21.44**	16.02**
No. of rows ear^{-1}	I(P_2xP_3)	9.87	8.53	6.30**	5.59**
	II(P_5xP_6)	9.73	13.52	-18.15**	5.17**
	III(P_1xP_4)	8.60	11.67	-13.98**	6.69**
No. of kernels row^{-1}	I(P_2xP_3)	23.73	17.87	22.10**	2.93**
	II(P_5xP_6)	25.47	20.13	15.07**	6.37**
	III(P_1xP_4)	19.07	14.30	11.40**	9.75**
100-kernel weight (g)	I(P_2xP_3)	30.69	31.30	-1.55	4.64**
	II(P_5xP_6)	23.10	27.77	-10.95**	6.87**
	III(P_1xP_4)	26.03	29.37	-11.59**	5.93**
Ear weight plant^{-1} (g)	I(P_2xP_3)	111.07	71.63	40.53**	13.33**
	II(P_5xP_6)	140.27	89.77	77.31**	9.22**
	III(P_1xP_4)	90.16	142.43	-103.96**	80.29**
Grain yield plant^{-1} (g)	I(P_2xP_3)	72.83	49.20	24.20**	2.48**
	II(P_5xP_6)	92.82	59.80	40.61**	6.85**
	III(P_1xP_4)	62.03	98.70	-67.43**	50.67**

** p≤ 0.01

Table (6): Heterosis %, potence ratio, inbreeding depression%, F_2-deviation (E1) and backcross deviation (E2) in the three crosses for maturity, yield and its components.

Trait	Cross	Heterosis Mp	Potance ratio	Inbreeding depression	Deviation E1	Deviation E2
Days maturity	I(P_2xP_3)	-6.28**	-2.13	-6.39**	3.19**	-3.50**
	II(P_5xP_6)	-5.89**	-1.48	-5.06**	2.02**	-2.82**
	III(P_1xP_4)	-9.14**	-2.53	-7.57**	2.55**	5.58**
No. of rows ear^{-1}	I(P_2xP_3)	42.03**	5.80	3.42**	1.49**	2.73**
	II(P_5xP_6)	33.03**	2.03	14.07**	-0.26*	-2.43**
	III(P_1xP_4)	57.24**	3.78	12.11**	0.97**	2.87**
No. of kernels row^{-1}	I(P_2xP_3)	61.38**	4.35	12.64**	2.14**	-2.31**
	II(P_5xP_6)	69.44**	5.94	26.56**	-2.34**	-1.98**
	III(P_1xP_4)	165.53**	11.59	41.31**	-4.49**	-4.63**
100-kernel weight	I(P_2xP_3)	14.21**	14.33	15.50**	-3.28**	-11.82**
	II(P_5xP_6)	33.29**	3.63	10.66**	0.62*	0.38
	III(P_1xP_4)	51.62**	8.58	12.83**	1.76**	-6.46**
Ear weight plant^{-1}	I(P_2xP_3)	139.93**	6.48	38.58**	-20.65**	-57.79**
	II(P_5xP_6)	103.88**	4.73	49.06**	-55.31**	-129.26**
	III(P_1xP_4)	114.54**	5.10	40.80**	-35.20**	-44.03**
Grain yield plant^{-1}	I(P_2xP_3)	161.95**	8.36	37.79**	-10.99**	-11.82**
	II(P_5xP_6)	128.22**	5.93	44.88**	-29.25**	-73.32**
	III(P_1xP_4)	154.53**	6.77	27.43**	5.98**	-19.21**

* p≤ 0.05; ** p≤ 0.01

Table (7): Parameters of gene effects relating to studied traits in the three crosses.

Trait	Cross	Gene action six parameters (Gamble procedure)					
		Main effect	Additive (a)	Dominane (d)	Add. X Add. (aa)	Add.xDom. (ad)	Dom.xdom. (dd)
Days to maturity	I(P_2xP_3)	111.67**	-2.78**	-26.78**	-19.75**	0.52	26.75**
	II(P_5xP_6)	109.89**	1.60**	-20.26**	-13.71**	6.02**	19.34**
	III(P_1xP_4)	107.79**	1.67**	-9.10**	0.97	-2.32**	-12.13**
No. of rows ear^{-1}	I(P_2xP_3)	12.62**	0.04	3.39**	-0.48	-0.62**	-4.99**
	II(P_5xP_6)	13.29**	0.27	0.01	-3.83**	2.16**	8.68**
	III(P_1xP_4)	14.00**	0.21	7.65**	1.85**	1.74**	-7.59**
No. of kernels row^{-1}	I(P_2xP_3)	29.33**	5.62**	-0.43	-13.20**	2.69**	17.82**
	II(P_5xP_6)	28.37**	-6.79**	21.24**	5.41**	-9.46**	-1.44
	III(P_1xP_4)	26.00**	2.07**	36.33**	8.71**	-0.32	0.55
100-kernel weight	I(P_2xP_3)	29.91**	0.92**	-6.10**	-10.51**	1.22**	34.16**
	II(P_5xP_6)	30.29**	5.35**	6.75**	-1.71	7.68**	0.95
	III(P_1xP_4)	36.61**	2.56**	-5.66**	-19.96**	4.22**	32.88**
Ear weight plant^{-1}	I(P_2xP_3)	134.61**	-15.56**	94.83**	-32.99**	-35.28**	148.58**
	II(P_5xP_6)	119.45**	21.69**	82.22**	-37.26**	-3.56**	295.78**
	III(P_1xP_4)	147.70**	33.60**	185.93**	52.73**	59.74**	35.32**
Grain yield plant^{-1}	I(P_2xP_3)	99.44**	-29.64**	119.11**	20.30**	-41.46**	3.35
	II(P_5xP_6)	95.98**	25.99**	68.18**	-29.67**	9.48**	176.32**
	III(P_1xP_4)	148.44**	33.16**	61.87**	-62.33**	51.49**	100.75**

** $p \leq 0.01$.

Additive x additive (aa) epistatic type of gene action was significant for all traits, except for the number of rows ear^{-1}, 100-kernel weight and days to maturity in the first, second and third cross, respectively. The 'additive x dominance gene' effects were significant for all traits, except for the days to maturity and the number of kernels row^{-1} in the first and third cross, respectively.' Dominance x dominance gene' effects were significant for all traits in the three crosses, except for the number of kernels row^{-1} in the second and third crosses, the 100-kernel weight in the second cross and the grain yield plant^{-1} in the first cross. The majority of dominance x dominance gene effects were significant for most traits. The absolute relative magnitudes of the epistatic gene effects to mean effects were rather variable depending on the studied cross and traits. Generally, the absolute magnitudes of the epistatic effects were larger than the mean effects and approach the dominance effects in most cases.

Therefore, it could be concluded that epistatic effect is important as a major contributor to the performance of these cases. These results agree with the concept that inheritance of a quantitative character is generally more complex than inheritance of a qualitative character. The significant values of epistasis in the three crosses were accompanied by significant estimates for E1 and E2.

The non-additive gene effects appears to be of primer importance in the inheritance of most traits, the large magnitude of both dominance and epistatic effects revealed that both types contribute in the expression of heterosis in most traits. These results well agree with those reported by Gamble (1962) from crosses between some inbred lines. Sentz

(1971) reported that dominance effects tended to be more important, and Fadhi (1978) stated that dominance gene effects had the first rank of gene action and (aa) type of epistasis had the second rank with regard to grain yield. On the other hand, some workers stated that both additive and dominance effects had similar magnitude El-Shouny *et al.* (2005). Most investigators reported that additive effects tended to be more important in the inheritance of yield (Hallauer 1971, El-Rouby and Galal 1972 and Shehata and Dhawan 1975).

Heritability in broad as well as narrow sense, genetical gain and genetic coefficient of variation (GCV%)for the studied traits are presented in Table 8. Utilizing the GCV% alone, however, is impossible to estimate the magnitude of heritable variation. The heritable portion of the variation could be found out with the help of heritability estimates and genetic gain under selection (Swarup and Chaugale, 1962).

High heritability values in broad-sense were detected for the three crosses, except grain yield plant^{-1} in the first cross. For the exceptional case, moderate heritability values were obtained. The highest estimate of heritability was 98.75% for ear weight plant^{-1} in the 3rd cross, while the lowest heritability (in broad-sense) was detected by grain yield plant^{-1} (59.60%) in the first cross. Heritability in a narrow sense was computed according to Mather's procedure on the basis of F_2 and back crosses. High heritability in narrow sense was detected for days to maturity in the third cross. Moderate to low heritability values in narrow sense were detected for the other cases in the three crosses. Non additive gene effects were found to be the major contributing factor in these traits. On these assumptions, heritability in the narrow-sense was expected to be low, the exception which was not realized in the present study. Comstock (1955) stated that the presence of epistatic gene effects causes an upward bias in the estimate of additive genetic variance. Gamble (1962) reported that genetic model assuming negligible epistasis may be an important source of bias in the estimate of additive genetic variance and that inclusion of epistasis in such models may decrease the amount of additive one. Such results are in agreement with those obtained by Warner (1952), El- Ebrashy (1961) and Fadhi (1978) who reported low values of heritability in the narrow-sense for grain yield plant^{-1}. On the other hand El-Shouny *et al.* (2005) and Abou-Deif (2007) obtained high to moderate heritability values for the traits of earliness.

Table (8) shows the genetic advance upon selection as the percentage of F_2 for all the studied traits in the three crosses. With the exception of the number of rows ear^{-1} in the first cross and the number of kernels row^{-1}, the results indicate that the predicted genetic advance expressed as the percentage of the mean was low for all traits. For the exceptional cases, moderate GA% was detected (Table 8). Johanson *et al.* (1955) and Rahman *et al.* (2015) reported that heritability estimates along with genetic gain are usually more useful than the heritability values alone in predicting the resultant effect for selecting the best individuals. On the other hand, heritability is not always associated with high genetic advance, but to make effective selection, high heritability should be associated with high genetic gain. In the present work, relative low genetic gain was found to be associated with rather moderate or low heritability estimates for most cases. Therefore, selection for these cases in these particular populations should not be effective for successful breeding purposes.

Table (8): Heritability percentage, genetic advance (Δg), genetic advance expected as a percentage of the mean for the studied traits and genetic coefficient of variation GCV% in the three crosses.

Cross	Parameter	Days to maturity	No. of rows ear^{-1}	No. of kernel row^{-1}	100 kernel weight	Ear weight plant^{-1}	Grain yield plant^{-1}
I(P_2xP_3)	h^2. Broad	89.24	82.12	65.92	78.46	92.50	59.60
	h^2. Narrow	55.49	60.85	41.77	40.79	18.29	21.45
	Δ g	3.57	2.64	2.08	2.75	4.95	2.36
	Δg%	3.20	20.89	7.10	9.18	3.68	2.38
	G.C.V%	2.65	15.10	6.70	9.68	9.39	4.15
II(P_5xP_6)	h^2. Broad	83.87	80.65	84.31	85.44	89.15	85.39
	h^2. Narrow	35.65	28.83	34.11	26.94	39.18	49.67
	Δ g	2.20	1.03	2.36	2.36	6.47	8.44
	Δg%	2.01	7.74	8.31	7.79	5.42	8.80
	G.C.V%	2.50	11.70	10.86	12.97	6.34	7.94
III(P_1xP_4)	h^2. Broad	93.76	85.06	89.75	83.13	98.75	98.03
	h^2. Narrow	81.09	43.66	60.95	42.94	52.43	17.40
	Δ g	9.63	1.69	6.33	2.53	19.33	5.30
	Δg%	8.94	12.05	24.33	6.91	13.08	3.57
	G.C.V%	5.18	12.35	18.36	7.13	12.04	9.85

For ear and grain yield plant^{-1}, high genetic gain was associated with low heritability values. In spite of the relative low heritability in the narrow sense computed in both traits, estimates of additive and 'additive x additive' genetic effects were highly significant. Therefore, it could be suggested that selection for these traits in subsequent generations is relatively more effective than in the early F_2 generation. It could be concluded that the highest genetic advance detected for both traits, in spite of low heritability estimates, may be due to a relatively big range of variability in these populations.

For number of kernels row^{-1} and the 100-kernel weight, moderate genetic advance was associated with low to moderate heritability values. In spite of the relative low or moderate heritability in the narrow sense computed in both traits, estimates of additive genetic effects were highly significant. Therefore, it could be suggested that selection for both traits in subsequent generations will be relatively more effective than the early F_2 generation. Relatively low genetic gain was associated with low heritability values in No. of rows ear^{-1} and plant height in the second cross. Hence, selection for these cases may be less effective.

Expected improvement of selection is directly proportional to heritability, and expected response to selection varies with the phenotypic standard deviation of population means. This is a measure of the total variability in the trait and therefore, it reflects the total response that could be realized by breeding techniques. It is possible to visualize a situation where the heritability is high, but because of little potential for improvement (low δ^2 ph) little response can be expected. On this basis, such a situation could be explained.

Generally, the variance in F_2, mean performance in the F_1 and GCV% increased with increasing GD as it was shown in cross P_1xP_4 followed by cross P_5xP_6 and then by cross P_2xP_3. This result was corresponding with correlation coefficient (r) between GD and each of grain yield plant^{-1} variance of F_2 (r= 0.98**) and mean performance of F_1 (r= 0.97**) and GCV% (r= 0.79**). Hence, ISSR

markers are a powerful, reliable, fast and inexpensive method for screening the genetic diversity between maize inbred lines.

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الملخص العربي

التباعد الوراثى لسلالات الذرة الشامية بأستخدام الواسمات الجزيئية ISSR و تداعياته على توريث الصفات الكمية

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قدر التباعد الوراثى لستة سلالات مرباه داخليا من الذرة الشامية بأستعمال الوسامات الجزيئية ISSR حيث كان عدد المعلمات الناتجة من استخدام عشر بادئات هو ٩٦ شظية حققت ٣٥ منهم اختلافات بنسبة ٣٦,٤٦%. و بتحليل تلك الشظايا وجد ان اعلى تشابه بين السلالات الابوية (٩٤,٣ %) كان بين السلالة المرباه داخليا الثانية و الثالثة .بينما تحقق اعلى اختلاف بين السلالة الاولى و الرابعة .ولدراسة توارث صفات التبكير فى النضج و المحصول و مكوناته تمت الدراسة على ثلاث هجن اختيرت على حسب مدى التشابه و الاختلاف بين ابويهم فتم اختيار اكثر الهجن تشابه للابوين وهو P2xP3 و الهجين الناشئ بين اكثر السلالات تباعدا و هو P1xP4 و اخر متوسط التباعد و هو الهجين P5xP6 و اقيمت تجربة حقلية لدراسة التحليل الوراثى للعشائر الستة للهجن الثلاثة تحت الدراسة فى مزرعة المحاصيل - كلية الزراعة – جامعة بنها .اجريت التجربة الحقلية فى عامين (٢٠١٣ و ٢٠١٤). و يمكن تلخيص اهم النتائج فيما يلى :كان التباين الوراثى و قوة الهجين و التدهور الراجع للتربية الداخلية معنويا لكل الصفات تحت الدراسة فى الثلاث هجن. و كانت قوة الهجين سالبة و معنوية لصفة عدد الايام حتى النضج بينما كانت قوة الهجين موجبة و معنوية فى صفات المحصول و مكوناته. و أظهر تحليل السياده وجود سيادة فائقة فى اتجاه الاب المبكر لصفة التبكير و لجهة الاب الافضل لصفات المحصول و مكوناته. كان الفعل المضيف و السيادى للجينات معنويا لمعظم الصفات تحت الدراسة فى الهجن المدروسة. كان التأثير التفوقى مساهم رئيسى فى اظهار الصفات المدروسة. كانت قيمة كفاءة التوريث بمعناها العريض عالية لكل الصفات فى الثلاث هجن عدا صفة محصول الحبوب/ نبات فى الهجين الاول بينما كانت قيمة كفاءة التوريث منخفضة لجميع الصفات المدروسة. حقق تباين الجيل الثانى و متوسط اداء الهجن فى الجيل الاول و قيمة التحسين الوراثى الناشىء عن انتخاب ٥% من نباتات الجيل الثانى زيادة كلما ازداد التباعد الوراثى بين الاباء الداخلة فى تكوين الهجين حيث كان اعلى تباعد وراثى بين الاب الاول و الرابع و اقل درجة تباعد كانت بين الاب الثانى و الثالث بينما كان التباعد متوسط بين الاب الخامس و السادس. كانت قيمة الارتباط معنوية و موجبة بين التباعد الوراثى المقدر بأستخدام الوسامات الجزيئية ISSR و كل من تباين الجيل الثانى و متوسط الجيل الاول و النسبة المئوية للتحسين الوراثى و كانت قيمة الارتباط r = ٠,٩٨ ،٠,٩٧ ،و ٠,٧٩ على الترتيب لصفة محصول الحبوب/ نبات. و لهذا السبب يمكن القول بأن الواسمات الجزيئية ISSR يمكن استخدامها كطريقة قوية و موثوقة و سريعة و غير مكلفة لفحص التباعد الوراثى بين سلالات الذرة الشامية.