Genetic Diversity in Bread Wheat as Revealed by RAPD Markers and Its Relationship to Leaf Rust and Hybrid Performance

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD) technique was used as a tool to determine the combining ability and heterosis among six-parental diallel cross of common Egyptian wheat. Analysis of variance of yield detected highly significant differences among the progenies. General combining ability (GCA) and Specific combining ability (SCA) were successfully used to determine the differences among the crosses. Three Egyptian wheat cultivars were highly susceptible to leaf rust, while the other three were resistant (monogenic lines carrying different *Lr* genes). Crosses revealed resistant to leaf rust were derived from the cross between resistant x resistant (*Lr37, 38,* and *39*) and between resistant x susceptible (Sakha 8 x *Lr*37, and Sakha 93 x *Lr*37). Nei's genetic distance ranged from 0.73 to 0.87 among the four genotypes selected based on their reaction to leaf rust. The correlation of Nei's genetic distance with SCA for grain yield and heterosis ranged from 0.62 to 0.89. These results indicated that the level of SCA and heterosis depends on the level of genetic diversity between wheat genotypes examined. RAPD technique was effective in detecting a markers ($OPO\text{-}08_{353}$) that is linked to some effective genes for affecting leaf rust (*Lr37, 38,* and *39*) under Egyptian condition that could be used as a maker to screen the materials in segregating populations.

Key words: genetic diversity, RAPD, diallel, bread wheat, leaf rust

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop in the world (www.FAO.org) as well as in Egypt, Moreover, It has been considered as the first strategic food crop in the winter season. The increase in the Egyptian wheat production is not sufficient to meet the demand of the growing population. In wheat breeding programs, the choice of parents is the most important step in the development of varieties adapted to biotic and abiotic stresses. Significant level of heterosis have been reported in a number of self-pollinated crops, and were first observed in wheat by (Freeman 1919). The magnitude of the heterotic effect is determined by the performance of parents and hybrids. One of the important application of DNA markers is its ability to predict heterosis in hybrids. Evaluation of hybrids for heterosis or combining ability in the field is both expensive and time-consuming. For this reason, other parameters such as pedigree information, qualitative and quantitative traits and biochemical data Leonardi *et al.*, (1991) and Wang *et al.*, (1992) were adopted to study heterosis. DNA markers have also been extensively used to correlate genetic diversity and heterosis in several crops such as maize Parentoni *et al.*, (2001), rice Zhao *et al.*, (1999), oat (Moser and Lee 1994), barley Melchinger *et al.*, (1990) and chickpea Sant *et al.*, (1999). El-Maghraby *et al.*, (2005) summarized the results of some Egyptian germplasm related to water-stress by using Simple Sequence Repeats (SSR) markers. They also reported the occurrence of significant mid and high-parent heterosis for yield and its components. In diversity studies, SSRs and RAPDs were shown to provide significant information on polymorphisms Plaschke *et al.*, (1995); Prasad *et al.*, (1999). In wheat, the possibility of introducing hybrids has been greatly enhanced by the discovery of effective chemical hybridizing agents. In Egypt, wheat rust diseases are the main factor for eliminating and decreasing the longevity of Egyptian wheat cultivars. Out of the three rust diseases of wheat, the most common one is the leaf or brown rust caused by *Puccinia recondita* f. sp. tritici. Epidemics of this disease can lead to severe losses of grain yield and decreased nutritional quality. In Egypt, crop losses due to leaf rust infection could be up to 50% Abdel Hak *et al.*, (1980). More than 45 different resistance genes derived from wheat and related species have been described McIntosh *et al.* (1995) and were used in wheat breeding(Johansson and Lupton, 1987). There are two main breeding strategies to improve leaf rust resistance: pyramidization of the major resistance (*Lr*) genes conferring complete resistance and/or the accumulation of minor resistance genes conferring quantitative resistance. Molecular markers have been developed for marker-assisted selection in breeding (Feuillet and Keller 1998); EL-Maghraby *et al.*, (2006). Nevertheless the diallel technique developed by (Griffing 1956) is used to give detailed genetic analysis after one generation and can provide valuable knowledge of genetic variance.

The present study was undertaken to examine the relationship of genetic diversity among six-parental diallel crosses of Egyptian wheat measured using RAPD technique and using the data to calculate the amount of specific combining ability and heterosis.

MATERIALS AND METHODS

Plant material and experimental design

A diallel was made among six parental genotypes of common wheat (*T. aestivum* L) including three bread wheat cultivars (Gemmiza 7, Sakha 8, and Sakha 93) and three monogenic lines carrying different *Lr* genes (*Lr* 37, *Lr* 38, and *Lr* 39). These genotypes were selected for their wide range of leaf rust resistance and other characteristics (Yield, yield components, heading and maturity). The cross number and pedigree of the F_1 derived from the diallel is presented in Table (1). Ten plants per plot (2 meters long and plants within row were 20 cm apart allowing a total of 10 plants per row) of the parents and the 15 F_1's were grown using randomized block design with three replication at Nubaria Agricultural Research Station, Agriculture Research Center, Giza, Egypt, in the years 2006 and 2007 under the recommended conditions of irrigation and fertilizer. The soil type was calcareous sandy. The dry method of planting was used and weed control was performed as hand weeding.

Table 1: Cross number and pedigree of the 15 F_1 hybrids derived from the diallel of six bread wheat (*Triticum aestivum* L.) genotypes

No	Crosses (parent *i* x parent *j*)
1	Gemmiza 7x Sakha 8
2	Gemmiza 7x Sakha 93
3	Gemmiza 7x Lr 37
4	Gemmiza 7x Lr 38
5	Gemmiza 7x Lr 39
6	Sakha 8x Sakha 93
7	Sakha 8x Lr 37
8	Sakha 8x Lr 38
9	Sakha 8x Lr 39
10	Sakha 93x Lr 37
11	Sakha 93x Lr 38
12	Sakha 93x Lr 39
13	Lr 37x Lr 38
14	Lr 37x Lr 39
15	Lr 38x Lr 39

Genetic analysis

The general and specific combining ability (GCA and SCA, respectively) analysis was conducted following Griffing's Model 1 Method 2 (Griffing, 1956). All calculations were performed using the MSTAT-C package (1991). Mid-Parents (MPH) Heterosis was computed as percent deviation from mean values of the parents. Reaction to leaf rust at adult stage, infection type and disease severity data for leaf rust has been recorded according to the scale of Stubbs *et al.,* (1986). The six parental genotypes and their 15 F_1 crosses were subjected to artificial infection with a mixture of leaf rust spores pathotypes at late tillering and early booting stage provided by Wheat Pathology Research Section, Plant Pathology Research Institute, ARC, Giza, Egypt. The genetic materials were surrounded by mixture of wheat genotypes highly susceptible to leaf rust spreader rows.

Molecular analysis

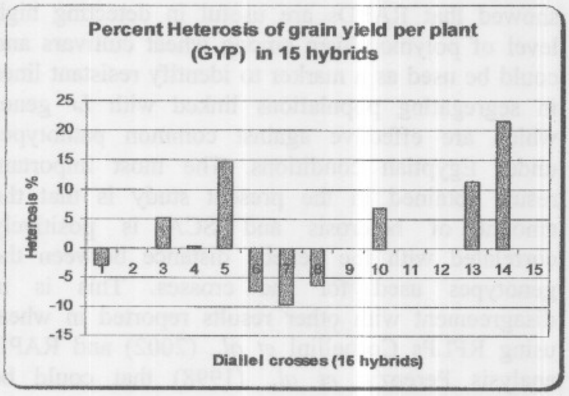
DNA from seven–day-old seedling of the parental cultivars and F_1's was extracted according to modified CTAB procedure (Murray and Thompson, 1980). RAPD primers (Operon Technologies, USA) were used to create the molecular marker data are presented in Table (2). Only clear and stable bands polymorphic were considered in the analysis. (Nei's 1972) genetic distance was calculated to measure genetic diversity among the six wheat genotypes using NTSY-pc software, version 2.02 (Rohlf, 1993). Cluster analysis of parental genotypes was based on Nei's values using the Unweighted Pair-Group Method with Arithmetical average (UPGMA) and the relationships among cultivars were visualized using a dendrogram. MPH and SCA for agronomic and quantitative traits of the 15 hybrids were correlated with the genetic diversity estimates based on RAPD markers.

Table 2: Random primers used for RAPD analysis and their corresponding sequence

Primer pair ID	Sequence 5' ⟶ 3'
OPA-08	GTGACGTAGG
OPB-07	GGTGACGCAG
OPD-08	GTGTGCCCCA
OPE-06	AAGACCCCTC
OPE-09	CTTCACCCGA
OPG-09	CTGACGTCAC
OPG-04	AGCGTGTCTG
OPO-08	CCTCCAGTGT
OPZ-012	TCAACGGGAC
OPZ-013	GACTAAGCCC

RESULTS AND DISCUSSION

Analysis of variance of grain yield in the diallel experiment showed highly significant differences due to genotypes indicating genetic diversity among genotypes. Under study, grain yield of the parental genotypes ranged from 30.5 (*Lr* 39) to 78.6 (Sakha 8) gm per plant. The heterosis of grain yield per plant (GYP) in F_1 exceeded their mid-parents in crosses number 2, 3, 5, 9, 10, 11, 12, 13, 14, and 15 Figure (1). The highest significant positive heterosis for grain yield per plant were recorded for (Sakha 8 X *Lr*39), (Sakha 93 X *Lr*38), (Sakha 93 X *Lr*39) and (*Lr*38 X *Lr*39) over mid parent. Highly significant negative heterosis was observed in Gemmiza 7 x Sakha 8, Sakha 8 x Sakha 93, Sakha 8 x *Lr*37, and Sakha 8 x *Lr* 38. Analysis of variance indicated that GCA and SCA mean squares were highly significant for grain yield. The highest SCA for GYP was observed in crosses 9, 11, 14, and 15 Figure (1). High negative SCA was found in cross number 6 (Sakha 8 x Sakha 93). On the other hand, estimation of GCA effects showed different trends and some cases were significant Table (3). Sakha 8 gave the highest significant positive GCA effects, while Sakha 93, *Lr*38, and *Lr* 39 gave the highest significant negative GCA effects. All crosses exhibited either significant positive or negative SCA.



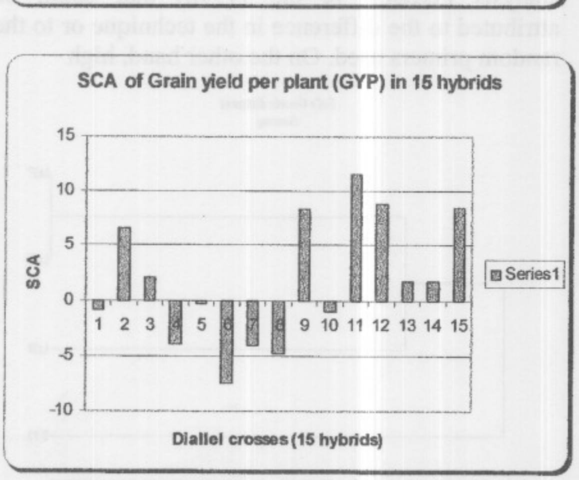


Fig. 1: Percent heterosis and SCA of GYP (*y*-axis) in 15 crosses (*x*-axis) developed from six genotypes of common Egyptian wheat (*T. aestivum* L.)

Table 3: Mean parental yield (g/plant) and general combining ability effects (GCA) for grain yield

Genotype	Mean yield (g/plant)	GCA
Gemmiza 7	70.66	-1.806
Sakha 8	78.60	4.415
Sakha 93	61.99	-9.268
Lr 37	50.09	-0.287
Lr 38	40.58	-6.540
Lr 39	30.57	-13.584
LSD *P* 0.05	**11.21**	**2.953**
***P* 0.01**	**14.99**	**3.952**

Leaf rust reaction scores are presented in Table (4), out of the six parents. Gemmiza 7, Sakha 8, and Sakha 93 showed highly susceptibility (susceptible group) reaction to leaf rust. However, genotypes *Lr*37, *Lr*38, and *Lr*39 showed different levels of resistance (resistant group) to leaf rust under the same artificial inoculation. Crosses between the susceptible genotypes showed high levels of susceptibility to leaf rust and crosses between resistant genotypes resulted in the resistant F_1 hybrids. The highest level of resistance was that recorded for the cross *Lr*37 x *Lr*39 followed by that of the cross *Lr*38 x *Lr*39 and the cross *Lr*37 x *Lr*38.

A total of 72 RAPD loci were identified using 10 random primers with four genotypes including three resistant genotypes (*Lr*37, *Lr*38, and *Lr*39) and Sakha 93 as a susceptible genotype with an average of 7.2 loci per primer. Among these identified RAPD loci, 27 loci (37.5%) were polymorphic. Nei's genetic distances (GD) was measured by using the 72 polymorphic RAPD alleles and ranged from 0.73 to 0.87 Figure (2). Four RAPD loci (OPA-08_{1179}, OPD-08_{1318}, OPG-04_{1332}, and OPO-08_{353}) generated amplifications that were present in the three resistant parental lines (*Lr*37, *Lr*38 and *Lr*39) but were absent in the susceptible one; Sakha 93 Figure (3). In their F_1's it was present in some hybrids absent in others, only the RAPD locus OPO-08_{353} was present in the three hybrids Table (5). The relationship of Heterosis and SCA for GYP with genetic distance, measured using RAPD markers, for the diallel is presented in Table (6). The amount of heterosis and SCA were highly correlated with Nei's GD. Heterosis was also significantly correlated with SCA.

The present investigation was carried out to identify parental common wheat genotypes and hybrids that have good performance for grain yield and resistant to leaf rust. The development and cultivation of varieties with high yield and

Table 4: Leaf rust reaction for parental genotypes and F1 hybrids

Genotypes	Leaf rust reaction
Gemmiza 7	90 S
Sakha 8	70 S
Sakha 93	100 S
Lr 37	50 R
Lr 38	50 MR
Lr 39	0
Gemmiza 7x Sakha 8	100 S
Gemmiza 7x Sakha 93	100 S
Gemmiza 7x Lr 37	10 S
Gemmiza 7x Lr 38	20 S
Gemmiza 7x Lr 39	40 S
Sakha 8x Sakha 93	60 S
Sakha 8x Lr 37	10 MR
Sakha 8x Lr 38	10 S
Sakha 8x Lr 39	20 MS
Sakha 93x Lr 37	5 MR
Sakha 93x Lr 38	10 MS
Sakha 93x Lr 39	10 MS
Lr 37x Lr 38	70 R
Lr 37x Lr 39	0
Lr 38x Lr 39	20 R

resistance to rusts and leaf in particular is the most effective means of wheat program and wheat farmers. A diallel among six wheat genotypes, three commercial cultivars as a susceptible group and three monogenic lines as a resistant group, was made to study the general combining ability, heterosis, and specific combining ability in relation to RAPD technique. The diallel cross revealed significant differences in both the phenotypic value of the F_1 hybrids and the amount of heterosis among the genotype combinations. The heterosis trend was positive for grain yield per plant for some crosses, indicating that genetic gain from simple breeding should be obtainable Nanda *et al.*, (1983). The results suggested that grain yield per plant is controlled by loci with both additive and non-additive gene effects under this investigation. This study suggested that the best combinations for grain yield per plant were (Sakha 8 X *Lr*39), (Sakha 93 X *Lr*38), (Sakha 93 X *Lr*39) and (*Lr*38 X *Lr*39) over mid parents. Previous results were in the same trend with an earlier findings (Borghi and Perrenzin 1994), (Abd El-ATY and Katta 2002). Combining ability has been shown to be an inherited character Satter *et a*l., (1992). Our study demonstrated that, although the amount of heterosis was independent of yield values, it was larger in crosses between more distantly related parents compared with closely related genotypes EL-Maghraby *et al.*, (2005). The best combinations for grain as listed above were resulted from cross between resistant group (*Lr*37, *Lr*38, and *Lr*39) as a cross between *Lr*38 x *Lr*39 or from the cross between resistant x susceptible as Sakha 8 X *Lr*39, Sakha 93 X *Lr*38, and Sakha 93 X *Lr*39.

Although the great success which have been received from DNA markers and used to group genotypes Peterson *et al.*, (1994); Zhang *et al.*, 1995), a little information is available about the relationships between genetic diversity and heterosis in wheat Perenzin *et al.*, (1998). If a simple, efficient, inexpensive and reliable method could be used to predict heterosis before expensive field testing, much of the field work associated with making crosses and field evaluation would be eliminated and hybrid breeding programs would be accelerated. For this reason, we focused on the relationship between heterosis and F_1 performance accompanied by leaf rust resistant with the level of genetic diversity between parents measured by RAPD markers.

The use of SSR markers for DNA fingerprinting and variety identification with emphases on common wheat was reviewed by (Gupta and Varshney 2000). Identifying SSR markers linked to *Lr26* resistance gene was an objective of the investigation of El-Maghraby *et al.*, (2006) in a cross between the resistant *Lr26* (monogenic Line) and Gemmiza 7 as a susceptible variety under Egyptian condition. This investigation showed that RAPDs are useful in detecting high level of polymorphism among wheat cultivars and could be used as a marker to identify resistant lines in segregating populations linked with *Lr* genes which are effective against common pathotypes under Egyptian conditions. The most important result obtained in the present study is that the amount of heterosis and SCA is positively correlated with the genetic distance between the genotypes used for the crosses. This is in disagreement with other results reported in wheat using RFLPs Corbellini *et al.*, (2002) and RAPD analysis Perenzin *et al.*, (1998) that could be attributed to the difference in the technique or to the random primers used. On the other hand, high

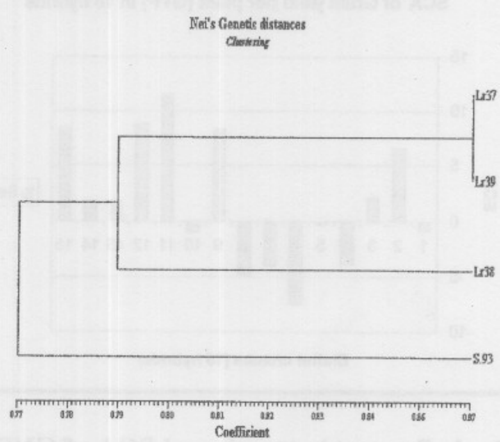


Fig. 2: Dendrogram obtained from UPGMA cluster based on RAPD data from the four wheat genotypes

Fig. 3: RAPD markers detecting polymorphism between parental genotypes (1st, 3rd, 5th, and 7th, lanes) and linked to *Lr* resistant genes in F_1 hybrids(2nd, 4th, and 6th lanes

Table 5: Performance of RAPD marker loci in F_1 hybrids and their parents

Locus	Hybrid 1			Hybrid 2			Hybrid 3		S-93
	Lr-37	F_1	S-93	Lr-38	F_1	S-93	Lr-39	F_1	
$OPA\text{-}08_{1179}$	+	+	-	+	-	-	+	-	-
$OPD\text{-}08_{1318}$	+	+	-	+	+	-	+	-	-
$OPG\text{-}04_{1332}$	+	+	-	+	+	-	+	-	-
$OPO\text{-}08_{353}$	+	+	-	+	+	-	+	+	-

Table 6: Pearson correlations among Nei's genetic distances (Nei's GD), Heterosis (H), and specific combining ability (SCA) for grain yield per plant (GYP)

	Nei 's GD	H	SCA
Nei's GD	1.000		
H	0.629**	1.000	
SCA	0.653**	0.895**	1.000

** Correlation is significant at P=0.01

correlations between heterosis and DNA-based genetic distances have been reported in other crops such as maize (Smith and Melchinger 1990), barley Melchinger *et al.*, (1994) and rice Zhang *et al.*, (1994). The high correlation depend on the high levels of heterosis Corbellini *et al.*, (2002). However, while the correlation is significant, selection of crosses solely on RAPD data would miss superior combinations. For instance, Sakha 93 X *Lr*37 showed lower heterosis than *Lr*37 x *Lr*39, although the former has a higher genetic diversity. Heterosis is presumably caused by directionally dominant loci and/or reduced homozygosity at deleterious recessive loci (Falconer, 1989). Significant correlation reported in this investigation between heterosis, SCA and genetic diversity suggest an effect of overall heterozygosity on the amount of heterosis. The negative values inamount of heterosis observed in the genotype combinations that share low genetic distances might be caused by individuals with similar genetic backgrounds.

In the present study, four RAPD loci ($OPA\text{-}08_{1179}$, $OPD\text{-}08_{1318}$, $OPG\text{-}04_{1332}$, and $OPO\text{-}08_{353}$) generated amplifions that were present in the three resistant parental lines (*Lr*37, *Lr*38, *Lr*39) but were absent in the susceptible parent Sakha 93. Only the RAPD locus $OPO\text{-}08_{353}$ was present in the three hybrids resulted from crosses between Sakha 93 with each of *Lr*37, *Lr*38 and *Lr*39. However, It could be suggested that $OPA\text{-}08_{1179}$, $OPD\text{-}08_{1318}$, and $OPG\text{-}04_{1332}$ were not able to identify most of the hybrids from the parental lines/cultivar involved in this study, since RAPD markers are dominant, that should be reflected in the hybrids. The reason for missing this amplicon from the parents to offspring was justified by smith *et al.* (1990). Similar results were obtained by Luo *et al.* (2002) who studied the inheritance of RAPD markers in an interspecific hybrid of grape (*Vitis quinquangularis* and *V. vinifera*) and El-Maghraby *et al.*, (2006) who studied the inheritance of the resistant gene *Lr*26 in F_2 bread wheat generation using SSR markers .

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الملخص العربى

الإختلافات الوراثية المقدرة بالدلائل الجزيئية العشوائية فى قمح الخبز وعلاقتها بصدأ الأوراق وقوة الهجين

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[1]مركز البحوث الزراعية ــ وزارة الزراعة

[2] قسم الوراثة ــ كلية الزراعة ــ جامعة الإسكندرية

باستخدام تقنية تحليل الهجن الدائرية تم اجراء كل الهجن الممكنة بين ستة من التراكيب الأبوية بينهم ثلاثة من أصناف قمح الخبز التجارية وهى جيزة ٧ & سخا ٨ & سخا ٩٣ والتى تصاب بصدأ القمح البرتقالى (صدأ الأوراق) وثلاثة من السلالات المقاومة لصدأ الأوراق والتى تم تعريف الجينات المقاومة بها وهى (*Lr37, Lr38,* and *Lr39*).

هدف الدراسة:

١. تحديد الاختلافات الوراثية بين التراكيب الوراثية الأبوية باستخدام تقنية الدلائل الجزيئية العشوائية وامكانية استخدام الاختلافات الوراثية بين الآباء كأداة للتنبؤ بقوة الهجين والقدرة الخاصة على الائتلاف فى الجيل الأول.

٢. التعرف على الهجن التى تتميز بقوة هجين عالية بالاضافة الى احتوائها على جينات المقاومة ذات الفعالية تحت ظروف البيئة المصرية لامكانية استخدامها كمصدر لانتاج نباتات مقاومة فى برنامج التربية.

النتائج:

أظهر تحليل التباين وجود اختلافات معنوية عالية بين التراكيب الوراثية تحت الدراسة بالنسبة للقدرة العامة والخاصة على الائتلاف وكذلك أوضح أهمية القدرة العامة على الائتلاف بالنسبة للآباء تحت الدراسة لصفة المحصول والصفات الاقتصادية الأخرى.

أظهرت بعض الهجن الناتجة قدرة عالية على مقاومة صدأ الأوراق بدرجات مقاومة مختلفة وكذلك ذات قوة هجين عالية بالنسبة لمحصول الحبوب وكذلك تفاوتت الهجن بين القدرة الخاصة على الائتلاف بالسلب أو الايجاب. وكانت الهجن التى أظهرت مقاومة عالية ناتجة من التهجين بين السلالات المقاومة معا (*Lr37) Lr38, Lr39*) فى حين أن السلالات التى أظهرت درجات مختلفة من المقاومة كانت ناتجة من التهجين بين السلالات الثلاث المقاومة وبين الصنف سخا ٩٣ (سخا٩٣ x *Lr37,*) وكذلك(سخا٨ x *Lr37*) ولذا تم اختيار الصنف التجارى سخا ٩٣ والسلالات المقاومة (*Lr37, Lr38, Lr39*) *Lr38, Lr39*)التى تحتوى على جينات مختلفة للمقاومة لصدأ الأوراق لتقدير الاختلافات الوراثية بينها باستخدام تقنية الدلائل الجزيئية العشوائية.

وأظهرت النتائج المتحصل عليها باستخدام تقنية الدلائل الجزيئية العشوائية وجود اختلافات وراثية بين التراكيب الوراثية الأبوية والتى تراوحت بين ٠,٧٣ الى ٠,٨٧ وذلك باستخدام عدد ١٠ دلائل جزيئية عشوائية باجمالى عدد ٧٢ اليل وكان متوسط عدد الأليلات لكل موقع هو ٧,٢ . أوضح تحليل الشجرة وقوع السلالات المقاومة رقم ٣٧ & ٣٩ فى مجموعة واحدة بدرجة تماثل تصل إلى ٠,٨٧ ووقعت السلالة المقاومة رقم ٣٨ فى مجموعة أخرى بدرجة تماثل وصلت إلى ٠,٧٩ مع المجموعة الأولى. فى حين أحتل الصنف سخا ٩٣ المجموعة الثالثة بدرجة تماثل وصلت إلى ٠,٧٣ مع المجموعتان السابقتان.

وبتحليل التلازم بين الاختلافات الوراثية المقدرة بين التراكيب الوراثية الأبوية وقوة الهجين المتحصل عليها فى الجيل الأول والقدرة الخاصة على الأئتلاف وجد أن هناك ارتباط عالى بينهم بالنسبة لصفة محصول الحبوب فى الكثير من الهجن الناتجة مما يدل على إمكانية إستخدام الاختلافات الوراثية المقدرة بين الآباء تحت الدراسة بإستخدام الدلائل الجزيئية كأداة للتنبؤ بقوة الهجين والقدرة الخاصة على الخلط فى الجيل الأول مما يفتح الباب لامكانية حصر الاختلافات بين التراكيب الوراثية المحتملة فى برنامج التربية قبل اجراء التهجينات فى الحقل لتحديد أفضل الهجن توفيرا للوقت والمال والجهد الناتجة عن إستخدام الطريقة التقليدية فى أختيار الآباء واجراء التهجينات اللازمة.