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Verification of varietals purity in maize seeds using rapd-pcr technique

Attia, A. N.* ; M. I. EL-Emery and A. Fayed, Eman ****

*** Agronomy Dept., Fac. Agric., Mans. Univ., Egypt**

**** Seed Technology Res. Dept., Agric. Res. Center, Egypt**

ABSTRACT

Laboratory experiments Randomly Amplified Polymorphic DNA (RAPD-PCR) was carried out during the 2010 season. RAPD-PCR products from five maize genotypes generated by random primer A03 with molecular weight of (1327, 1033, 904, 727, 595, 538, 487, 412, 360, 331, 280, 245 and 200 b.p.), primer B05 with molecular weight of (1327, 1033, 904, 727, 595, 538 and 487), primer B09 with molecular weight of (1948, 1513, 1233, 1020, 844, 578, 435, 354, 293 and 243 b.p.) primer B11 with molecular weight of (1232, 1038, 917, 726, 651, 574, 507, 455, 395, 328, 303, 263 and 218 b.p.) and primer B16 with molecular weight of (1554, 1203, 1001, 865, 657 and 518 b.p.). DNA pattern of genotypes using primers A03, B05, B09, B11 and B16 produced 13, 7, 10, 13 and 6 bands, respectively. RAPD-PCR products from the five maize genotypes generated by the random primers as visualized on 1% Agarose gel electrophoresis stained with 10 mg/ml ethidium bromide. The DNA was extracted from maize leaves tissue using Dellaporta extraction. (M)100 bp Marker, lanes from 1-15 is different five Maize genotypes.

INTRODUCTION

Maize (Zea mays) is one of the important staple food crops all over the world. Seed quality have a major important on prospective crop yield while seed carry the genetic traits. Seed quality affected by many factors among them, genetic purity of seed purity problem has received very low attention in maize coexistence studies, and has not been considered in prediction tools that simulate pollen flow and out-crossing between maize fields, (Dietiker *et al*, 2010). While the genetic purity of the seed planted must equal or exceed the final product purity standard required. Genetic purity of hybrid maize affected by many factors such as the isolation of the seed production fields, the removal of the female parental tassel, the cleaning of the harvest and processing machinery.

In all major crops, genetic distances (GDs) based on reliable molecular marker data have been found to reflect accurately the degree of pedigree relationships between genotypes (Melchinger 1999). In maize, several studies reported highly significant correlations between GDs based on molecular markers and the coefficient of co ancestry (Lübberstedt *et al*. 2000).

Molecular markers that reveal polymorphism at the DNA level have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity. The use of molecular markers for diversity analysis can also serve as a tool to discriminate between closely related individuals from different breeding sources (Lombard *et al*. 2000; Métais *et al*. 2000; Sun *et al*. 2001).

Random Amplified Polymorphic DNAs (RAPDs) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams *et al*., 1990 and Welsh and McClelland, 1990). RAPDs produce DNA profiles of varying complexity, depending on the primer and template used. Each amplification product is expected to result from the existence of two annealing sites in inverted orientations, 3`ends facing each other, within amplifiable distance (Williams *et al*., 1990). Polymorphisms could be caused by differences in nucleotide sequences at the priming sites (such as point mutations), or by structural rearrangements within the amplified sequence, (e.g., insertions, deletions, inversions) (Welsh and McClelland, 1990). RAPD analysis has been used in many applications and various organisms, especially in the plant sciences (Sharma and Mohapatra, 1996).

RAPDs has great advantages, since no prior knowledge of the target sequences is required for the design of primers, requires only nanogram amount of DNA, results are directly read from agarose gels, and the entire genome is screened (Williams *et al*., 1990). However, the nature of DNA sequences involved in RAPD fragments is little known, as many different kinds of sequences are probably involved (Schierwater, 1995). Indeed, in those studies where this question has been addressed, repetitive DNA has often been detected (Paran and Michelmore, 1993 and Kostia *et al*., 1996), which may limit the applicability of RAPDs (Hopkins and Hilton, 2001).

Drinic *et al* (2004) determined genetic diversity of maize inbred lines of different origin on the basis of RAPD markers and examined usefulness of RAPD markers for assigning inbred lines to heterotic groups. The results agree with another reports that stated that the level of polymorphism for RAPD markers is high in maize. Cluster analysis based on generic distance calculated from RAPD data showed clear grouping of inbred lines into main heterotic groups. To fill this gap, the aim of this study was to estimate the level of polymorphism among five maize genotypes i.e. inbred lines S7 and S61(The parents of Single cross10) as well as Single Cross 10 (SC10) from three different sources for verification of varietals purity in maize seeds.

MATERIALS AND METHODS

Genetic Materials :

This study was carried out during 2009 and 2010 seasons at the Farm of Faculty of Agriculture, Mansoura Univ., and Laboratories of Seed Tech. Res. Sec., Field Crops Research Institute, ARC . The main objective of this study was to estimate the level of polymorphism among five maize genotypes i.e. inbred lines S7 and S61 (the parents of Single cross10) as well as Single Cross 10 (SC10) from three different sources for verification of varietal purity in maize seeds.

These genotypes were as follows:

1-S63 Inbred line (Male inbred line) .
2-S7 Inbred line (Female inbred line).
3-S.C.10 Controlled produce with researcher.

4-S.C.10 Produce with researcher according the traditional method for hybrid seed production.

5- S.C.10 commercial seeds.

Single cross 10 (S.C.10) Controlled produce with researcher:

The technique of inbreeding requires careful attention to prevent natural crossing (according to Poehlman,s method, 1987). Ear shoots on the plants to be selected (Female inbred line, S7) are covered with a glassine or parchment shoot bag 1 or 2 days before the silks emerge. When the silks have emerged and the tassel is shedding pollen, the shoot bag is lifted slightly, the shoot is cut back with a sharp knife about 2-3cm below the tip of the husk, and the shoot bag is replaced. By the following day the silks will grow out and form a brush about 2-4 cm in length, permitting uniform distribution of the pollen over the silks. At the same time that the silks are cut back, the tassel(male inbred line, S63) is covered with a kraft paper bag to catch the pollen. The following day the tassel bag containing fresh pollen is removed and, after removing the shoot bag, the pollen is quickly poured over the fresh silks. Care should be taken to avoid contamination with foreign pollen. The tassel bag is placed over the shoot, securely fastened, and labeled with information about the cross. Individual plants generally labeled by numbers and letters.

Steps in control selfing and crossing maize:

(A) Ear shoot of Female inbred line S7 emerging from the leaf sheath. Shoots are bagged at this stage to prevent pollination.

(B) Glassine bag; in place over ear shoot to protect silks from wind-blown pollen. Shoots are covered 1 or 2 days before the silks emerge.

(C) Ear shoot cut back on the day previous to pollination. The shoot bag is replaced immediately.

(D) Tassel bag is placed over tassel on day previous to pollination.

(E) Silk brush grown out ready for pollination. The brush provides a uniform growth of fresh silks on which to place the pollen. Pollen collected in the tassel bag is dusted over the silk brush.

(F) Tassel bag fastened in place over the shoot to protect the developing ear.

(G) Apron in which pollinating equipment is carried. Supplies include a sharp paring knife, shoot bags, wax pencil, field notebook, and tassel bags.

Single cross 10 (S.C.10) Produce with researcher according the traditional method for hybrid seed production:

In the commercial production of single-cross seed, the two inbreds to be crossed are planted in separate rows in an isolated field. A planting pattern in common use for single-cross seed production is one pollen parent (S63) row to four seed parent (S7) rows (1:4).

Genomic DNA extraction from leaves of Zea mays:

Samples collected from young leaves on ice and stored at/or below –70oC until used. Grind 100 mg of leaves using mortar and pestle. Add 640 μL of CTAB extraction buffer plus 160 μL of CTAB to the ground leaves and mix with a mortar and pestle. Pour the slurry into a clean 2 ml Eppendorf

tube. Incubate at 60oC for 30 min. Add 500 μL of chloroform isoamylalcohol and mix gently by inverting the tubes 20-25 times to form an emulsion. Spin at 100,000 g for 6 min in a tabletop centrifuge at room temperature. Transfer the top aqueous phase to a new Eppendorf tube. A second chloroform: isoamylalcohal extraction may be performed if the aqueous solution is cloudy. Add 2/3 volume of cold (-20oC) isopropanol and refrigerate (4-6oC) for 15–20 min or until DNA strands begin to appear. Spin at 10,000 g for 6 min. Pour off the supernatant and wash the pellet with 200 μL of 5 M ammonium acetate plus 600 μL of absolute ethanol. Spin at 10,000 g for 6 min. Completely remove ammonium acetate and ethanol by air pump. Dissolve in 100 μL TE. Treat with 1 μL RNAse per 100 μL DNA solution and incubate at 37oC for 45 min. Quantify DNA for all samples using a fluorometer for adjusting sample DNA concentration to 15 ng/μL. Keep the DNA at –70oC for long-term period or –20oC for short-term storage according to Leroy and Leon (2000).

Genomic DNA amplification using RAPD.PCR.

Amplification of genomic DNA was made on perken Elmer DNA cycler using arbitrary decamer primers A03, B05, B09, B11 and B16 which are presented in Table1.

Table1: Oligonucleotide primers used in the study.

Primer name	Sequence (5' → 3')
0P- A03	5′ AGTCAGCCAC 3′
0P- B05	5′ TGCGCCCTTC 3′
0P-B09	5′ TGGGGGACTC 3′
0P- B11	5′ GTAGACCCGT 3′
0P- B16	5′ TTTGCCCGGA 3′

Amplification of genomic DNA was performed in 25 μL reaction volumes containing 1-2 units of Tag polymerase, 10 mM tris HCl, 25 mM Kcl, 2 mM MgCl2, 0.2 mM of each 4 NTP, 25 ng of primers and 40 ng of template DNA. The cycle program included an initial 1 min denaturation at 94^{o}C, followed by 36 cycle of 15 sec at 94^{o}C, 30 sec at 42 and 1 min at 72^{o}C. PCR product was extended at 72^{o}C for 7 min final RAPD fragments were separated electrophorically on 1-5% agarose gel in 1 x TBE buffer, stained with ethidium bromide and photographed on a u.v transilluminator using a digital camera.

The similarity between parental inbred lines were determined according to Jaccard's (1908) Similarity coefficient as follows:

$$Sij = 2\,M/(\varepsilon i + \varepsilon j)$$

Where: M = number of matching band, εi = Total number of band in the first lan

and εj = Total number of band in the second lan.

The main objectives of this study were to estimate the level of polymorphism among five maize genotypes i.e. inbred lines S7 and S61(The parents of Single cross10) as well as Single Cross 10 (SC10) from three different sources for verification of varietals purity in maize seeds**.**

RESULTS AND DISCUSSION

In this connection, RAPD–PCR protocol plays a major role in many of the processes that affect many things. Random primers are used in these reactions and they are very useful. When little or no information is known about the species in animals and plant applications the utilization of RAPD–PCR reactions would be useful. The data obtained in Tables 2 and 3 and Figure 1 showed that with the primer A03 many specific bands appeared in genotype No.4 with molecular size of 1327 b.p., 1033 b.p. with genotypes No.1, 2 and 4, 904 b.p. with genotype No.5, 245 b.p. with genotypes No.3 and No.4, 200 b.p. with genotypes No.3 and 4. On the other hand, there are nine common bands which appeared in all genotypes. These bands were No.4, 5, 6, 7, 8, 9, 10 and 11.These bands have molecular sizes of 727, 595, 538, 487, 412, 360, 331 and 280 b.p., respectively. The bands which have molecular sizes of 1033, 245 and 200 b.p. were present in more genotypes. There is one band which has molecular size of 1327 b.p. appeared in one genotype No.4 and one band which has molecular size of 904 b.p. appeared in one genotype No.5. The obtained results were in common agreement with the results obtained by Drinic *et al* (2004) and Fayed (2009).

Table 2: RAPD primer PCR analysis with primer A03.

Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13
MW	1327	1033	904	727	595	538	487	412	360	331	280	245	200
Genotype 1	*	1033	*	727	595	538	487	412	360	331	280	*	*
Genotype 2	*	1033	*	727	595	538	487	412	360	331	280	*	*
Genotype3	*	*	*	727	595	538	487	412	360	331	280	245	200
Genotype4	1327	1033	*	727	595	538	487	412	360	331	280	245	200
Genotype 5	*	*	904	727	595	538	487	412	360	331	280	*	*

Genotype 1: Inbred line S7.
Genotype 2: Inbred line S63.
Genotype 3: S.C.10 Controlled produce with researcher.
Genotype 4: S.C.10 Produce with researcher according the traditional method for hybrid seed production.
Genotype 5: S.C.10 commercial seeds.

Table 3: Similarity degree using Jaccardi's Coefficient using primer A03 for studied five maize genotypes.

Genotypes	1	2	3	4	5
1	-	1	0.84	0.86	0.89
2	-	-	0.84	0.86	0.89
3	-	-	-	0.91	0.84
4	-	-	-	-	0.76

The obtained results from RAPD–PCR analysis with B05 primer obtained in Tables 4 and 5 and Figure 2 showed that many specific bands appeared in genotypes No.2 and 4 with molecular size of 1280 b.p., 1096 b.p. with genotypes No. 2, 3, 4 and 5, 737 b.p. with genotypes No. 2, 3, 4 and 5, 642 b.p. with genotypes No. 2, 3, 4 and 5, 559 b.p. with genotypes No. 2, 3, 4 and 5. On the other hand, there are four common bands which appeared in all genotypes. These bands were No. 6 ,7, 8 and 9. These

bands had molecular sizes of 454, 376, 316 and 257 b.p. respectively. The bands which have molecular size of 1280, 1096, 737, 642 and 559 b.p. were present in more genotypes. Similar results were reported by Drinic *et al* (2004), EL-Diasty (2007) and Tong-ming *et al* .(2011).

Table 4: RAPD primer PCR analysis with primer B05.

Band No.	1	2	3	4	5	6	7
MW	1327	1033	904	727	595	538	487
Genotype 1	-	-	-	-	-	538	487
Genotype 2	1327	1033	904	727	595	538	487
Genotype3	-	1033	904	727	595	538	487
Genotype4	1327	1033	904	727	595	538	487
Genotype 5	-	1033	904	727	595	538	487

Table 5: Similarity degree using Jaccardi's Coefficient using primer B05 for studied five maize genotypes.

Genotypes	2	3	4	5
1	1	1	0.80	1
2	-	1	0.80	1
3	-	-	0.80	1
4	-	-	-	0.80

The molecular sizes of the obtained bands from RAPD–PCR analysis with B09 primer are found in Tables 6 and 7 and Figure 3, and showed Bands 4,5,6,7,8,9 and 10 which have molecular sizes 1020, 844, 578, 435, 354, 293 and 243 b.p., respectively. Meanwhile bands 1,2 and 3 with molecular sizes 1948, 1513 and 1233 b.p. appeared in all genotypes except inbred line S7 (genotype 1).

All of these results were in agreement with the results obtained by Yu and Nguyen (1994), Drinic *et al* (2004), EL-Diasty (2007) and Tong-ming *et al*. (2011).

Table 6: RAPD primer PCR analysis with primer B09.

Band No.	1	2	3	4	5	6	7	8	9	10
MW	1948	1513	1233	1020	844	578	435	354	293	243
Genotype 1	-	-	-	1020	844	578	435	354	293	243
Genotype 2	1948	1513	1233	1020	844	578	435	354	293	243
Genotype3	1948	1513	1233	1020	844	578	435	354	293	243
Genotype4	1948	1513	1233	1020	844	578	435	354	293	243
Genotype 5	1948	1513	1233	1020	844	578	435	354	293	243

Table 7: Similarity degree using Jaccardi's Coefficient using primer B09 for studied five maize genotypes.

Genotypes	2	3	4	5
1	0.96	1	0.96	0.96
2	-	0.96	0.92	0.92
3	-	-	0.96	0.96
4	-	-	-	0.92

The obtained molecular weights from RAPD–PCR analysis with B11 primer are presented in Tables 8 and 9 and Figure 4. Total number of Bands was 13 bands. The molecular size of these bands ranged between 218-1232 b.p. It could be also noticed that one specific band appeared in genotype No.5 with molecular size of 303 b.p. and two specific bands with genotypes 2 and 4 with molecular size of 218 b.p. On the other hand, there are eleven common bands which appeared in all genotypes. These with molecular sizes of 1232, 1038, 917, 726, 651, 574, 507, 455, 395, 328 and 263 b.p., respectively. The results also cleared that the number of bands obtained in each genotype were ranged between 11–12 bands in each genotype.

Table 8: RAPD primer PCR analysis with primer B11.

Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13
MW	1232	1038	917	726	651	574	507	455	395	328	303	263	218
Genotype 1	1232	1038	917	726	651	574	507	455	395	328	-	263	-
Genotype 2	1232	1038	917	726	651	574	507	455	395	328	-	263	218
Genotype 3	1232	1038	917	726	651	574	507	455	395	328	-	263	-
Genotype 4	1232	1038	917	726	651	574	507	455	395	328	-	263	218
Genotype 5	1232	1038	917	726	651	574	507	455	395	328	303	263	-

Table 9: Similarity degree using Jaccardi's Coefficient using primer B11 for studied five maize genotypes .

Genotypes	2	3	4	5
1	0.62	0.67	0.62	0.67
2	-	0.94	1	0.94
3	-	-	0.94	1
4	-	-	-	0.99

The obtained molecular weights from RAPD–PCR analysis with primer obtained in Tables 10 and 11 and Figure 5 showed that many specific band appeared in genotypes No.1, 2, 3 and 5 with molecular sizes Ranged between 1554 and 518 b.p. On the other hand, there are four common bands which appeared in all genotypes. These bands were No.2, 3, 4 and 5. These bands have molecular sizes of 1203, 1001, 865 and 657 b.p., respectively. The bands which have molecular sizes of 1554 b.p. and 518 b.p were present in most genotypes except the genotype No.4.

Table 10: RAPD primer PCR analysis with primer B16.

Band No.	1	2	3	4	5	6
MW	1554	1203	1001	865	657	518
Genotype 1	1554	1203	1001	865	657	518
Genotype 2	1554	1203	1001	865	657	518
Genotype 3	1554	1203	1001	865	657	518
Genotype 4	-	1203	1001	865	657	-
Genotype 5	1554	1203	1001	865	657	518

Table 11: Similarity degree using Jaccardi's Coefficient using primer B16 for studied five maize genotypes.

Genotypes	2	3	4	5
1	0.82	0.82	0.82	0.82
2	-	1	1	1
3	-	-	1	1
4	-	-	-	1

Genetic similarity matrix among the five studied genotypes was calculated, the genetic similarity matrix ranged 83% to 95% (Table 12). Maximum (96%) and minimum (83%) genetic similarity coefficients were observed in genotypes 3 and 5 and genotypes 1 and 4, respectively. These results agreed with those reported by Williams *et al*. 1990; EL-Diasty (2007) and Fayed, Eman (2009).

All used primers produced polymorphic bands. Before achievement of calculations, unclear bands were deleted and didn't calculated The five RAPD primers produced 51 bands in the five genotypes under study, 17 of them were polymorphic and 34 were monomorphic (Table13). Primers A03 and B05 produced 5 polymorphic bands, meanwhile primers B11 and B16 produced 2 polymorphic bands. Primers with higher polymorphic bands are more efficient in studying genetic diversity and discrimination the genotypes.

Table 12: Proximity matrix among the five maize genotypes based on RAPD analysis.

case	Matrix file input			
	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Genotype 2	0.892	-	-	-
Genotype 3	0.878	0.945	-	-
Genotype 4	0.833	0.946	0.935	
Genotype 5	0.878	0.945	0.956	0.891

Table 13: Levels of polymorphism based on RAPD analysis.

Bands / Primer	Total bands	Polymorphic bands	Monomorphic bands	Polymorphic%
OP-A03	13	5	8	38.5
OP-B05	9	5	4	55.6
OP-B09	10	3	7	30.0
OP-B11	13	2	11	15.4
OP-B16	6	2	4	33.3
Total	51	17	34	33.3

The dendrogram based on RAPD analysis (Figure 6) separated the studied genotypes into two main clusters, the first cluster included all the studied genotypes except genotype 1 While it in a separate cluster. RAPD can identified markers for cultivars 4 and 5. Cultivar 5 has two cultivar-specific RAPD markers at 904, 303 b.p. of primers A03 and B11. While, cultivar 4 has one marker at 1327 b.p. of primer A03. All of these results were in agreement with the results obtained by Tong-ming *et al* (2011) who observed that the band number of the F1 hybrid was exactly equivalent to the number of the common bands and the specific bands of the two parents, Indicating that the difference of band patterns was a genetic trait controlled by the nuclear genes. The F1 electrophoregram could be predicted by those of the two parents. The band pattern of the F1 hybrids was identical with that produced from mechanically mixed extract of the two parent inbreds and this procedure could be used in corn cultivar identification and as a test for genetic purity. Similar results were reported by Drinic *et al*. (2004); EL-Diasty (2007) and Fayed,Eman (2009).

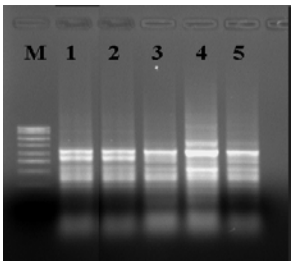

Fig. 1: AO3

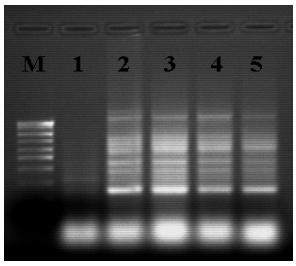

Fig. 2: BO5

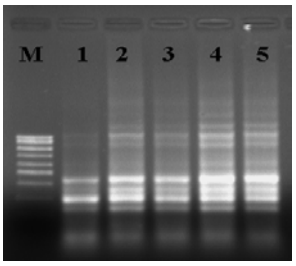

Fig. 3: BO9

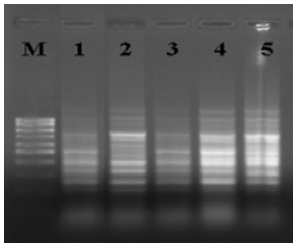

Fig. 4: BO11

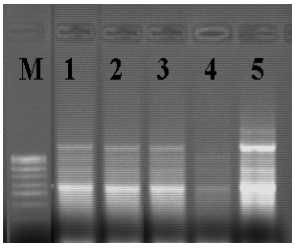

Fig. 5: BO16

Figs. 1, 2, 3, 4 and 5: RAPD finger- printing of the five maize genotypes generated by the five primers (AO3, BO5, BO9, BO11 and BO16).

Lines from left to right: M=Marker (BPL 50 bp. Ladder); Lanes from 1 through 5 are: Genotypes 1, 2, 3, 4 and 5.

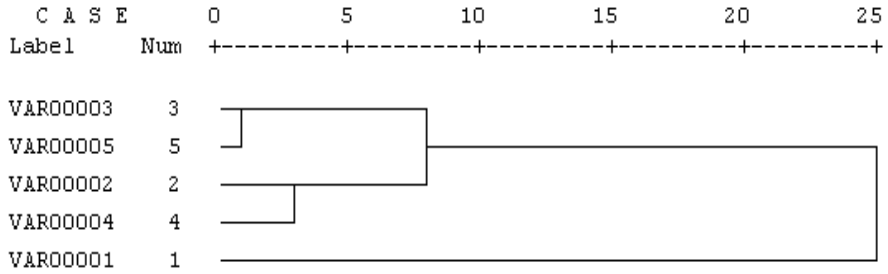

Fig. 6: Dendrogram of the genetic distances between the five maize genotypes based on RAPDs analysis.

CONCLUSION

In conclusion, the set of markers used here provides a positive assessment to the ability of RAPD marker to produce unique DNA profiles of maize genotypes. The data obtained can be used for varietals survey and the construction of a database of all maize varieties grown in Egypt, providing also additional genetic information of the agronomic and quality characteristics of maize varieties.

There are many problems in producing single cross 10 of maize for following reasons:

1- The high genetic proximity between the parental inbred lines about 89%.
2- The genetic purity in the parents is low because the heterozygosity in the female parent is about 5 %, while in the male parent is about 13-17%.
3- The heterozygosity in S.C.10 which produced from three ways is about 4-7%.
4- There is high genetic proximity (95%) between the female inbred line (S7) and S.C.10 which produced from three ways under studies, and low similarity (83-87%) between the male inbred line (S63) and S.C.10 which produced from three ways under studies so there is a heterozygosity because of (the female parent's detasseling isn't completely 100% and pollination is un accomplished, pollen may be carried by the wind from other parent, or both of them) and the high genetic proximity between the parental inbred lines.

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التحقق من النقاوة الصنفية فى تقاوى الذرة الشامية باستخدام تكنيك RAPD–PCR

أحمد نادر السيد عطية* ، محمود إبراهيم العميرى و إيمان على فايد****

*** قسم المحاصيل – كلية الزراعة – جامعة المنصورة - مصر.**

****قسم بحوث تكنولوجيا البذور – مركز البحوث الزراعية – مصر.**

تم استخدام تكنيك RAPD-PCR وذلك باستخدام خمسه بادئات عشوائية للتحقق من النقاوة الوراثية للذرة الشامية (هجين فردى 10) المنتج بطريقتين (التهجين اليدوي و الطريقة العادية لإنتاج الهجن) وكذلك الهجين فردى 10 التجاري. أجريت تجربتان حقليتان خلال موسمي 2009 و 2010 في مزرعة كلية الزراعة جامعه المنصورة، فى الموسم الأول 2009 تم زراعة الآباء وتم تجديد السلالات وإنتاج الهجين الفردي وفى الموسم الثاني 2010 تم زراعة السلالات النقية (الآباء) والهجين الفردي وتم عمل تحليل البصمة الوراثية للخمسة تراكيب وراثية في معامل قسم بحوث تكنولوجيا البذور بالجيزة مركز البحوث الزراعية. ويمكن تلخيص أهم النتائج فيما يلى:-

أظهرت النتائج أن نسبه عدم التشابه الوراثي بلغت 33.3% وأن أعلى نسبه عدم تشابه 55.6% تم الحصول عليها باستخدام المعلمات الجزيئية op-B05 وكان أقلها 15% وذلك باستخدام المعلمات الجزيئية op-B11 .

أعلى نسبة قرابة وراثية 95% كانت بين السلالة الأم S7 والهجين الفردي وتراوحت درجه القرابة بين الأب S63 والهجين الفردي من (83%-87%) وبلغت درجه القرابة الوراثية بين السلالتين الآباء 89%.

قسم التحليل الشجيرى التراكيب الوراثية الى مجموعتين رئيسيتين حسب درجة القرابة شملت المجموعة الأولى التراكيب الوراثية الثانى والثالث والرابع والخامس والمجموعة الثانية التركيب الوراثي الأول.

من خلال هذه الدراسة فإنه يمكن استخدام تكنيك RAPD-PCR للتحقق من النقاوة الوراثية للذرة الشامية وأنه يجب على مربى الذرة الشامية الانتخاب من قاعدة وراثية عريضة وزيادة النقاوة الوراثية للسلالات المستخدمة في إنتاج الهجن.

قام بتحكيم البحث

أ.د / عبد الرحيم عبد الرحيم ليله	**كلية الزراعة – جامعة المنصورة**
أ.د / ابراهيم فتحى مرسل	**مركز البحوث الزراعية**