

ADVANCEMENT OF AFLP TECHNIQUES IN SOME MAIZE (*ZEAMAYS L.*) LINES*Pál PEPÓ – Zoltán BÓDI*

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Introduction

The use of molecular markers to enhance plant breeding efforts is being widely studied. Even though marker-assisted selection now plays a prominent role in the field of plant breeding, examples of successful, practical outcomes are rare. Garcia et al. (2004) compared relative efficiencies of RAPD, RFLP, AFLP and SSR markers to find the most suitable one for maize diversity studies. The dominant markers (AFLP and RAPD) had small CV values indicating skewed distribution while the co-dominant markers gave high CV values. The number of RFLP and AFLP loci used was enough to give CV values of below 5 %, while the SSRs and RAPD loci gave higher CV values.

In spite of the fact that Random Amplified Polymorphic DNA (RAPD) is suitable for determination of genetic relationship (Carvallo et al., 2004) and selecting herbicide tolerant/resistant maize lines (Szabó and Pepó 1998); increasing attention was paid to survey genetic divergence among maize inbred lines (Marshan et al., 1998). AFLPs detect polymorphisms more efficiently in comparison to RAPD, due to the larger number of loci assayed in a single PCR reaction (Wu and Dai, 2000).

Much research has been done concerning the amount of variation for AFLP markers in maize inbred lines, examined the usefulness of AFLP markers for assigning inbred lines to heterotic groups, compared the genetic similarity (GS) based on AFLP markers (Hajósné Novák et al., 1999; Lübberstedt et al., 2000; Nagy et al., 2000, 2003). Barbasa et al. (2003) found that AFLP molecular marker is efficient in assigning maize lines to heterotic groups and that AFLP-based genetic distance (GD) is suitable for predicting the maize single cross performance for intrapopulation crosses of broad-based populations. AFLP markers were applied in maize to compare genetic distance and hybrid performance (Ajmone et al., 1998; Pejic et al., 1998). The aim of our research was to estimate the genetic divergence/genetic distance using AFLP analysis.

Methods

Maize inbred lines applied were originated from earlier mutation breeding program (Table 1.) It was described by Pepó and Tóth (2003).

Table 1. Maize lines used for AFLP analysis

Lines	Genotypes pedigree	Type of irradiation	Dose (Gy)
UDL 1	F ₁ (NK-PX) M ₂ 1 B-3	Co ⁶⁰ -fn	15-7,5
UDL 4	F ₁ (Pi 3978 SC) M ₃ 23 B-4	fn	5
UDL 5	F ₁ (Pi 3764 MTC) M ₃ 54/2 B-4	fn	5
UDL 6	F ₁ (Pi 3478) M ₂ 82 B2-2	fn	5

Co⁶⁰= γ radiation source, fn = fast neutron (cyclotron)

The genomic DNA of the leaves was extracted following the method described by Hoisington et al. (1994), and was isolated by QIAGEN DNeasy Plant Mini Kit. AFLP analysis profiles were performed as described by Vos et al. (1995). The genomic DNA (400 ng) of each inbred line was isolated and digested simultaneously at 37 °C for 1 hour by the EcoRI and for 20 minutes at 67 °C by MseI adapters, 0,4 mM ATP, 10 mM Tris-HCl pH 7,5, 10 mM Mg-

acetate, 50 mM K-acetate and 1 μ l of DNA ligase (T4). Next, 5 μ l of diluted DNA were amplified in a PTCTM-100 thermocyclator (Programmable Thermal Controller). Amplified products were applied in polyacrylamide gel and submitted to electrophoresis.

Results and discussion

For AFLP analysis we applied 7 primer combinations which were the following: P-C/M-CA, P-T/M-CA, P-A/M-CT, P-C/M-CT, P-A/M-CC, P-A/M CG P-A/M-CA. We had very dense DNA fingerprinting using the PCR-based AFLP techniques. We estimated that the number of polymorph bands totalled 145 which means 21 ones in average for a particular primer combinations. The highest number of bands were found in P-T/M-CA (32) furthermore we found the following polymorphic bands in the following primers: P-C/M-CA (30), P-C/M-CT (26), P-A/M-CC (24), P-A/M-CT (21), P-A/M-CG (18), P-A/M-CA (15).

We had better results using primer combinations. The three AFLP variants amplified 208 bands out of them 70 were polymorphic. The different combinations provided the following results: Mse11-EcoRI 44/128, Mse12-EcoRI 7/38, Mse13-EcoRI 19, which means that in average 23 bands was polymorph out of 69 amplified bands.

Morphological observations are continuing during the whole vegetation period and ecological conditions affect these characters to a large extent. Because of it Smith and Smith (1989) found phonological characters not suitable to estimate genetic distance among inbred lines. The genetic similarity by Veress and Matók (1999) can be established with morphological parameters together with isoenzyme patterns. We got a dendrogram (Figure 1.) based on 28 morphological characters of CPVO-TP/2/2. Our result indicated that there are two clusters: UDL5/UDL6 and UDL1/UDL4 based on their genetic distance.

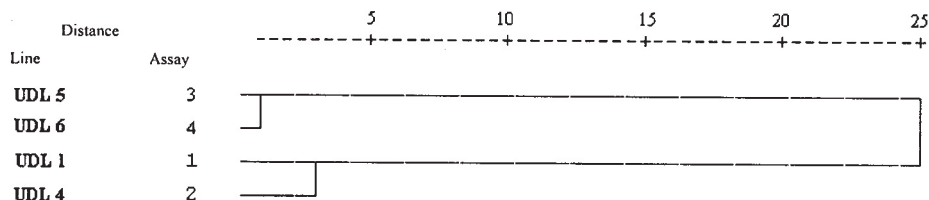


Figure 1: Dendrogram obtained from cluster analysis based on the morphological descriptions (DUS) of four maize inbred lines

We use cluster analysis and the dendrogram - obtained from it - based on the AFLP amplification patterns of four maize inbred lines which is shown in Figure 2.

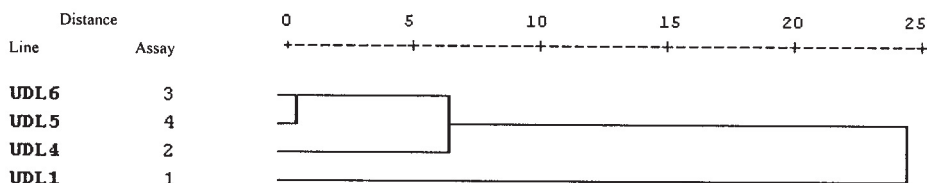


Figure 2. Dendrogram obtained from cluster analysis based on the AFLP amplification patterns of four maize inbred lines.

Based on dendrogram we can cluster two groups: I: UDL1 and II.: UDL4, UDL5, UDL6 which are different from cluster based only in morphological characters. These features are modified by ecological condition but the AFLP based genetic similarity (GS) are not sensitive to ecological factors and can be easily reproduced. The genetic distance (GD) among the four inbred lines based on Jaccard index was the following: UDL4-UDL1:0,75; UDL6-UDL1:0,78; UDL5-UDL1:0,74; UDL6-UDL4:0,59; UDL5-UDL4:0,57; UDL5-UDL6:0,51).

Conclusion

We can conclude that the AFLP markers provide a large number of polymorphic bands. Therefore, by applying a few primer combinations, we can detect the genetic variation. Furthermore we can easily demonstrate the genetic distance using this molecular method. AFLP seems to be most efficient technique for comparing genetic similarities/differences among different genotypes.

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