

**RELATIONSHIP BETWEEN HYBRID PERFORMANCE AND GENETIC DIVERSITY BASED ON ISSR-PCR MARKERS IN PEPPER**

*(Capsicum annuum. L.)*

BY

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**ABSTRACT**

*Capsicum annuum* is widely cultivated around the world. The enormous genetic diversity available for pepper breeding has facilitated the development of new varieties and hybrids. Several pepper breeders coincide in that the level of heterosis exhibited by pepper hybrids is directly related with the genetic distance between their parental lines. Therefore, it is important to develop reliable techniques for the estimation of genetic distance. The search for superior hybrid parents in pepper breeding programs is commonly based on the estimation of the General Combining Ability (GCA) and Specific Combining Ability (SCA) of inbred lines. However, the application of this procedure is expensive and time consuming. The development of DNA based molecular markers represents an alternative procedure for the identification of promising parental lines for high performance of hybrid production. The Inter Sequence Repeat Polymerase Chain Reaction (ISSR-PCR) markers have been widely used for the estimation of genetic distance among closely related individuals. Thus molecular markers, such as ISSR-PCR, could be used for germplasm classification and clustering. This could be a valuable information for heterosis prediction. The aim of this research was to study the relationship between the genetic distances, measured using ISSR markers among parental lines, and the heterosis observed as yield, of the F<sub>1</sub> hybrids as estimations of GCA, SCA. Heterosis were performed using seven elite lines and their F<sub>1</sub> hybrids. The 28 genotypes (7 lines and 21 hybrids) were distributed in the field following a complete block design with three replicates. The genotypes tested were statistically different for fruit yield/plant. Among all the hybrids and parental lines, the F<sub>1</sub> (P<sub>4</sub> × P<sub>6</sub>) produced the highest yield. Also, GCA and SCA were statistically significant, with P<sub>6</sub> showing the highest GCA effect, and the F<sub>1</sub> (P<sub>6</sub> × P<sub>7</sub>) the highest SCA. The F<sub>1</sub> (P<sub>4</sub> × P<sub>6</sub>) showed the highest heterosis (108.95%). Genetic distances calculated by ISSR markers produced a dendrogram with seven nodes for the parental lines. However, the correlation between the matrix of genetic distances among parental lines and the matrix of heterosis gave positive significant correlation ( $r = 0.574$ ).

**Key Words:** *Capsicum annuum* — ISSR\_PCR — cluster analysis — genetic diversity — heterotic group.

**INTRODUCTION**

Pepper (*Capsicum annuum*) is widely cultivated around the world as well as in Egypt. The main producers are Hungary, India, Mexico, China, and Korea. The

more probable ancestor of *Capsicum annum* is the "Piquin" pepper (*C. annum* var. *aviculare*). Piquin is extensively distributed from South America up to the south of The United States. However, probably the main area of domestication includes Central America and México (De Witt and Bosland, 1996).

The enormous genetic diversity available in Egypt has facilitated the development of new varieties and hybrids. The methods used for pepper breeding in Egypt include pedigree, single seed descend, and a combination of both (Hassan, 1993).

Plant breeders working with several species have reported a direct relationship between the level of heterosis exhibited by the F<sub>1</sub>, and the divergence between their parents (Lee *et al.*, 1989; Sekhon and Gupta, 1995). Therefore, estimation of genetic distance can be useful for prediction of high performance crossings (Smith *et al.*, 1990).

Furthermore, diallelic designs can be used to estimate the genetic components of phenotypic variation. However, the application of these techniques is expensive and time consuming. The use of molecular markers has been proposed as an alternative procedure (Toby *et al.*, 1999). Molecular markers based on polymorphisms of the DNA are specially useful for this enterprise, because they are not affected by the environment (Tatineni *et al.*, 1996).

The Random Amplified Polymorphic DNA (RAPD-PCR) and Inter Sequence Repeat Polymerase Chain Reaction (ISSR-PCR) technique has been widely used to quantify the genetic variation due to its simplicity and power to detect differences, even among closely related individuals, in species of *Brassica* (Jain *et al.*, 1994), *Pisum* (Hoey *et al.*, 1996), *Wheat* (Liu *et al.*, 1999) and *Pepper* (Geleta *et al.* 2004 and Kumar *et al.*, 2001). Additionally, this type of markers is extremely useful to estimate genetic distances, mainly due to their productivity in terms of number of markers per essay, sensibility, quickness, and possibility of automation (Laucou *et al.*, 1998).

The main aim of this research was to determine the relationship between genetic distance, based on ISSR-PCR markers, and the heterosis for fruit yield in pepper. In addition, *GCA* and *SCA* were quantified to study several parental lines and the heterosis shown by their F<sub>1</sub> hybrids.

## MATERIALS AND METHODS

This research was conducted at the Agricultural Experimental Station of the Genetics Department, Faculty of Agriculture at Moshtohor, Benha University, during summer seasons of 2004 to 2006.

### Plant Material:

Seven pepper parental germplasm (*Capsicum annum*. L.) i.e. P<sub>1</sub>: (PI:15925601\_U.S.A.), P<sub>2</sub>: (PI: 5927201\_U.S.A.), P<sub>3</sub>: (PI:16736101\_Turkey), P<sub>4</sub>: (PI: 13582401\_Afghanistan), and P<sub>5</sub>: (PI: 13587301\_Pakistan) were kindly provided by University of Georgia, Plant Genetic Resources Conservation Unit, U.S.A., while

seeds of the lines P<sub>6</sub>: (YellowWax\_U.S.A.) and P<sub>7</sub>: (Aswany\_Local) were obtained from the germplasm preservation laboratory of the Horticulture Department, Faculty of Agriculture, Moshtohor, Benha University.

**Field Experiments:**

Crosses were made among the parental germplasm and a half diallel set (21 hybrids) was obtained. The 28 genotypes (Seven parental lines and 21 hybrids) were distributed in the field, following a complete block design with three replicates. Agricultural practices were applied as recommended for pepper production. Plant height (PH), fruit length(FL), fruit diameter(FD), fruit weight (FW),fruit number per plant (FN) and fruit yield per plant (FY) were used for the morphological characterization of the all genotypes.

**DNA extraction:**

Young leaves were collected in 1.5 ml eppendorf tube, quickly frozen in liquid nitrogen and ground with konte pestles into fine powder. DNA was extracted according to Doyle and Doyle, (1990) mini preparation protocol. The purity of extracted DNA was tested on 1% agarose gel using 0.5x TE (Tris EDTA) buffer and stained with 10 mg/ml ethidium bromide. The gel was exposed to UV-light and photographed. Optimizations of the working dilutions were made using various dilution ratios. Finally, the dilution produced amplification with the ISSR primer and three samples for screening was in ratio of 1:1000 after determining the concentration with a TD-700Fluorometer.

**ISSR-PCR:**

ISSR-PCR was carried out according to (Williams *et al.*, 1990). The primers used were 11 to18 mer oligonucleotide; nine primers were selected as potentially useful. The codes and sequences of the used primers are shown in Table (1).

**Table (1): Name and sequences of the used primers with ISSR molecular markers.**

ISSR Primer	Nucleotide sequence 5' to 3'
814 <sub>A</sub>	(CT) <sup>8</sup> TG
844 <sub>A</sub>	(CT) <sup>8</sup> AC
844 <sub>B</sub>	(CT) <sup>8</sup> GC
17898 <sub>A</sub>	(CA) <sup>8</sup> AC
17899 <sub>A</sub>	(CA) <sup>8</sup> AG
HB <sub>9</sub>	(GT) <sup>8</sup> GG
HB <sub>11</sub>	(GT) <sup>8</sup> CC
HB <sub>12</sub>	(CAC) <sup>8</sup> GC
HB <sub>13</sub>	(GAG) <sup>8</sup> GC

PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200µM), MgCl<sub>2</sub> (1.5mM), 1x buffer, primer(0.2µM), DNA (50ng),Taq DNA polymerase (2units).Amplification was carried out in a thermo Cycler programmed for 94°C for 3min(one cycle); followed by 94°C for 30sec,40°C for 45 sec and 72°C for 1 min (35 cycle), 72°C for 10 min (one cycle) then 4°C (infinite).

Amplification products (25µl) were mixed with 3µl loading buffer and separated on 1.3% agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 1kb plus DNA ladder marker.

#### Data analysis:

The statistical design applied was the Method-2 / Model-1 of Griffing (1956). Heterosis was estimated as the deviation of the  $F_1$  from the average of its two parents (Bhatt, 1971).

The obtained data of ISSR analysis was entered in a computer file as binary matrices where "0" stands for the absence of a band and in each individual sample. Similarity coefficients were calculated according to dice matrix (Nei and Li, 1979; Rohlf, 1993). Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

## RESULTS AND DISCUSSION

### 1. Performance of parental lines and hybrids:

Highly significant differences among genotypes were detected for plant height, fruit length, fruit diameter, fruit weight, fruit number and fruit yield per plant (Table-2), indicating abundant genetic variability for fruit yield per plant. The highest yielding parent was  $P_1$ , even it was statistically similar to  $P_6$ ; Parental line  $P_7$  presented the lowest fruit yield per plant. Among all the hybrids and parental lines, the  $F_1$  ( $P_4 \times P_6$ ) produced the highest yield (Table -3).

As can be seen in Table-2, the mean squares of *GCA* and *SCA* were highly significant; in accordance with Sprague and Tatum (1942). These results suggest the presence of additive and dominance effects in the genetic control of fruit yield.

Table (2): Analysis of variance (mean squares) for six characters and estimated combining ability for fruit yield / plant character in pepper.

S.O.V	d.f	PY	FN	FW	FD	FL	pH
Replication	2	140.75	201.04	71.49	0.001	0.061	2.118
Genotypes	27	269269.67	67488.31**	86832.42**	1.713**	16.477**	792.041**
GCA	6	186.38**					
SCA	21	25460.90**					
Error	54	88929.50**					

The lines  $P_4$  and  $P_6$  showed the highest general combining ability; the highest specific combining ability corresponded to  $P_6 \times P_7$ ; however,  $P_4 \times P_6$ ,  $P_1 \times P_5$ ,  $P_2 \times P_3$ , and  $P_2 \times P_3$ , produced hybrids of similar yield to  $P_6 \times P_7$ .

As can be seen in Table-3, the  $F_1$  ( $P_4 \times P_6$ ) showed the highest heterosis with 108.29.5%, and a relatively high *SCA* effects. The heterosis values found in this study are well over others reported in pepper (Milerue and Nikornpun, 2000; Owen, 1992).

As mentioned before,  $P_4$  and  $P_6$  exhibited the highest *GCA* effect for fruit yield. However, these lines just participated in three out of six of the best hybrids. On the other hand,  $P_3$  with an intermediate *GCA* effect, produced two out of six of the

best hybrids in terms of heterosis and in one out of five of the highest values of *SCA*. These results suggest that the *SCA* effects were important in controlling these traits.

In the present investigation, the mean values of the hybrids were significantly larger for FY, FN, FW, FD, FL and PH, when compared with the mean of parental lines (Mid-parent) indicating that heterosis was present for these traits.

Comparatively, FY showed a greater level of heterosis in most of  $F_1$  hybrids. However, the parental performance (additive gene action) contributed less to  $F_1$  performance. The hybrid mean was significantly lower than the mean of the parental lines for FW, revealing the lack of dominance and positive heterosis for this character.

Some characters, such as FY, FD and PH, demonstrated average in the desired direction indicating the presence of true heterosis. The reason for the  $F_1$  hybrids showing PH for these characters might be explained by desirable genetic complementation between the inbred genotypes (Dubreuil *et al.* 1996).

In this study, although it was not consistent for all hybrids, generally hybrids obtained from very closely or distantly related parents showed low heterosis but crosses produced from parents of intermediate divergent classes tended to show higher heterosis for FY, FL and FW; this suggests that intermediate divergence of parental lines could give higher heterosis for these traits. Liu *et al.* (1999) reported similar results in wheat.

## **2. Genetic relationship using Inter Sequence Repeat Polymerase Chain Reaction analysis (ISSR-PCR):**

### **a. ISSR-PCR analysis.**

In the present study, the genetic variability among different genotypes of pepper based on ISSR-PCR analysis has been studied.

Screening nine random primers with seven genotypes of pepper resulted in nine primers that produce different polymorphic bands and amplified DNA fragments as shown in Tables (4) and Fig.(1).

The nine primers used in the study generated a total of 104 amplification products, among which 100 were found to be polymorphic; this resulted in 96.15% polymorphism. All the primers produced polymorphic amplification products, however, the extent of percent polymorphism varied with each primer (81.82 to 100%).

The PCR products of primer  $844_A$  ranged from two bands in  $P_6$  and  $P_7$  to eight bands in  $P_1$  (Tables 4 and Fig.1A); this primer produced two monomorphic bands in all genotypes; the other bands were polymorphic as they were present in some genotypes and absent in the other. Some genotypes had some specific bands and could be used to distinguish among them; for instance  $P_4$  has one positive specific marker at (*M.W.*) of 473.33bp.

The PCR products of primer  $844_B$  and analysis of these products are illustrated in Fig.1B and Tables(4); this primer produced 3-8 bands for the studied genotypes; and one positive specific marker was found in  $P_1$  with *M.W.* of 400.0bp.

Table (3): Performance of pepper parents and elite crosses based on character means and estimates of heterosis%, gca and sca effects for fruit yield / plant.

Genotypes	Plant Height (cm)		Fruit Length (cm)		Fruit Diameter (cm)		Fruit Weight (gm.)		Fruit Number / plant		Fruit Yield / plant (gm.)			
	Mean	Heterosis%	Mean	Heterosis%	Mean	Heterosis%	Mean	Heterosis%	Mean	Heterosis%	Mean	Heterosis%	GCA effect	SCA effect
P <sub>1</sub>	91.50		5.45		1.75		378.33		300.0		1372.97		-72.79	
P <sub>2</sub>	61.33		5.95		3.33		24.33		19.0		769.55		83.25	
P <sub>3</sub>	104.67		11.50		2.09		79.67		65.0		759.55		-2.45	
P <sub>4</sub>	103.00		2.85		2.19		225.33		200.0		758.55		207.48	
P <sub>5</sub>	99.67		2.95		2.19		545.67		440.0		802.33		-68.76	
P <sub>6</sub>	65.67		9.55		2.35		69.67		50.0		1003.90		190.64	
P <sub>7</sub>	102.67		2.75		0.95		590.33		460.0		510.33		-337.38	
P <sub>1</sub> XP <sub>1</sub>	116.00	51.79	7.55	32.46	2.35	-7.48	120.55	-40.12	100.0	-37.30	1214.33	13.36		36.07
P <sub>1</sub> XP <sub>2</sub>	107.00	9.01	7.44	-12.26	1.95	1.56	217.45	-5.04	195.0	6.85	1095.67	2.76		2.77
P <sub>1</sub> XP <sub>3</sub>	114.00	17.22	5.99	44.34	1.55	-0.21	235.52	-21.97	210.0	-16.0	1178.00	10.53		-124.61
P <sub>1</sub> XP <sub>4</sub>	106.00	10.89	7.75	84.52	1.70	-13.71	223.65	-51.59	193.0	-43.24	1210.00	11.49		182.74
P <sub>1</sub> xP <sub>1</sub>	99.33	26.39	7.97	6.27	1.75	-14.63	225.55	0.69	195.0	11.43	1165.44	-1.94		-120.89
P <sub>1</sub> xP <sub>2</sub>	111.53	14.86	4.43	8.05	1.33	-1.48	319.00	-34.14	295.0	-22.37	785.55	-16.58		23.92
P <sub>1</sub> XP <sub>5</sub>	81.67	-1.60	9.55	10.61	3.45	27.31	87.33	67.94	70.0	66.67	1355.00	77.23		105.06
P <sub>1</sub> XP <sub>6</sub>	99.67	21.30	4.75	7.95	3.95	43.12	149.00	19.36	135.0	23.29	1468.55	92.19		9.68
P <sub>1</sub> XP <sub>7</sub>	124.00	54.04	6.57	47.64	2.15	-22.10	150.55	-99.75	139.0	-39.43	1344.55	71.08		162.92
P <sub>2</sub> XP <sub>1</sub>	72.00	13.39	6.80	-12.26	3.59	25.70	66.00	46.81	55.0	59.42	1185.33	33.34		-256.96
P <sub>2</sub> XP <sub>2</sub>	107.00	30.49	5.55	27.59	1.65	-22.89	255.00	-17.03	200.0	-16.49	859.55	34.32		-56.78
P <sub>2</sub> xP <sub>1</sub>	119.00	14.60	7.44	3.62	2.22	-26.49	221.65	46.79	201.00	51.70	1369.65	80.44		-3.19
P <sub>2</sub> xP <sub>2</sub>	120.00	17.45	4.57	-36.79	2.19	-27.48	333.00	7.02	295.00	16.83	1115.85	42.89		18.58
P <sub>2</sub> xP <sub>3</sub>	86.00	0.98	8.87	-15.76	1.80	-18.92	109.40	46.51	95.0	65.22	1292.00	46.53		-63.67
P <sub>2</sub> xP <sub>4</sub>	105.55	1.81	6.35	-10.94	0.98	-35.53	350.55	4.64	300.0	14.29	768.75	21.07		-59.56
P <sub>2</sub> xP <sub>5</sub>	96.00	-5.27	3.47	19.66	2.58	17.81	295.00	-23.48	250.0	-21.88	1239.75	58.85		-67.78
P <sub>2</sub> xP <sub>6</sub>	81.00	-0.04	7.53	21.45	2.33	2.64	235.00	59.32	210.0	68.0	1835.55	108.29		271.28
P <sub>2</sub> xP <sub>7</sub>	101.55	1.25	4.63	65.36	1.25	-20.38	585.55	43.58	460.0	39.39	945.85	-2.38		-85.39
P <sub>3</sub> xP <sub>1</sub>	80.67	-2.42	3.97	-36.48	2.25	-0.88	230.00	-25.24	200.0	-18.37	1133.33	25.49		-152.03
P <sub>3</sub> xP <sub>2</sub>	105.67	4.45	2.27	-20.35	1.47	-6.37	335.55	-40.96	310.0	-31.11	618.55	-5.76		-144.45
P <sub>3</sub> xP <sub>3</sub>	115.33	35.83	6.50	5.69	1.47	-10.91	675.75	104.75	500.0	96.10	1343.70	77.48		322.25
LSD <sub>H</sub>	1.544	0.423	0.518	0.142	0.116	0.100	2.965	2.568	3.465	3.010	3.377	2.927		
LSD <sub>H</sub>	2.057	0.563	0.689	0.189	0.154	0.134	3.949	3.420	4.614	4.050	4.497	3.898		

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The result of ISSR analysis using primer 814<sub>A</sub> were illustrated in Fig.1C and Table (4); the total number of bands varied with a lowest number of one band in P<sub>2</sub>,P<sub>5</sub> and P<sub>7</sub> to a highest number of seven bands in P<sub>3</sub>; there was one monomorphic band in all genotypes and four positive specific markers were found in P<sub>1</sub> and P<sub>3</sub>, with *M.W.* of 672.55,490.28,453.97 and 400bp, respectively.

The PCR products of primer 17898<sub>A</sub> ranged from one band in P<sub>4</sub> to six bands in P<sub>1</sub> (Tables 4 and Fig.1D); this primer produced one monomorphic band in all genotypes; some genotypes had some specific bands and could be used to distinguish among them; for instance P<sub>6</sub> has one positive specific marker at *M.W.* of 658.236bp.

The PCR products of primer 17899<sub>A</sub> and analysis of these products are illustrated in Fig.1E and Table(4); this primer produced 3-8 bands for the studied genotypes; this primer don't produce any monomorphic bands in all genotypes. Three positive specific markers were found in P<sub>2</sub> and P<sub>7</sub> with *M.W.* of 475.07, 429.60 and 311.37bp), respectively.

**Table (4): Number of amplified fragments and specific markers of seven pepper genotypes based on ISSR-PCR analysis with nine primers.**

Genotypes		ISSR Primers								
		844A	844B	814A	17898A	17899A	HB9	HB11	HB12	HB13
	TAF	11.0	9.0	8.0	9.0	14.0	9.0	12.0	21.0	11.0
	MB	2.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
	PB	9.0	9.0	7.0	8.0	14.0	9.0	12.0	21.0	11.0
	PB%	81.82	100.0	87.50	88.89	100.0	100.0	100.0	100.0	100.1
P <sub>1</sub>	AF	8.0	7.0	4.0	6.0	8.0	4.0	5.0	8.0	7.0
	SM	0.0	1.0	2.0	0.0	0.0	0.0	1.0	5.0	0.0
P <sub>2</sub>	AF	6.0	3.0	1.0	3.0	8.0	5.0	3.0	4.0	8.0
	SM	0.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0	1.0
P <sub>3</sub>	AF	5.0	4.0	5.0	2.0	8.0	3.0	5.0	7.0	3.0
	SM	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	1.0
P <sub>4</sub>	AF	2.0	7.0	0.0	1.0	0.0	4.0	3.0	6.0	3.0
	SM	1.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
P <sub>5</sub>	AF	4.0	6.0	1.0	3.0	3.0	5.0	6.0	11.0	3.0
	SM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
P <sub>6</sub>	AF	2.0	6.0	0.0	5.0	4.0	5.0	3.0	8.0	2.0
	SM	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.0
P <sub>7</sub>	AF	2.0	7.0	1.0	3.0	7.0	5.0	3.0	11.0	2.0
	SM	0.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0
Total	AF	29.0	40.0	12.0	23.0	38.0	31.0	28.0	55.0	28.0
	SM	1.0	1.0	4.0	1.0	3.0	3.0	3.0	6.0	3.0

TAF=total amplified fragment., MP= monomorphic bands., PB=polymorphic bands.  
AF=amplified fragment, SM=specific marker.

The result of ISSR analysis using primer HB<sub>9</sub> were illustrated in Fig.1F and Tables(4). The total number of bands varied with a lowest number of three bands in P<sub>3</sub> and a highest number of five bands in three genotypes; there were three positive specific markers were found in P<sub>2</sub>,P<sub>4</sub> and P<sub>7</sub> with *M.W.*459.09, 481.82 and 693.25bp.

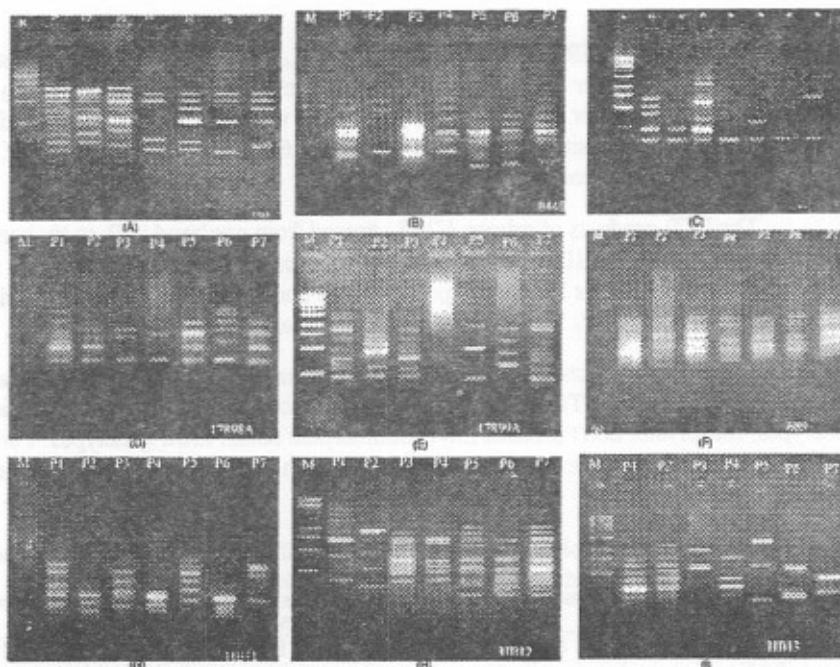


Fig. (1): DNA polymorphism of the seven pepper genotypes using ISSR\_PCR with nine primers.

The result of ISSR analysis using primer *HB<sub>11</sub>* were illustrated in Fig. 1G and Tables(4); the total number of bands varied with a lowest number of three bands in *P<sub>2</sub>, P<sub>4</sub>, P<sub>6</sub>* and *P<sub>7</sub>*, a highest number of six bands in *P<sub>5</sub>*. Three positive specific markers were found in *P<sub>1</sub>, P<sub>4</sub>* and *P<sub>7</sub>* with *M.W.* 400.0, 368.56 and 553.66bp, respectively.

The PCR products of primer *HB<sub>12</sub>* ranged from four bands in *P<sub>2</sub>* and found 11 bands in *P<sub>5</sub>* and *P<sub>7</sub>* (Tables 4 and Fig. 1H); this primer don't produce any monomorphic bands in all genotypes; some genotypes had some specific bands and could be used to distinguish among them; for instance *P<sub>1</sub>* and *P<sub>6</sub>* had six positive specific markers at *M.W.* of (918.79, 814.45, 771.44, 700.0, 400.0 and 270.59bp), respectively.

The result of ISSR analysis using primer *HB<sub>13</sub>* were illustrated in Fig. 1I and Tables(4); the total number of bands varied with a lowest number of two bands in two genotypes *P<sub>6</sub>* and *P<sub>7</sub>* to a highest number of eight bands in *P<sub>2</sub>*; this primer does not produce any monomorphic band in all genotypes. However, three positive specific markers were found in *P<sub>2</sub>, P<sub>3</sub>* and *P<sub>5</sub>* with *M.W.* 824.96, 791.92 and 612.8273bp, respectively.

ISSR-PCR analysis was performed using primers to obtain markers to assist selection for some of the aforementioned traits in pepper. Primers 844<sub>A</sub>, 844<sub>B</sub>, 814<sub>A</sub>, 17898<sub>A</sub>, 17899<sub>A</sub>, HB<sub>9</sub>, HB<sub>11</sub>, HB<sub>12</sub> and HB<sub>13</sub> produced ISSR fragments with different lengths could be used as markers to assist selection for some of the yield related traits in pepper as shown in Tables (5).



Table (5): Trait specific ISSR markers in the seven pepper genotypes.

Molecular Marker	Band Number	MW <sub>(bp)</sub>	Trait Morphological	Parents
844A	3	473.33	PH	P <sub>4</sub>
844B	1	400.0	FW, FN, FY	P <sub>1</sub>
814A	1	672.55	PH, FL, FD	P <sub>1</sub> , P <sub>3</sub>
	2	453.97		
17898A	2	658.23	FL, FD, FY	P <sub>6</sub>
17899A	2	475.07	FD	P <sub>2</sub>
	3	429.60		
	6	311.37	PH, FW, FN	P <sub>7</sub>
HB9	3	459.09	FD	P <sub>2</sub>
	4	481.82	PH	P <sub>4</sub>
	1	693.25	PH, FW, FN	P <sub>7</sub>
HB11	3	400.0	FW, FN, FY	P <sub>1</sub>
	1	368.56	PH	P <sub>4</sub>
	2	553.66	PH, FW, FN	P <sub>7</sub>
HB12	1	918.79	FW, FN, FY	P <sub>1</sub>
	2	814.45		
	3	771.44		
	4	700.0		
	8	400.0		
HB13	7	270.59	PH, FW, FN	P <sub>6</sub>
	1	824.96	FD	P <sub>2</sub>
	1	791.92	PH, FL, FD	P <sub>3</sub>
	1	612.82	PH	P <sub>5</sub>

Some of these ISSR products could be used in markers assisted selection programs. Primer 844<sub>A</sub> produced an amplified fragment with length of 473.33 bp related to plant height and could be used as marker to assist selection for this trait. On other hand, primers 844<sub>B</sub> and 17898<sub>A</sub> produced fragment with length of 400.0 and 658.23 bp, respectively, which could be used as negative marker in selection for FW, FN, FL, FD and FY traits, in accordance with (Geleta *et al.* 2004 and Kumar *et al.*, 2001).

**b. Nei's similarity coefficient.**

Similarity indices and consensus tree were developed on the basis of the scorable banding patterns of the seven pepper genotypes using the nine ISSR primers as shown in Table (6); the two most closely related genotypes P<sub>1</sub> and P<sub>2</sub> with highest genetic distances ranged from (0.475). On the other hand, the two most closely related genotypes P<sub>4</sub> and P<sub>7</sub> with low genetic distances ranged from (0.176); the average of similarity among genotypes was (0.336).

**c. Cluster analysis.**

Nei's genetic distance (Fig.2) showed that the genetic distances for each genotype combination ranged from (0.24) to (0.48) and the studied genotypes formed two main clusters. The first main cluster separated at genetic similarity of (0.32) and

created four sub clusters, the first subcluster included an individual genotype P<sub>3</sub> at genetic similarity of (0.40), the second subcluster included P<sub>1</sub> and P<sub>2</sub> at genetic similarity of (0.48), the third subcluster included P<sub>5</sub> and P<sub>7</sub> at genetic similarity about (0.44) and the fourth subcluster included an individual cultivar P<sub>6</sub> at genetic similarity of (0.36). In reaction to second main cluster separated at genetic similarity of (0.24) and created one subcluster, included an individual cultivar P<sub>4</sub> at genetic similarity of (0.24).

The aforementioned results confirmed that ISSR profiling is a powerful method for identification and molecular classification which agreed with (Geleta *et al.* 2004 and Kumar *et al.*, 2001).

Table (6): Genetic distances among the seven pepper parents as estimated using ISSR data.

Genotype	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>	1.0						
P <sub>2</sub>	0.475	1.0					
P <sub>3</sub>	0.403	0.358	1.0				
P <sub>4</sub>	0.210	0.200	0.176	1.0			
P <sub>5</sub>	0.299	0.220	0.333	0.333	1.0		
P <sub>6</sub>	0.333	0.278	0.327	0.239	0.327	1.0	
P <sub>7</sub>	0.333	0.328	0.259	0.196	0.431	0.373	1.0

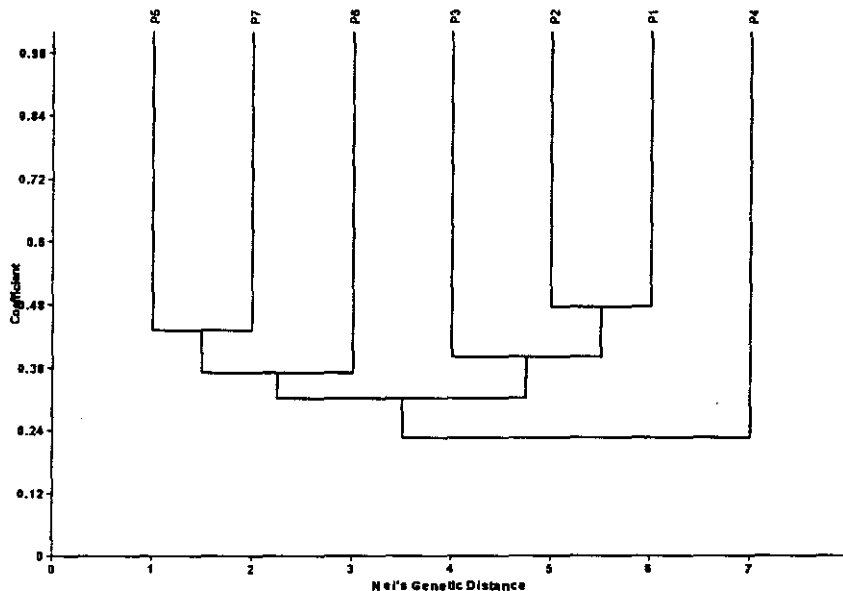


Fig. (2): Dendrogram obtained from UPGMA cluster based on ISSR data from the seven pepper genotypes.

**3.Genetic relationship between genetics distance and heterosis:**

The correlation coefficients between the Nei's genetic distances matrix and the heterosis matrix was medium positive significant correlation ( $r = 0.574$ ).

The correlation coefficients of ISSR distance with SCA effects of FY was low negative significant correlation( $r = -0.022$ ). It is assumed that the SCA effect expressed by a hybrid is related to the genetic distance between its parental lines (Lee *et al.* 1989).

The genetic distances among parental lines are related with the heterosis of their hybrids (Table-7). However, P<sub>4</sub> and P<sub>6</sub> were the more divergent parents and produced the highest fruit yield and the second highest heterosis values. This shows an isolated tendency, since it was observed that parental lines with smaller divergence had low values of heterosis (Sekhon and Gupta, 1995).

Similar results have been obtained in maize Dudley *et al.* (1991). Also, Barbosa *et al.* (1996) and Burkhamer *et al.* (1998) reported low correlations between genetic distances and heterosis in wheat. Charcosset *et al.* (1991) indicate that the detection of molecular markers which are not close to the genes responsible for the assayed trait may decrease the correlation between genetic distances and heterosis. Bernardo (1992), mention that it is essential to identify a specific marker related to the segments of the genome which determine the expression of the traits of interest to find a high correlation between genetic distances and heterosis.

**Table (7): Genetic distances and heterosis values of seven pepper genotypes and their F<sub>1</sub> hybrids.**

Genotypes	Genetic Distances						
	P1	P2	P3	P4	P5	P6	P7
P1	1.0	0.475	0.403	0.210	0.299	0.333	0.333
P2	0.134	1.0	0.358	0.200	0.220	0.278	0.328
P3	0.028	0.772	1.0	0.176	0.333	0.327	0.259
P4	0.105	0.922	0.804	1.0	0.333	0.239	0.196
P5	0.115	0.771	0.429	0.589	1.0	0.327	0.431
P6	-0.019	0.333	0.645	1.083	0.255	1.0	0.373
P7	-0.166	0.343	0.211	-0.024	-0.058	0.775	1.0
<b>Heterosis Values</b>							

It may be expected that genetic distances, calculated using molecular markers, will become a useful way to predict heterosis until genes controlling important traits are placed on highly saturated genetic linkage maps and the adequate markers, those strongly linked, can be chosen to calculate the genetic distance.

The results of this study showed that genetic distances, in general, correlated medium with heterosis and hybrid performance. Previous studies in various crop species such as maize Benchimol *et al.* (2000), rice (Kwon *et al.* 2002), wheat (Burkhamer *et al.* 1998), alfalfa (Riday *et al.* 2003) and chickpea

(Sant *et al.* 1999) also showed low or medium correlations of genetic distance with heterosis.

Although higher levels of heterosis for the majority of characters studied show the possibility of hybrid breeding in pepper, extreme divergence for characters such as fruit-related traits is not desirable.

However, if the objective of a breeding programme is, for example, to develop a hybrid of medium sized-fruit, smaller-and larger-fruited parents can be used in the crossing programmes.

In this study, both morphological and ISSR distances differentiated pepper lines into heterotic groups from which superior hybrids can be derived. However, the method expressed little or no promise for predicting F<sub>1</sub> performance for most characters studied.

The parental genotypes used in this study had diverse morphological backgrounds and were from different market types. As each type of pepper must conform to its own unique set of characteristics in order to be commercially acceptable, pepper hybrid breeding should deal with parental lines of similar varietal groups (market types) unless the breeding programme is to develop hybrids or pure lines of different forms.

#### REFERENCES

- Barbosa-Neto, J.F., Sorrells, M.E. and Cisar, G. (1996): Prediction of heterosis in wheat using coefficient of parentage and RFLP based estimates of genetic relationship. *Genome*. 39: 1142- 1149.
- Benchimol, L. L., de Souza, C. L., Garcia, A. A. F., Kono, P. M. S., Mangolin, C. A. and Barbosa, A. M. (2000): Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid performance determined by RFLP markers. *Plant Breeding*. 119: 491-497.
- Bernardo, R. (1992): Relationship between single cross performance and molecular marker heterozygosity. *Theor. Appl. Genet.* 83: 628-634.
- Bhatt, G. M. (1971): Heterotic performance and combining ability in a diallel cross among spring wheat's (*T. aestivum* L.). *Austr. J. of Agric. Res.*, 22:359-369.
- Burkhamer, R.L., Lanning, S.P., Martens, R.J., Martin, J.M., and Talbert, L.E. (1998). Predicting progeny variance from parental divergence in hard red spring wheat. *Crop Sci.* 38: 243-248.
- Charcosset, A., Lefort, B.M. and Gallais, A. (1991): Relationship between heterosis and heterozygosity at marker loci: a theoretical computation. *Theor. Appl. Genet.* 81: 571-575.
- De Witt, D. and Bosland, P.W. (1996): Peppers of the World. An Identification Guide. Ten Speed Press. Berkeley, California. pp 219.
- Doyle, J.J. and Doyle, L.H. (1990): Isolation of plant DNA from fresh tissue. *Focus*. 12: 13-15.

- Dubreuil, P., Dufour, P., Krejci, E., Cause, M., De Vienne, D., Gallais, A. and Charcosset, A. (1996): Organization of RFLP diversity among inbred lines of maize representing heterotic groups. *Crop Sci.* 36: 790—799.
- Dudley, J.W., Saghai-Maroo, M.A. and Rufener, G.K. (1991): Molecular markers and the grouping of parents in maize breeding programs. *Crop Sci.* 31: 718—723.
- Geleta, M.T., Labuschagne, M.T. and Viljoen, C.D. (2004): Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. *Plant Breeding.* 123:467-473.
- Griffing, B. (1956): Concept of general and specific ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9: 443-452.
- Hassan, A.A. (1993): Breeding of vegetable crops. In Arabic. El-Dar El- Arabia Li Nashr Wa Tawzee. Cairo, Egypt. pp.799.
- Hoey, B.K., Crowe, K.R., Jones, V.M. and Polans, N.O. (1996): A phylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. *Theor. Appl. Genet.* 92: 92-100.
- Jain, A., Bhatia, S., Banga, S.S., Prakash, S. and Lakshmikiran, M. (1994): Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationships to heterosis. *Theor. Appl. Genet.* 88: 116-122.
- Kumar, D.L., Kathirvel, M., Rao, G.V. and Nagaraju, J. (2001): DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. *Forensic Science International.* 116:63-68.
- Kwon, S. J., Ahn, S. N., Jeong, E. G., Hwang, H. G., Choi, H. C. and Moon, H. P. (2002): Relationship between genetic divergence and hybrid performance in japonica rice grown in a cold water-irrigated field. *Euphytica.* 128: 389-396.
- Laucou, V., Haurogn, K., Ellis, N. and Rameau, C. (1998): Genetic mapping in pea. 1. RAPD-based genetic linkage map of *Pisum sativum*. *Theor. Appl. Genet.* 97: 905—915.
- Lee, M., Godshalk, E.B., Lambey, K.R. and Woodman, W.L. (1989): Association of restriction length polymorphism among maize inbreds with agronomic performance of their crosses. *Crop Sci.* 29:1067-1071.
- Liu, Z. Q., Pei, Y. and Pu, Z. J. (1999): Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. *Plant Breeding.* 118: 119-296.
- Milerue, N., and Nikornpun, M. (2000): Studies on Heterosis of Chili (*Capsicum annum* L.). *Kasetsart J. Nat. Sci.* 34: 190-196.
- Nei, M. and Li, W. H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA,* 76: 5269-5273.
- Owens, K. (1992): Breeding pepper varieties for commercial processing. Proceedings of the conferences on chili pepper production in the tropics. Kuala Lumpur. 195-201pp.
- Riday, H., Brummer, E. C., Cambell, T. A. and Luth, D. (2003): Comparison of genetic and morphological distance with heterosis between *Medicago sativa* and subspecies *falcata*. *Euphytica.* 131: 37-45.

- Rohlf, F. J.(1993): NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.70. Exeter Software, Setauket, New York.
- Sant, V. J., Patankar, A. G., Strode, N. D., Mhase, L. B., Sainani, M. N., Deshmukh, R. B., Ranjekar, P. K. and Gupta, V. S. (1999): Potential of DNA markers in detecting divergence and in analyzing heterosis in Indian elite chickpea cultivars. *Theor. Appl. Genet.* 98: 1217-1225.
- Sekhon, M.S. and Gupta, V.P. (1995): Genetic distance and heterosis in Indian mustard: developmental isozymes as indicators of genetic relationships. *Theor. Appl. Genet.* 91: 1148-1152.
- Smith, O.S., Smith, J.S.C., Bowen, S.L., Tenborg, R.A. and Wall, S.R. (1990): Similarities among a group of elite maize inbreds as measured by pedigree, F<sub>1</sub> grain yield, grain yield, heterosis, and RFLPs. *Theor. Appl. Genet.* 80: 833-840.
- Sprague, G.F. and Tatum, L.A. (1942): General vs. specific combining ability in single crosses of corn. *J. Amer. Soc. Agron.* 34: 923-932.
- Tatineni, V., Cantrell, R.G. and Davis, D.D. (1996): Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Sci.* 36: 186-192.
- Toby, A.J., Abberton, T.M., Michaelson-Yeates, T.M. and Foster, J.W. (1999): Relationships between genetic distance measured by RAPD-PCR and heterosis in inbred lines of white clover (*Trifolium repens* L.). *Euphytica* 107: 159-165.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V.(1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531- 6535.

### العلاقة بين أداء الهجن والتنوع الوراثي على أساس المعلمات الوراثية لتتابعات البيئية المتكررة لتفاعل المتسلسل البوليمرات في الفلفل

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يزرع الفلفل على نطاق واسع حول العالم مع وجود تنوع وراثي هائل متوفر لمربي الفلفل مما سهل من تطوير إنتاج أصناف وهجن وراثية جديدة والعديد من مربى الفلفل تعتمد على دور قوة الهجين في إنتاج هجن الفلفل وذلك بتقدير المسافة الوراثية بين السلالات الأبوية المتفوقة مستندة في ذلك على حساب القدرة العامة على الإبتلاف والقدرة الخاصة على الإبتلاف بين السلالات المرباه داخليا من الفلفل فإن تطبيق هذه الطريقة يحتاج إلى تكاليف مادية باهظة وفترة زمنية طويلة وحدث تطوير في استخدام المعلمات الوراثية الجزيئية على مستوى دانا يعتبر طريقة بديلة للتفريق بين السلالات الأبوية المتفوقة والواعدة وراثيا في إنتاج الهجن. إستخدام المعلمات الوراثية بواسطة تكتيك التتابعات البيئية المتكرره لتفاعل المتسلسل البوليمرات كانت تستخدم بكثرة لتقدير المسافة الوراثية بين الأفراد وثبقي القرابة والصلة هكذا هناك معلمات وراثية كثيرة مثل تكتيك التتابعات البيئية المتكررة لتفاعل المتسلسل البوليمرات يمكن أن يستعمل توصيف وتصنيف الأصول الوراثية النباتية في مجموعات متقاربة وهذا يمكن أن يعطى معلومات ثمينة للتنبؤ بقوة

الهجين ولقد كان الهدف الرئيسى لهذا البحث هو دراسة العلاقة بين المسافة الوراثية المقاسة باستعمال تحليل المعلمات الوراثية الناتجة من التتابعات البيئية المتكررة لتفاعل المتسلسل البوليمرات فمابين السلالات الأبوية وقيم قوة الهجين نصف الدائرى لسبع سلالات أبوية مبشرة وقد تم دراسة ٢٨ تركيب وراثى مختلف عبارة عن ٧ سلالات أبوية و ٢١ هجين تم زراعته وتقييمها فى الحقل فى تصميم قطاعات كاملة العشوائية فى ٣ مكررات والتحليل الإحصائى للتراكيب الوراثية المختلفة أوجد اختلافات فى محصول ثمار الفلفل/ نبات بين كل الهجن والسلالات الأبوية كان هجين الجيل الأول (٦×٤) الأعلى إنتاجا فى محصول الثمار على الرغم من أن القدرة العامة والخاصة على الإبتلاف تكون معنوية مع الأب رقم ٦ حيث شوهد أعلى تأثير للقدرة العامة على الإبتلاف وشوهد أعلى تأثير للقدرة الخاصة للإبتلاف كان مع الهجين (٧×٦) ولقد حقق الهجين (٦×٤) أعلى تأثير لقوة هجين ١٠٨,٩٥%. المسافة الوراثية المحسوبة بواسطة تحليل المعلمات الوراثية الناتجة من التتابعات البيئية المتكررة لتفاعل المتسلسل البوليمرات أنتجت دندوجرام بسبع عقد تقع داخله السبع سلالات أبوية وعن حساب العلاقة بين معدل التشابه الوراثية فيما بين السلالات الأبوية وقيم قوة الهجين الناتجة كان هناك ارتباط وراثى معنوى موجب ٠,٥٧٤.