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PREDICTION OF HETEROSIS AND COMBINING ABILITY IN SOME YELLOW MAIZE CROSSES VIA SEROLOGICAL ANALYSIS AND MOLECULAR GENETIC MARKERS

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ABSTRACT

5X5 half diallel crosses along with their parental maize inbred lines were evaluated for grain yield per plant to detect heterosis and combining ability. Meantime, serological analysis (double diffusion gel technique) using the extracted saline soluble protein of inbred lines grains was carried out to identify general combining ability (GCA). In addition, DNA of the five inbred lines and their ten F1's were analyzed via PCR-RAPD technique in an attempt to find out some molecular genetic markers associated with heterosis and combining ability results obtained from the field. Comparing field and serological precipitation results, it was indicated that the double diffusion gel technique was a satisfactory tool for the prediction of general combining ability in yellow maize. It was difficult to distinguish the pattern of association between specific combining ability, heterosis values and serological analysis. When comparing field and molecular genetic markers results, it was found that PCR-RAPD technique could be a useful tool for the identification and characterization of the five inbred lines of yellow maize. This technique could generally agreed with the actual field performance of the crosses and as molecular genetic markers associated with hybrid vigor. It was possible to elucidate reliable molecular genetic markers associated with heterosis and specific combining ability in yellow maize.

Key words: ***Yellow maize, Prediction, Heterosis, Combining ability, Serological analysis, PCR-RAPD technique, Molecular genetic markers.***

INTRODUCTION

Serological tests were used in many studies to predict heterosis and combining ability in order to save time and field testing efforts. *Abdel-Tawab et al(1978)* revealed that the double diffusion gel technique was a satisfactory tool for the prediction of general combining ability in maize. *Abdel-Tawab et al(1990)* indicated that the immuno diffusion gel technique was effective in the identification of general combining ability in maize inbreds and for the partial differentiation between the high and low specific combining ability and heterosis. *Esmail et al (1999)* indicated that the immuno diffusion gel

technique could be used effectively for the identification of general combining ability in cotton varieties.

The randomly amplified polymorfic DNA (RAPD) assay, which detects nucleotide sequence polymorphisms by means of the polymerase chain reaction (PCR) and a single primer or arbitrary nucleotide sequence , has become extremely a useful tool for identifying maize genotypes and to assess genetic diversity. Therefore, development of a reliable method for development of heterotic groups and predicting hybrid performance without testing thousands of single cross combinations was the goal of numerous studies, using molecular and phenotypic markers (*Ajmone et al (1993), Lanza et al (1997), El-Khishin et al (2003) and Fahmi et al (2003)*).

The objective of this investigation was to study the possibility of predicting heterosis and combining ability in yellow maize *via* immuno diffusion gel technique and molecular genetic markers.

MATERIALS AND METHODS

5X5 half diallel crosses along with their parental maize inbred lines were previously evaluated under normal and drought environments (*Abdel-Sattar and Ahmed (2004)*). The genetic material used in this part included new five yellow maize (*Zea mays L.*) inbred lines (AY1,AY2,AY3,AY4 and AY5), representing a wide range of diversity for several agronomic characters. These lines were developed by the first author through the breeding program at Dept. of Genetic and Cytology, National Research Center. These lines were derived from the single crosses (S.C.) 150, S.C.151, S.C.158, the three-way cross (T.W.C.)352 and the double cross (D.C.)Dahab x Sabeiny, respectively.

In 2003 season, all possible cross combinations excluding reciprocals were made among the five inbred lines giving a total of 10 crosses. On 15th May 2004, the five parents and their ten F_1 crosses were evaluated at the Experimental Farm of the National Research Center, at Shalakan, Kalubia Governorate, Egypt in a randomized complete block design with three replications. The experimental plot included three rows of five meters long and 70 cm wide. Planting was in hills spaced at 25 cm apart. The common agricultural practices of growing maize were applied properly as recommended for the district. At harvest grain yield per plant was recorded on a sample of 10 guarded plants in the middle row of each plot. General and specific combining ability variances and effects were obtained by employing *Griffing's (1956)* diallel cross analysis, method 2 model I .

In the serological study, the inbred lines AY1,AY2 and AY4 were used to immunize rabbits and produce antisera. The method of *Hsieh and Fery (1972)* was applied. The double diffusion gel technique (*Ouchterlony(1968)*) was used to predict general combining ability.

In the molecular genetic study, ten random primers were used for RAPD analysis, provided by Operon Technology (USA), with the following sequences:

Primer code	Sequence
A03	5'AGTCAGCCAC'3
A04	5'ATTCGGGCTG'3
A07	5'GAAACGGGTG'3
A09	5'GGGTAACGCC'3
A10	5'GTGATCGCAG' 3
B07	5'GGTGACGCAG'3
B11	5'GTAGACCCGT'3
O15	5'TGGCGTCCTT'3
O16	5'TCGGCGGTTC'3
D11	5'AGCGCCATTG'3

Ten grains from each of the five inbred lines and their ten F_1 crosses were germinated in plastic pots in the dark for two weeks. Total genomic DNA was extracted following the method of *Bushra et al (1999)*. PCR was performed in 30-µl volumes tubes according to *Williams et al (1990)*. Amplification was carried out in a DNA thermocycler (MWG-BIOTECH Primuse) programmed for 47 cycles as follows: 94°C/4 min (1cycle), 94°C/1 min, 36°C/ 1 min and 72°C/2 min (45 cycles); (1cycle) 72°C for 7min ,then, 4^0 C infinitive.

RESULTS AND DISCUSSION

Serological analysis

The reactions of antigen-antiserum in the double diffusion gel technique are given according to the following ranks, which were based on the degree of sharpness of the serological precipitation:

0= no precipitation, 1 = very faint precipitation, 2 = faint precipitation,

3 = clear precipitation, 4 = sharp precipitation .

The double diffusion gel technique was carried out using three antisera, two good combiners (AY1 and AY4) and one poor combiner (AY2) based on field performance results against the five tested inbred lines. Each testing antiserum was repeated three times.

The results of the serological reactions for the three antisera are presented in Figure (1) and Table (1). The good combiners antisera AY1 andAY4 showed clear or sharp precipitation reactions with all tested inbred lines as they marked by rank 3 or 4 .

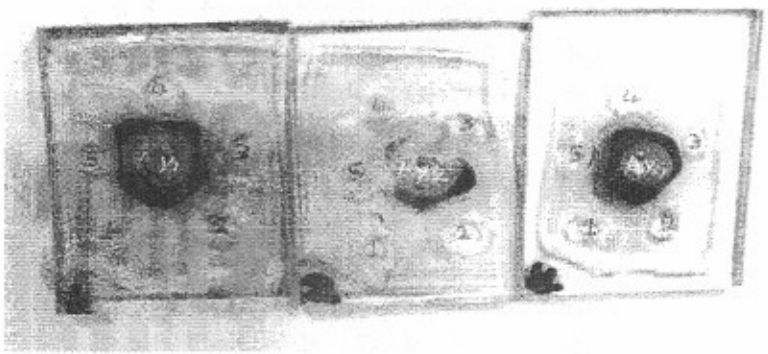

Figure 1 . Double diffusion reaction of AY1, AY2 and AY4 with the five inbred lines of yellow Maize .

Table 1. Serological reactions of three inbred lines antisera against the five inbred lines and the field estimates of GCA effects for grain yield per plant in gram of yellow maize.

No.	Inbred lines	Serological reactions			GCA effects
		AY1	AY2	AY4	
1	AY1	4	2	4	8.18**
2	AY2	3	2	3	-6.01**
3	AY3	3	0	2	-4.20*
4	AY4	2	1	4	9.32**
5	AY5	1	0	4	-7.30**
	Average ranks	2.6	1.0	3.4	

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

In contrast, the poor combiner antiserum AY2 showed no precipitation reaction with the two antigens AY3 and AY5 and faint or very faint precipitation reaction with the other tested inbred lines AY1, AY2 and AY4. The differences between the three sets of precipitation reactions (Table 1) were positively associated with the estimates of general combining ability effects which were -6.01 for the poor combiner AY2 and (8.18 and 9.32) for the good combiners AY1 and AY4, respectively. In the same time, the average serological ranks of poor (AY2) and good (AY1 and AY4) combiners were 1, 2.6 and 3.4, respectively (Table 1). It is evident that, the differences between the three sets of reactions were clear cut and indicated that the double diffusion gel technique used in this study was a satisfactory tool for the prediction of general combining ability in yellow maize. This was in agreement with the results of *Abdel-Tawab et al (1978 and 1990)* who reported that, in maize the good combiner antiserum generally gave relatively sharp and clear precipitation reactions with most of the inbred lines, while the poor combiner was characterized by the occurrence of rather very faint or no precipitation reaction with the antigen of most inbred lines, and concluded that the double diffusion gel technique was a satisfactory tool for the prediction of general combining ability in maize. *Esmail et al (1999)* found that the immuno diffusion gel technique could be used effectively for the identification of general combining ability in cotton.

The relationships between specific combining ability, heterotic values and double diffusion tests for grain yield per plant are present in Table (2). In case of AY1 as antiserum, negative association was obtained between each of specific combining ability effects, heterotic estimates and serological ranks. The specific combining ability effects and heterosis estimates for the crosses AY1XAY2, AY1XAY3, AY1XAY4 and AY1XAY5 were –3.46, -9.60, 38.29 and 1.51 and –3.54, -6.25, 32.29 and –3.13, respectively. In the meantime, these crosses gave clear or faint precipitation reactions. When AY2 was used as antiserum the same trend was noticed (Table 2), where the crosses AY1XAY2, AY2XAY3, AY2XAY4 and AY2XAY5 gave ranks 2, 0, 1 and 0, respectively for precipitation reactions and recorded SCA effects of –3.46, 24.59, 2.06 and 22.02, respectively and gave heterosis values of –3.54, 37.84, 18.38 and 35.81%, respectively.

Table 2. Better parent heterosis(%) and SCA effects for grain yield per plant and serological reactions for the good combiners (AY1 and AY4) and the poor combiner (AY2) of yellow maize.

Crosses	Heterosis %	SCA effects	Serological reactions
			AY1antiserum
AY1XAY2	-3.54	-3.46	3
AY1XAY3	-6.25	-9.60	3
AY1XAY4	32.29**	38.55**	2
AY1XAY5	-3.13	-1.51	1
			AY4 antiserum
AY4XAY1	32.29**	38.55**	4
AY4XAY2	18.38**	2.06	3
AY4XAY3	26.96**	11.92**	2
AY4XAY5	33.58**	24.02**	4
			AY2 antiserum
AY2XAY1	-3.54	-3.46	2
AY2XAY3	37.84**	24.59**	0
AY2XAY4	18.38**	2.06	1
AY2XAY5	35.81**	22.02**	0

** significant at the 0.01 probability level.

In contrast, when AY4 was used as antiserum the opposite trend was noticed (Table 2), where the crosses AY1XAY4, AY2XAY4, AY3XAY4 and AY4XAY5 gave ranks of 4, 3, 2 and 4, respectively for precipitation reactions and recorded SCA effects of 38.55, 2.06, 11.92 and 24.02, respectively and heterosis values of 32.29, 18.38, 26.96 and 33.58%, respectively. In that case the positive correlation was obtained .

From these results, we found inconsistency between the precipitation reactions of the three inbred lines antisera and the estimates of SCA effects and heterosis. So that, it was difficult to distinguish the pattern of association between SCA effects, heterosis values and serological analysis. This result was in agreement with the results of *Abdel-Tawab et al (1978)* who found that, it was difficult to distinguish the pattern of association between heterosis and serological analysis. In contrast, *Urano and Arai (1956)*, indicated that the

egree of heterosis for grain yield of maize was significantly and negatively ssociated with the amount of precipitation.

Iolecular genetic markers

The DNA of the five inbred lines and their ten F1's were tested against ten 0-mer random primers to study the possibility of predicting heterosis and ombining ability. Four out of the ten primers developed PCR products with ıe samples of DNA, i.e.(A07, B11, O15 and O16). Banding pattern for the ɔur primers were illustrated in Figure(2)and scored as present (1) or absent)) as shown in Table(3). For PCR reaction with the primer A07, a 520bp band 'as shown to be present for the five inbred lines while it was absent for the ten ybrids. Six bands at molecular weight 1182bp, 1153bp, 1041bp, 553bp, 340bp ıd 290bp were shown to be present for the most hybrids while they were ɔsent for the five inbred lines. With respect to primer B11 the inbred line AY1 ıowed five bands one of them, a 670bp band, is unique band for this inbred ne and the other four bands resembled one or more of the other inbred lines. he inbred lines AY2 and AY3 exhibited four bands one of them, 610bp and 10bp, respectively, is a unique band and the other three bands resembled the :her inbred lines. The inbred lines AY4 and AY5 contained five bands one of .em, 1250bp and 1213bp,respectively, is a unique band and the other four ınds resembled the other inbred lines. Similar results were detected for 'imers O15 and O16 where each inbred line of maize was distinguished with ıe unique band from each primer as follows:

The inbred line AY1 was distinguished with 863bp and 360bp bands from 'imers O15 and O16, respectively. The inbred line AY2 was characterized ith 1130bp and 1205bp bands from primers O15 and O16, respectively. The bred line AY3 was distinguished with 1272bp and 1141bp bands from 'imers O15 and O16, respectively.

The inbred line AY4 was distinguished with 1286bp and 897bp bands ɔm primers O15 and O16, respectively. The inbred line AY5 was ıaracterized with 1340bp and 1175bp bands from primers O15 and O16, spectively.

From these results, we conclude that, PCR-RAPD technique could be a eful tool for the identification and characterization of the five inbred lines of llow maize. These results are in agreement with those obtained by *Ajmone et (1993), Lanza et al(1997), Moller and schaal (1999), Sun et al (2001), El-hishin et al (2003)*and *Fahmi et al (2003)*. They indicated that RAPD chnique can be used as a tool for determining the extent of genetic diversity nong maize inbred lines.

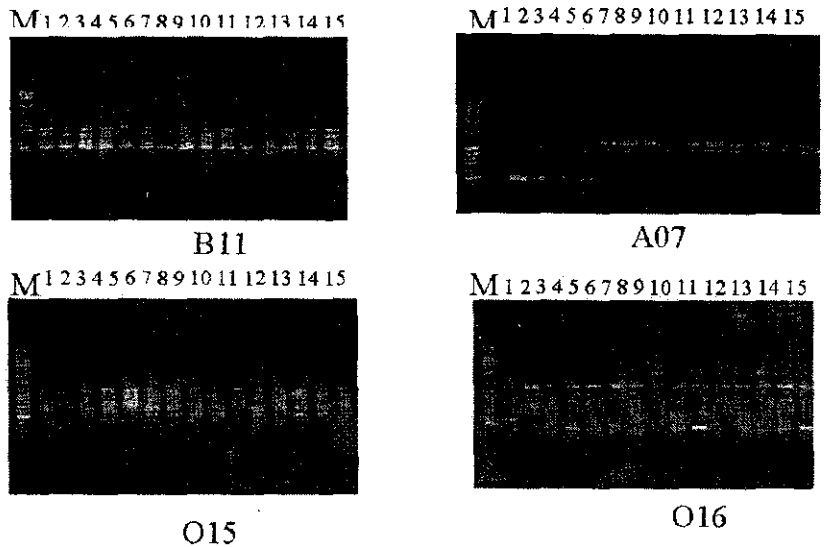

Fig. 2. DNA Polymorphisms using RAPD Markers generated by four Random Primers (A 07, B11, O15 and O16), M= Marker, 1-- 5 = the five inbred lines and 6-- 15 = the ten hybrids of Yellow Maize

In a trail to predict heterosis and specific combining ability via PCR-RAPD technique, two primers (O15 and O16) out of the four primers could be considered as reliable molecular markers positively linked with heterosis and SCA as follows:

With respect to PCR reaction with the primer O15 (Fig. 2), the three hybrids (AY1XAY2, AY1XAY3 and AY1XAY5) contained four bands, the same number which found in their parents, one of them (515bp) is a unique band and the other three bands resembled their respective parents (Table 3). In the same time, these three hybrids showed negative heterosis and SCA effects (Table 4). The other seven hybrids, except the hybrids AY1XAY5 and AY4XAY5, showed higher number of bands which exceeded the number of bands present in their respective parents (Table 3). In the same time, these hybrids showed significant positive heterosis and SCA effects (Table 4).

Similar results were detected for the primer O16, where the three hybrids (AY1XAY2, AY1XAY3 and AY1XAY5) exhibited five bands which didn't exceed the number of bands of their parents and associated with field performance which showed negative heterosis and SCA effects (Table 4). The other seven hybrids, except the hybridAY2XAY4, exhibited higher number of bands which exceeded the number of bands present in their respective parents (Table3). In the same time, these hybrids showed significant positive heterosis and SCA effects (Table 4).

Table 3. DNA polymorphism using randomly amplified polymorphic DNA, A07,B11,O15 and O16 primers (P.) for the five inbred lines and their ten F1's showing number of the band (B.no.), relative mobility (Rm), molecular weight (Mw) and the total numer of bands/ each colum (T.).

P.	B. No	R.m	M.W bp	The Five Inbred lines					The ten Hybrids									
				1	2	3	4	5	1x2	1x3	1x4	1x5	2x3	2x4	2x5	3x4	3x5	4x5
A07	1	0.28	1182	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
	2	0.31	1153	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
	2	0.36	1041	0	0	0	0	0	1	1	1	0	1	1	1	1	0	1
	4	0.42	553	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0
	5	0.45	520	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
	6	0.52	340	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
T.	7	0.61	290	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
				1	1	1	1	1	4	6	2	1	2	3	4	2	1	2
B11	1	0.29	1250	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
	2	0.31	1213	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	3	0.34	1150	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0
	4	0.36	1093	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0.38	910	0	0	1	0	0	1	1	1	0	0	1	1	0	0	0
	6	0.41	823	1	1	0	1	1	0	0	1	1	1	0	0	1	1	1
	7	0.44	670	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1
	8	0.45	610	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0
	9	0.47	530	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
T.	10	0.52	430	5	4	4	5	5	1	1	0	1	1	1	1	0	1	1
									1	1	1	1	1	1	1	1	1	1
									4	4	4	4	5	5	4	4	4	5
O15	1	0.32	1340	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
	2	0.35	1286	0	0	0	1	0	0	0	1	0	1	1	1	1	1	1
	3	0.36	1272	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1
	4	0.37	1130	1	1	1	1	1	0	0	1	0	0	0	1	1	0	0
	5	0.38	1080	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1
	6	0.40	863	1	1	1	1	1	0	0	0	0	1	0	0	0	0	1
	7	0.43	650	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0
	8	0.45	593	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0
	9	0.47	515	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0
T.	10	0.51	381	4	4	4	4	4	1	1	1	1	0	1	1	1	1	0
									0	0	1	0	0	1	1	1	1	0
									4	4	7	4	4	6	6	7	6	4
O16	1	0.29	1266	0	0	1	1	1	0	1	1	0	0	0	1	1	0	0
	2	0.32	1205	0	1	0	0	0	1	0	1	1	1	1	1	1	1	1
	3	0.34	1175	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	4	0.35	1141	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
	5	0.37	983	1	0	0	1	0	0	1	1	1	1	0	1	1	1	1
	6	0.39	915	0	1	1	1	1	0	0	0	0	1	0	0	0	0	1
	7	0.40	897	1	1	1	1	1	1	0	0	0	1	1	0	0	0	1
	8	0.44	833	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
	9	0.48	430	1	5	5	5	4	1	1	1	1	1	1	1	1	1	1
T.	10	0.53	360						0	0	0	1	0	0	0	0	1	0
									5	5	6	5	7	5	6	6	6	7

Table 4. Better parent heterosis(%) and SCA effects for grain yield per plant.

No.	Crosses	Heterosis	SCA effects
1	AY1XAY2	-3.54	-3.46
2	AY1XAY3	-6.25	-9.60
3	AY1XAY4	32.29**	38.55**
4	AY1XAY5	-3.13	-1.51
5	AY2XAY3	37.84**	24.59**
6	AY2XAY4	18.38**	2.06
7	AY2XAY5	35.81**	22.02**
8	AY3XAY4	26.96**	11.92**
9	AY3XAY5	33.78**	20.87**
10	AY4XAY5	33.58**	24.02**

** significant at the 0.01 probability level.

It is evident therefore that, these two PCR-RAPD products could generally agree with the actual field performance of the crosses. This indicates that, it is quite possible to elucidate reliable molecular genetic markers associated with heterosis and specific combining ability in yellow maize. Some studies detected positive association between parental genetic distance based on DNA marker and hybrid yield (*Smith et al (1990)* and *Enoki et al (2002)*). Many other studies, however, reported low correlation between parental distance based on DNA marker and hybrid performance (*Salama et al (2001)* and *Fahmi et al (2003)*)

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التنبؤ بقوة الهجين والقدرة على التآلف فى بعض هجن الذرة الصفراء من خلال التحليلات السيرولوجية والواسمات الوراثيه الجزيئية

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١ – قسم الوراثة والسيتولوجى_المركز القومى للبحوث_الجيزة_مصر .

٢ – قسم المحاصيل_كلية الزراعة_جامعة عين شمس–القاهرة–مصر .

يهدف البحث إلى دراسة إمكانية التنبؤ بظاهرة قوة الهجين والقدرة على الإئتلاف فى بعض هجن الذره الصفراء من خلال التحليلات السيرولوجية و الواسمات الوراثية الجزيئية توفيرا للوقت والمجهود والإعتمادات المالية التى تتطلبها الإختبارات الحقلية وذلك بإستخدام خمسة سلالات مربـاة داخليا وكافة الهجن التبادلية دون العكسية (عشرة هجن)،حيث تم تقييمها فى موسم ٢٠٠٤ بمحطة البحوث والتجارب الزراعية بالمركز القومى للبحوث_شلقان_قليوبية فى تصميم تجريبى قطاعات كاملة العشوائية من ثلاثة مكررات وتم تحليل النتائج بطريقة جريفنج ١٩٥٦ (الموديل الاول_ الطريقة الثانية) لصفة إنتاجيه النبات من الحبوب (جرام).

تم حقن الجلوبيولين المستخلص من بذور الأباء فى مجموعة من الأرانب للحصول على الأجسام المناعية لها ثم إستخدام طريقة التفاعل بين (الأنتيجن / الأنتيسيرم) لقياس درجة الترسيبات السيرولوجية ومقارنتها بالنتائج الحقلية للوقوف على مدى إمكانية التنبؤ بالقدرة العامة على التالف للسلالات الداخلة فى التفاعل . و تم دراسة قوة الهجين والقدرة الخاصة على التالف من خلال اختبار مستخلص **DNA** بإستخدام تقنية **PCR-RAPD** فى محاولة لتحديد الواسمات الجزيئية الخاصة بقوة الهجين. تم مقارنة القيم المتحصل عليها من التجربة الحقلية لصفة محصول الحبوب ونتائج الترسيبات السيرولوجية والواسمات الجزيئية المتحصل عليها لمعرفة مدى إمكانية التنبؤ بقوة الهجين والقدرة العامة والقدرة الخاصة على التالف فى هجن الذرة الصفراء .

وقد أظهرت نتائج التحليلات السيرولوجية إمكانية إستخدام هذه الطريقة فى التنبؤ بالقدرة العامة على الائتلاف بينما كان من الصعب تحديد نمط العلاقة بين التحليلات السيرولوجيه وقوة الهجين والقدرة الخاصة على التالف ، وقد وجد أن تقنية **PCR-RAPD** يمكن أن تكون أداة فعالة للتعرف على وتوصيف سلالات الذرة المرباة داخليا ، كما وجد أنه يمكن لهذه التقنية أن تتفق بوجه عام مع الاداء الفعلى للهجن فى الحقل كواسمات وراثية جزيئية مرتبطة بقوة الهجين ، وبصفة عامة فقد أثبتت الادلة الوراثية الجزيئيه المتحصل عليها فى هذه الدراسة أنها يمكن أن تستعمل كأدلة للتنبؤ أو التحقق من الاداء الفعلى للسلالات المرباة داخليا أو الهجن الناتجة منها.