J. Agric. Sci. Mansoura Univ., 33(5): 3405 - 3411, 2008

FINGER PRINTING FOR SOME MAIZE INBRED LINES THROUGH RAPD-PCR TECHNIQUE

Abd El-Hadi, A. H. ; A. M. El-Adl ; Kawther S. Kash and M.Z.M. El-Diasty

Genet. Dept., Fac. of Agric., Mansoura University, Egypt.

ABSTRACT

In this investigation 11 inbred lines of maize were used. These inbred lines were crossed among them to obtain 30 F_1 hybrids according to factorial mating design. DNA finger printing was made by using RAPD-PCR for all 11 maize inbred lines. The two primers, XD_8 and XD_9 were used in this technique. The results showed similarity between inbred lines. The results also revealed the presence of three common bands between all inbred lines at primer XD_8, while five common bands were obtained at primer XD_9. Cluster analysis for similarity degree between the 11 parental inbred lines was done and showed similarity degree between the within inbred lines. Phylsgenetic analysis showed high similarity coefficient between the inbred lines 6, 4 and 2, 3. However, there were high genetic distances obtained between inbred lines No: 4, 5, 6, 7 and 11.

INTRODUCTION

Mackill *et al*. (1996) found that the 21 RAPD primers produced 103 bands of which 43 were polymorphic. In the same time, Lanza *et al*. (1997) evaluated the genetic diversity of 18 maize inbred lines. They also determined the correlation between genetic distance and single-cross hybrid performance. They used RAPD0PCR technique with 32 primers. They indicated that cluster analysis divided the samples to three distanict groups. Prevast and Mipkinson (1999) concluded that ISSR-PCR provides a quick, reliable and highly informative system for DNA finger printing. Phillip *et al*. (2000) used of random amplified polymorphic DNA (RAPD) markers for evaluating seed purity. They stated that genomic DNA isolated from single unregimented seed was found to be suitable for RAPD analysis. They illustrated that the RAPD data showed that the parental lines were not very closely related. Shieh and Thseng (2002) evaluated the genetic diversity of 13 maize inbred lines and determined the correlation between genetic distance and single cross hybrid performance. They employed DNA (RAPD), PCR technique. They indicated that 13 inbred lines of maize could be classified into distinct heterotic groups. There was no significant heterosis values of grain dry weight. McGregor *et al*. (2006) found that several DNA marker systems and associated techniques are available are available for finger printing plant germplasm. They also investigated that PCR based DNA fingerprinting. They also added that techniques differ in the mean number of profiles generated per primer (or primer pair) per cultivar.

MATERIALS AND METHODS

Genomic DNA extraction from leaves of mays were conducted according to Laroy and Leon (2000). Amplification of genomic DNA was made on perken Elmer DNA cycler using arbitrary December primers, XD_8

and XD_9 which are presented in Table 1. The genomic DNA amplification using RAPD-PCR were made according Changxin *et al*. (2003). The similarity between parental inbred lines were determined according to Jaccard's (1908) similarity coefficient as follows:

$$S_{ij} = 2\ M/(\varepsilon_i + \varepsilon_j)$$

Where:

M ; number of matching band.

ε_i : total number of band in the first lan.

ε_j : total number of band in the second lan.

Table 1: Oligonucleotide primers used in the study.

Primer	Sequence (5` ⟶ 3`)	GC %
XD_8	GAAGGCATCC	60
XD_9	GAAGTGGTCC	60

RESULTS AND DISCUSSION

PAPD-PCR protocol plays a major vole 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 plant applications the utilization of RPD-PCR reactions would be useful. The obtained data in Tables 2 and 3 and figure 1 showed that with the primer XD_8 the total number of band obtained were 15 bands. The molecular size of these bands ranged from 1541.144 b.p. It could be also regarded that many specific bands appeared in inbred line No. 6 with molecular size of 1541 b.p., 1434 b.p. with line No. 3 and 1421 b.p. with line No. 5. On the other hand, these common bands were obtained with band No. 5 and the two bands No. 7 and No. 8. These bands had molecular size of 1071, 722 and 734, respectively.

Table 2: RAPD–PCR analysis of DNA polymorphic using XD_8 primer with different *Zea mays* inbred lines.

Band number	b.p.	1	2	3	4	5	6	7	8	9	10	11
1	1541	•	•	•	•	•	1	•	•	•	•	•
2	1434	•	•	1	•	•	•	•	•	•	•	•
3	1421	•	•	•	•	•	•	1	•	•	•	•
4	1141	•	•	•	•	1	•	1	•	•	•	•
5	1071	1	1	1	1	1	1	1	1	1	1	1
6	1016	•	1	•	•	1	•	1	•	•	1	•
7	772	1	1	1	1	1	1	1	1	1	1	1
8	734	1	1	1	1	1	1	1	1	1	1	1
9	529	1	•	•	1	•	1	•	1	1	•	1
10	513	1	1	•	1	1	•	•	1	1	1	1
11	449	•	•	1	1	•	•	1	•	•	1	•
12	144	1	1	1	•	•	•	•	•	1	•	1
Total		6	6	6	6	6	5	7	5	6	6	6

The results also revealed that the number of bands obtained in each inbred line ranged from 5 to 7 bands. These bands which have molecular size 513, 448 and 144 b.p. were present in more inbred lines.

Table 3: RAPD primer PCR analysis with primer XD_8.

Band number	b.p.	1	2	3	4	5	6	7	8	9	10	11
1	1541	•	•	•	•	•	1541	•	•	•	•	•
2	1434	•	•	1434	•	•	•	•	•	•	•	•
3	1421	•	•	•	•	•	•	1421	•	•	•	•
4	1141	•	•	•	•	1141	•	1141	•	•	•	•
5	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071
6	1016	•	1016	•	•	1016	•	1016	•	•	1016	•
7	772	772	772	772	772	772	772	772	772	772	772	772
8	734	734	734	734	734	734	734	734	734	734	734	734
9	529	529	•	•	529	•	529	•	529	529	•	529
10	513	513	513	•	513	513	•	•	513	513	513	513
11	449	•	•	449	449	•	•	449	•	•	449	•
12	144	144	144	144	•	•	•	•	•	144	•	144

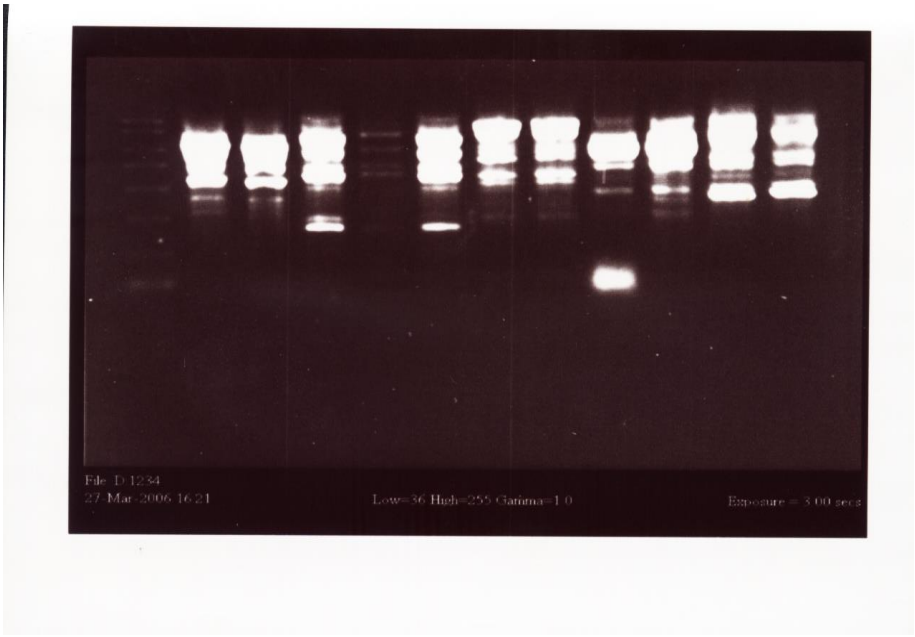

Fig. 1: Agarose gel electrophorasis of PCR amplification f eleven maize inbred lines by XD_8 primer.

Data in Figure 3 showed a similarity degree between inbred lines using cluster analysis. The obtained similarity degree was 0.36 in the first group, while sub groups had high similarity degree (0.6 – 0.88) as soon as phylsgenetic diversity between the different 11 inbred lines examined by inserting RAPD data into Jaccard's similarity matrix and analysed by (lq A) to gave phylsgenetic tree.

The obtained results from RAPD-PCR analysis with XD_9 primer are present in Tables 4 and 5 and Figure 2. The results showed the presence of high similarity between studied inbred lines. There were 16 bands had a range of size between 685 to 132 b.p. The number of common bands between all inbred lines of *Zea mays*, L. were three bands: 610, 414 and 393 b.p. The obtained specific bands were 675 b.p. in the inbred lines 2, 7 and 11. In the same time, specific band with molecular size was 245 b.p. for inbred line No. 2. All of these results wee in agreement with the results obtained by Yu and Nyuyen (1994) who detected similar level between different rice cultivars (80%) of polymorphism in 9 samples gave 260 RAPD fragments. In this respect, Akimato *et al*. (1994) and Buso *et al*. (1998) revealed greater variation between four natural collected using isozyme and RAPD-PCR. Phylsgenetic relationship between and within 11 inbred lines of *Zea mays*, L. led to classify the inbred lines into three major groups depending on the genetic distance between each inbred line and the others.

RAPD analysis using two primers XD_8 and XD_9 were in agreement with Juff *et al*. (1993) who classified cultivars using cluster analysis of RAPD. The obtained data showed good similarity with their results, where they obtained. Minor variation within subgroups was observed for two types of markers. The results indicated that RAPD-PCR technique may be used for QTL mapping different types of plant.

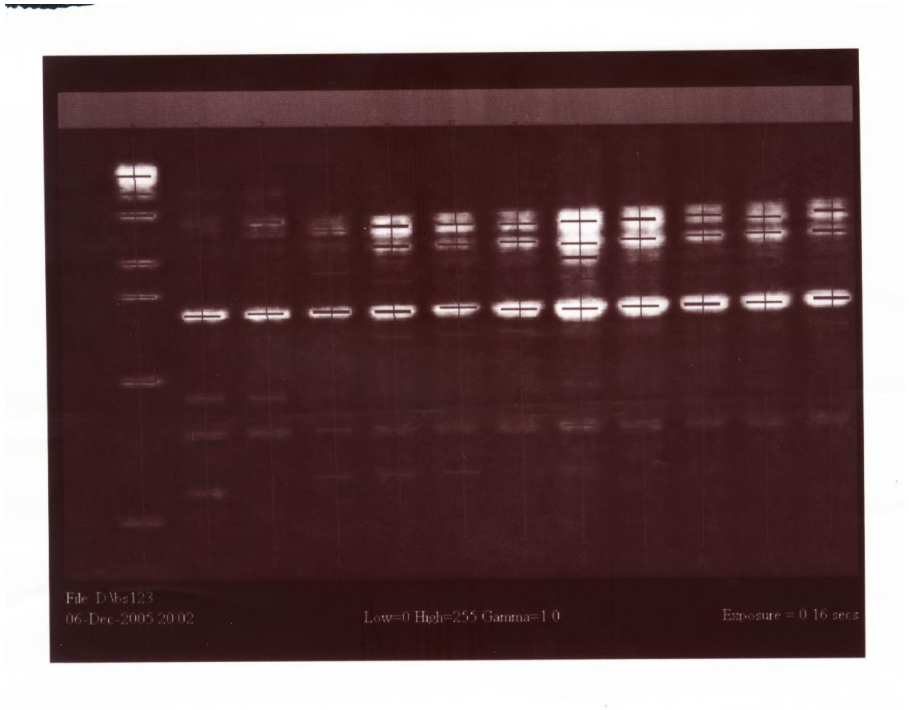

Fig. 2: Agarose gel electrophorasis of PCR amplification of eleven miaze inbred lines by DX_9 primer.

Table 4: RAPD–PCR analysis of DNA polymorphic using XD_9 primer with different *Zea mays* inbred lines.

Band number	b.p.	1	2	3	4	5	6	7	8	9	10	11
1	685	•	1	•	•	•	•	1	•	•	•	1
2	684	•	•	•	1	•	•	•	1	•	•	1
3	653	1	1	•	•	1	•	•	1	•	•	•
4	624	•	•	•	•	•	1	•	•	•	•	•
5	610	1	•	1	1	1	1	1	•	•	1	•
6	593	1	1	•	•	1	1	1	1	•	1	1
7	561	1	1	1	1	1	1	•	•	1	1	1
8	414	•	1	1	1	1	1	1	1	1	•	•
9	401	1	•	1	•	•	1	1	1	1	1	1
10	393	•	1	•	1	1	1	1	1	1	1	•
11	372	1	•	•	1	•	1	•	•	1	•	•
12	295	•	•	1	•	1	•	•	•	1	•	1
13	280	•	•	•	•	•	1	1	•	•	•	•
14	254	•	1	•	•	•	•	•	•	•	•	•
15	132	•	1	1	1	1	1	•	•	1	•	•
Total	132	6	8	6	7	8	10	7	6	7	5	6

Table 5: RAPD primer PCR analysis with primer XD_9

Band number	b.p.	1	2	3	4	5	6	7	8	9	10	11
1	1541	•	•	•	•	•	1541	•	•	•	•	•
2	1434	•	•	1434	•	•	•	•	•	•	•	•
3	1421	•	•	•	•	•	•	1421	•	•	•	•
4	1141	•	•	•	•	1141	•	1141	•	•	•	•
5	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071	1071
6	1016	•	1016	•	•	1016	•	1016	•	•	1016	•
7	772	772	772	772	772	772	772	772	772	772	772	772
8	734	734	734	734	734	734	734	734	734	734	734	734
9	529	529	•	•	529	•	529	•	529	529	•	529
10	513	513	513	•	513	513	•	•	513	513	513	513
11	449	•	•	449	449	•	•	449	•	•	449	•
12	144	144	144	144	•	•	•	•	•	144	•	144

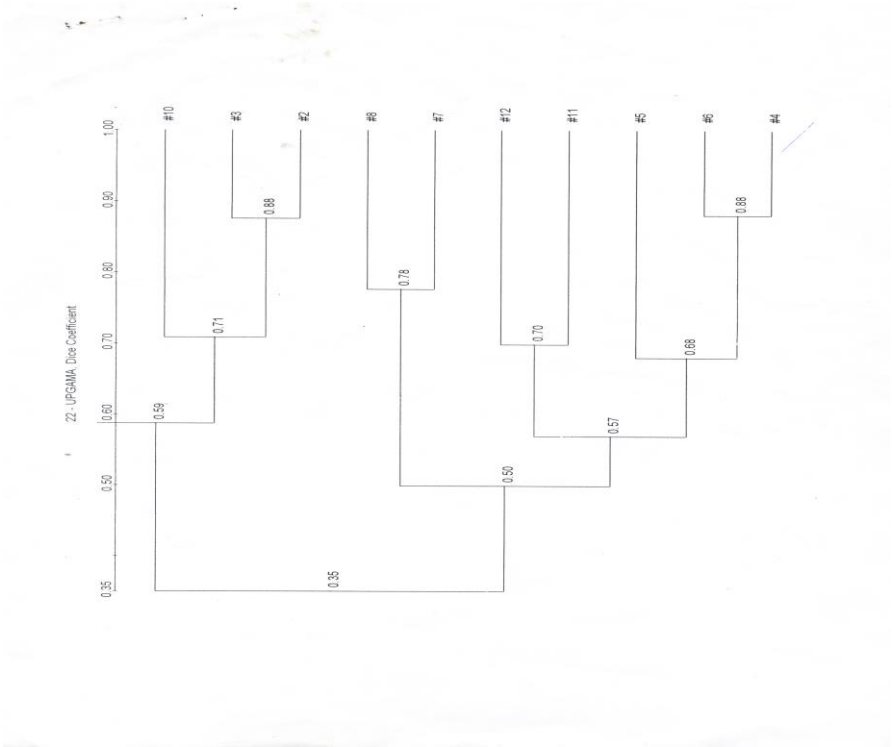

Fig. 3: Phylogenetic analysis of different maize inbred lines using PCR.

REFERECNES

Akimoto, M.; M. Ohara; Y. Shimamoto and H. Morishima (1994). Genetic structure of natural populations of oryza glumaepatula distributed in the Amazon basin. Rice. Res. Newsl., 11: 74 – 75.

Buso, G.S.C.; P.H. Rangel and M.E. Ferreira (1998). Analysis of genetic variability of South American wild rice populations (Oryza glumaepatula) with iso Zymes and RAPD markers. Mol. Ecol., 7: 107 – 117.

Chengxin Fu; Q. Yingxiong and K. Hanghui (2003). RAPD analysis for genetic diversity in changium smyrnioides (Apiaceae), an endangered plant. Bot. Bull. Acad. Sin, 44: 13 – 18.

Jaccard, P. (1908). Nouvelles reserches sur la distribution florale. Bull. Soc. Vaud Sci. Nat., 44: 223 – 270.

Huff, D.R.; R. Peakall and P.E. Smouse (1993). RAPD variation within and among natural populations of out-crossing buffalograss [Buchloe actyloides (Nutt.)]. Theor. Appl. Genet., 86: 927 – 934.

Lanza, L.L.B.; C.L. de Souza Jr.; L.M.M. Ottoboni; M.L.C. Vieira and A.P. de Souza (1997). Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. J. TAG Theoretical and Applied Gene., 94(8): 1023 – 1030.

Leroy, X.J. and K. Leon (2000). A rapid method for detection of plant genomic instability using unanchored-microsatellite primers. Plant Molecular Reporter 18: 283a-283g.

Mackill, D.J.; Z. Zhang; E.D. Redona and P.M. Colowit (1996). Level of polymorphism and genetic mapping of AFLP markers in rice. J. Genome, 39: 969 – 977.

McGregor, C.E.; C.A. Lambert; M.M. Greyling; J.H. Louw and L. Warnich (2006). A Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum,* L.) germplasm. J. Euphytica. 113(2): 135 – 144.

Phillip, A.C.; P.L. Bhalla, C.K. Lee and M.B. Singh (2000). RAPD analysis of seed parity in a commercial hybrid cabbage (Brassica Oleracea var. Capitata) Cultivar. J. Genome, 43: 317 – 321.

Prevost, A. and M.J. Wilkinson (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. J. TAG Theoretical and Applied Genetics. 98(1): 107 – 112.

Shieh and Thseng (2002Genetic diversity of Tainal-white maize inbred lines and prediction of single cross hybrid performance using RAPD markers.Euphytica 124 :307-313.

Yu, L.X. and H.T. Nguyen (1994). Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa*, L.). Theor. Appl. Genet., 87: 668 – 672.

البصمة الراثية لبعض السلالات النقية فى الذرة الشامية باستخدام تكنيك RAPD-PCR

أشـــرف حســـين عبـــد الهـــادى، علـــى مـــاهر العـــدل، كـــوثر ســـعد قـــش و محمد زكريا محمد الديسطى

قسم الوراثة – كلية الزراعة – جامعة المنصورة – جمهورية مصر العربية٠

تـم عمـل تحليـل البصـمة الوراثيـة لعـدد ١١ سـلالة مـن الـذرة الشـامية باسـتخدام تكنيـك RAPD-PCR وباستخدام عدد ٢ برمبير XD_9, XD_9 واللذان يتميز كل منهما باحتوائه على ٦٠% G/C٠

- أظهرت النتائج وجود درجة عالية من التماثل بين جميع السلالات التى تم استخدامها فى هذه الدراسة٠
- أظهرت النتائج وجود ٣ حزم مشتركة عند استخدام Primer XD_8 و٥ حزم مشتركة عند استخدام Primer $XD_9$٠
- تم تحليل درجة التماثل بين السلالات المستخدمة فى الدراسة باستخدام التحليل العنقودى لجميع السلالات، وقد أظهرت النتائج ارتفاع درجة التماثل بين معظم السلالات المستخدم فى الدراسة٠
- أظهرت نتائج التحليل الشهرى لدرجة القرابة بين السلالات المستخدمة بقسمة السلالات إلى ثلاث مجموعات درجة القرابة٠

وكانت أعلى السلالات فى درجة القرابة هى (٤، ٦) و (٢، ٣)، وأقل السلالات قرابة هى ١، ٢، ٣ مع السلالات ٤، ٥، ٦، ٧، ١١.