UDC 575.22: 633.15
Original scientific paper

CHARACTERISATION OF MAIZE INBRED LINES BASED ON MOLECULAR MARKERS, HETEROSIS AND PEDIGREE DATA

Jelena SRDIĆ, Snežana MLADENOVIĆ DRINIĆ, Zorica PAJIĆ and Milomir FILIPOVIĆ

Maize Research Institute „Zemun Polje“ Belgrade, Serbia

Srdić J., S. Mladenović Drinić, Z. Pajić, and M. Filipović (2007): *Characterization of maize inbred lines based on molecular markers, heterosis and pedigree data.* – Genetika, Vol. 39, No. 3, 355 - 363.

Information about the genetic diversity of inbred lines is essential in planning maize breeding programmes. Utilization of diverse parents in the process of hybridization has the greatest influence on producing high yielding hybrids.

The aim of this research was to determine genetic diversity of ten maize inbred lines of different origin on the basis of protein and RAPD markers and to compare these results with pedigree and grain yield heterosis data. Results of genetic distances, based on protein and RAPD markers were similar and in concurrence with the date on the origin of inbreds.

Usefulness of protein and RAPD markers for assigning inbreds to heterotic groups was examined by the cluster analysis. Cluster analysis based on protein markers, RAPD and heterosis showed clear grouping of lines into two main heterotic groups. Only few deviations were noticed,

Corresponding author: Jelena Srdić, Maize Research Institute, Slobodana Bajića 1
11180 Zemun-Belgrade, Serbia , phone: #381 11/ 3756-704, fax: #381 11/3756-707
e-mail: jsrdic@mrizp.co.yu

and those among inbreds not belonging to those heterotic groups. According to the observed results it could be concluded that grouping of inbred lines based on molecular markers, generally agrees with their pedigrees and that clusters are representatives of hetrotic groups. Very high and highly significant estimate of rank correlation coefficient between RAPD and heterosis (0,876**) also confirmed that.

Key word: cluster analysis, heterotic groups, molecular markers, RAPD

INTRODUCTION

Maize breeding programs depend on the understanding and knowledge of genetic diversity and relationship among inbred lines and breeding material. That is especially fundamental in assigning inbreds to heterotic groups and planning outstanding hybrid crosses.

There are several approaches in assessing genetic similarity between breeding material (i.e. inbred lines, hybrids, populations, landraces and races), which include analysis of pedigree and heterotic data, morphological data or molecular data, such as protein and DNA markers (LI et al., 2002).

Developing and selecting inbreds in classical breeding programs and evaluating hybrid performance from extensive yield trials is easy, but also costly and time consuming. It is also not possible to predict hybrid performance from inbred parent performance because of the high level of dominance for the grain yield. Moreover, the large number of possible hybrids produced from relatively small number of inbred parents does not allow the evaluation of all hybrids (HALLAUER, 1990). The use of genetic markers to assess the genetic divergence among pairs of inbred lines has been suggested as a means to overcome these drawbacks, allowing the prediction of single-cross hybrid performance (LANZA et al., 1997).

As specific gene products, proteins could indicate the genetic specificity of tested plant material, and therefore could be used as markers for characterization hybrids and inbred lines, as well as for seed genetic purity testing (KORANYI, 1989; DRINIĆ-MLADENOVIĆ and KONSTANTINOV, 2002). On the other hand these markers should be used only for obtaining preliminary information, while for the more precise results DNA base markers should be used.

The RAPD has been useful technique in studying polymorphism, identifying genes of interest and characterizing genetic resources. RAPD markers are used for characterization of maize inbred lines (HAHN et al., 1995) and hybrids (STOJŠIN et al., 1996).

The objective of this study was to assess genetic diversity based on protein and RAPD markers among ten inbred lines and examine usefulness of molecular markers for assigning inbred lines to heterotic groups.

MATERIAL AND METHODS

The study included 5 medium early and 5 medium late maize inbred lines, originating from *BSSS* (ZPPL 149, ZPPL 225 and ZPPL 15), *Lancaster* (ZPPL 151, 204, 200 and 80), *Wf 9* (ZPPL 148) heterotic groups, and 2 inbreds not related to either of them (B 97 and ZPPL 52). The 10 lines were crossed into 45 crosses in a diallel without reciprocal combinations. The experimental design was randomized complete block design with three replications in three environments. Heterosis was estimated as better-parent heterosis (KRALJEVIĆ-BALALIĆ et al., 1991).

The proteins were isolated from inbred germs according to WANG et al.(1994) and separated by polyacrylamide gel electrophoresis according to LEAMMLI (1970). The genomic DNA was isolated from inbred germs following the protocol of SAGHAI-MAROOF et al. (1984) and RAPD was performed using modified protocol of WILLIAMS et al. (1990). The amplified bands were scored based on 1/0 (presence/absence) system. Genetic distances among all possible pairs of inbred lines were estimated from protein and RAPD data according to NEI and LI (1979). Cluster analysis were carried out on the matrix of genetic distances using the unweighted pair group method with arithmetic averages (UPGMA) clustering algorithm. The dendograms were constructed with NTSYS-pc software (ROHLF, 2000). Correlations between GD and heterosis as well as GD/SCA, both based on protein and RAPD markers were calculated by *Spearman*'s rank correlation coefficient (ZAR, 1999).

RESULTS AND DISCUSSION

The analysis of embryo salt-soluble proteins showed that all studied genotypes had specific protein pattern. A total of 42 protein fractions of different molecular weight were observed and 76% of them were polymorphic. The shortest GD was found between inbred lines ZPPL 204 and ZPPL 200 (0,094). These lines had similar genetic background, with one common parent. Maximum GD was observed between two pairs of inbreds: ZPPL 148 and ZPPL 15, and between ZPPL 148 and ZPPL 52. These results are in agreement with pedigrees of the lines, for the line ZPPL 148 was derived from heterotic group *Wf 9*, ZPPL 15 obtained by recurrent selection from Iowa Stiff Stalk Synthetic and ZPPL 52 is a European dent line (Table 1).

Ten random 10-mer primers from Genosys Biotechnologies were used to amplify fragments from the DNA templates of 10 inbreds. A total of 68 RAPD fragments of different molecular weight were scored, 81% were polymorphic and gave 3 to 11 fragments per primer. The GD calculated from 45 combinations of 10 parental lines ranged from 0,124 in the combination ZPPL 15 and ZPPL 149 up to 0,674 between inbreeds ZPPL 148 and ZPPL 15, which was the same combination that had the maximum GD value based on protein markers. The combination ZPPL

204 x ZPPL 200 that had minimum GD based on protein markers had the second value based on RAPD (Table 2).

Table 1. Genetic distance among inbred lines based on protein markers

Inbreds	ZPPL 148	ZPPL 225	ZPPL 151	ZPPL 204	ZPPL 15	ZPPL 200	ZPPL 80	B 97	ZPPL 52
ZPPL 149	0,231	0,123	0,228	0,213	0,253	0,238	0,241	0,288	0,220
ZPPL 148		0,280	0,255	0,200	**0,359**	0,273	0,231	0,213	**0,359**
ZPPL 225			0,269	0,254	0,161	0,288	0,286	0,298	0,228
ZPPL 151				0,107	0,311	0,167	0,134	0,207	0,214
ZPPL 204					0,290	**0,094**	0,115	0,194	0,258
ZPPL 15						0,323	0,322	0,267	0,200
ZPPL 200							0,115	0,226	0,311
ZPPL 80								0,193	0,236
B 97									0,276

Table 2. Genetic distance among inbred lines based on RAPD

Inbreds	ZPPL148	ZPPL225	ZPPL151	ZPPL204	ZPPL15	ZPPL200	ZPPL80	B97	ZPPL52
ZPPL 149	0.221	0.198	0.320	0.440	**0.124**	0.325	0.272	0.289	0.310
ZPPL 148		0.260	0.391	0.420	**0.674**	0.582	0.632	0.160	0.318
ZPPL 225			0.528	0.532	0.306	0.381	0.425	0.352	0.372
ZPPL 151				0.148	0.438	0.268	0.165	0.330	0.442
ZPPL 204					0.561	**0.150**	0.234	0.432	0.375
ZPPL 15						0.613	0.592	0.421	0.512
ZPPL 200							0.430	0.398	0.578
ZPPL 80								0.415	0.552
B 97									0.173

Cluster analysis based on protein markers grouped inbred lines in two subclusters. These subclusters clearly divided inbreeds by their origin in two major heterotic groups. The subcluster I consisted of genotypes that belonged to *BSSS* heterotic group. The closest link was between lines ZPPL 149 and ZPPL 225, who had inbred ZPPL 15 attached to them. The inbred ZPPL 52- European dent line, was also grouped in this subcluster (Figure 1).

The subcluster II contained inbreds that belonged to *Lancaster* germplasm. Four inbreds of *Lancaster* germplasm were closely linked, among them the closest link was between two lines (ZPPL 204 and ZPPL 200) who had one common parent. Two inbreds B 97 and ZPPL 148, that didn't belong to this heterotic group, were loosely attached to this subcluster. According to previous results these lines showed better performances in combinations with *BSSS* lines, and that indicates their genetically adjacency to *Lancaster* group (Figure 1).

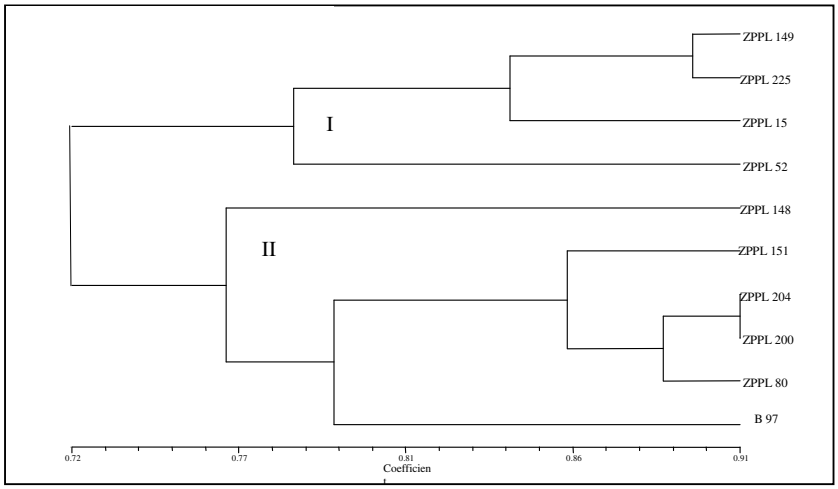

Figure 1. Cluster of GD of 10 inbred lines based on protein markers

The RAPD cluster also divided lines in two groups – *BSSS* lines and *Lancaster* type lines. Three *BSSS* inbreds formed the subcluster I together with the group of three unrelated lines (ZPPL 52, B 97 and ZPPL 148). The classification of these three unrelated lines was the major difference between clustering of GD of ten inbreds based on protein and RAPD markers. Subcluster II with *Lancaster* type inbreds looked mostly the same as the one formed by protein markers (Figure 2).

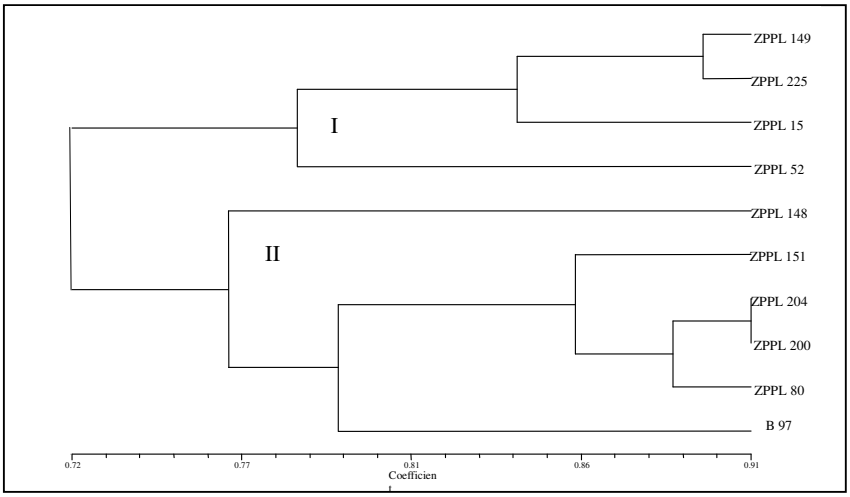

Figure 2. Cluster of GD of 10 inbred lines based on RAPD

The third cluster represents the date of grain yield heterosis. This cluster was performed in order to verify the accuracy of grouping lines into heterotic groups based on GD of inbreds derived by markers. This cluster is almost the same as the RAPD cluster and differs in grouping of unrelated inbreds, from the protein cluster (Figure 3).

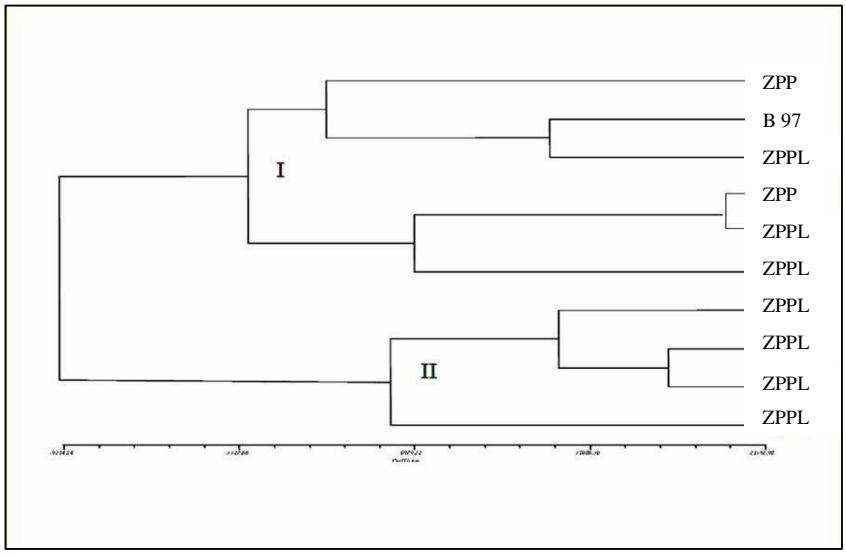

Figure 3. Cluster based on data for grain yield heterosis

The concurrence between the GD determined by both type of markers and heterosis was estimated by *Spearman*'s rank correlation coefficient. While the estimate of on between protein markers and heterosis was highly significant (r_s=0,422**), it was not very strong. The estimate of correlation between RAPD and heterosis was both highly significant and very strong (0,876**). This indicates that there is strong agreement between the genetic distance of inbreds based on RAPD markers and heterosis, while the protein markers could only be used for preliminary screening and grouping inbreds into heterotic groups without predicting hybrid performances.

Comparing the results of the cluster analysis obtained by protein markers and RAPD with the pedigree data of 30 ZP maize hybrids ERIĆ (2004), also determined higher concurrence between RAPD cluster and pedigree date.

Applying the RAPD method, LANZA (1997), reported that genetic divergence can be used to establish heterotic groups, though this method was not very efficient in predicting the performances of single crosses.

One of the major objectives in hybrid breeding is the determination of heterotic pools and simplifying the choice of parent lines for the production of high-yielding hybrids (LÜBBERSTEDT et al., 2000). Therefore molecular marker method offers a reliable and effective mean for assessing genetic diversity within and between maize populations (PEJIĆ et al, 1998; REIF et al., 2003).

CONCLUSION

The results of this study showed that the estimation of the genetic distance between maize inbred lines by different marker methods is in agreement with data on the origin of the inbreds, and also with the grain yield heterosis of their crosses. While the polymorphism of the protein markers could be used as a preliminary method for the characterization of inbred lines, RAPD markers detect larger genetic variability and are therefore more suitable for genetic research.

The DNA based markers represent a powerful tool in the assessment of the genetic diversity between inbred lines. Using them, field trials for the identification of promising heterotic patterns can be planned more efficiently based on the prior obtained information by markers, and that would make a great contribution to the efficiency of maize breeding.

Received April 18th, 2007
Accepted August 17th, 2007

REFERENCES

DRINIĆ-MLADENOVIĆ, S., K. KONSTANTINOV (2002): Korišćenje proteinskih markera za karakterizaciju i utvrđivanje genetičke čistoće semena kukuruza. Selekcija i semenarstvo, *8*, 1-8.

ERIĆ, I. (2004): Genetička karakterizacija ZP hibrida kukuruza (*Zea mays* L.) iz različitih ciklusa selekcije primenom molekularnih markera. Magistarski rad. Biološki fakultet, Beograd.

HAHN, V., K. BLANKENHORN, M. SCHWALL, A.E. MELCHINGER (1995): Relationship among early European maize inbreds: III. Genetic diversity revealed with RAPD markers and comparison with RFLP and pedigree data. Maydica, *40*, 299-310.

HALLAUER, A.R. (1990): Methods used in developing maize inbreds. Maydica, *35,* 1-16.

KRALJEVIĆ-BALALIĆ, M., S. PETROVIĆ, LJ. VAPA (1991): Genetika: teorijske osnove sa zadacima. Poljoprivredni i Prirodno-matematički fakultet, Univerzitet u Novom Sadu, Novi Sad, 322-32.

LANZA, L.L.B., C.L. SOUZA JR., L.M.M. OTTOBONI, M.L.C. VIEIRA, A.P. SOUZA (1997): Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. TAG, *94*, 1023-1030.

LEAMMLI, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, *227*, 680-85.

LI, Y., J. DU, T. WANG, Y.SHI, Y.SONG, J.JIA (2002): Genetic diversity and relationships among Chinese maize inbred lines revealed by SSR markers. Maydica, *47*, 93-101.

LÜBBERSTEDT, T., A.E. MELCHINGER, C. DUßLE, M. VUYLSTEKE, M. KUIPER (2000): Relationships among Early European Maize Inbreds: IV. Genetic Diversity Revealed with AFLP Markers and Comparison with RFLP, RAPD, and Pedigree Data. Crop. Sci. *40,* 783-791.

KORANYI, P. (1989): Simple purity checking of maize lines and hybrids by protein monomer analysis. Seed Sci. & Tehnology, *17*, 161-168.

PEJIĆ, I., P. AJMONE-MARSAN, M. MORGANTE, V. KOZUMPLICK, P. CASTIGLIONI, G. TARAMINO, M. MOTTO (1998): Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. TAG, *97*, 1248-1255.

REIF, J.C, A.E. MELCHINGER, X.C XIA, M.L. WARBURTON, D.A. HOISINGTON, S.K. VASAL, G. SRINIVASAN, M. BOHN, M. FRISCH (2003): Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Sci., *43*, 1275-1282.

ROHLF, F.J. (2000): NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.0 Exeter Software, Setaket, N.Y.

SAGHAI-MAROOF, M.A., K. M. SOLIMAN, R. A. JORGENSON, R.W. ALLARD (1984): Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA *81*, 8014-8018.

STOJŠIN, D., L. W. KANNENBERG, J. RAJNPREHT, P. K. PAUL, R. STOJŠIN (1996): Genetic relationship among commercial corn hybrids and parents based on RAPD analyses of pericarp and embryo DNA. Genetika *28*, 137-150.

WANG, C., K. BIAN, H. ZHANG, Z. ZHOU, J. WANG (1994): Polyacrilamide gel electrophoresis of salt soluble proteins for maize variety identifiction and genetic purity assessment. Seed Sci & Tehnology, *22*, 51-57.

WILLIAMS, J.G.K., A.R. KUBELIK, K.J. LIVAK, J.A. RAFALSKY, S.V. TINGEY (1990): DNA polymorphism amplimfied by arbitrary primers are useful as genetic markers. Nucleic Acid Res. *18*, 6531-6535.

ZAR, J.H. (1999): Biostatistical Analysis, 4th edition. Prenice-Hall, Inc., New Jersey, USA.

KARAKTERIZACIJA SAMOOPLODNIH LINIJA KUKURUZA NA OSNOVU MOLEKULARNIH MARKERA, HETEROZISA I POREKLA

Jelena SRDIĆ, Snežana MLADENOVIĆ-DRINIĆ, Zorica PAJIĆ
i Milomir FILIPOVIĆ

Institut za kukuruz „Zemun Polje“, Beograd, Srbija

I z v o d

Stvaranje visokoprinosnih hibrida kukuruza u direktnoj je zavisnosti od genetičke udaljenosti roditeljskih komponenti, te je za proces selekcije neophodno poznavanje genetičke divergentnosti samooplodnih linija kukuruza.

Cilj ovog rada bio je da se utvrdi genetička divergentnost 10 samooplodnih linija kukuruza različitog porekla na osnovu proteinskih i RAPD markera i da se ti podaci uporede sa podacima o heterozisu i njihovim poreklom. Izračunata genetička distanca na osnovu proteinskih i RAPD markera pokazala je podudarne rezultate, koji su takođe bili saglasnosti sa podacima o poreklu tih linija.

Klasterima na osnovu heterozisa, proteinskih i RAPD markera, linije su grupisane u dve osnovne heterotične grupe. Primećeno je nekoliko odstupanja i to kod linija koje po poreklu nisu pripadale tim heterotičnim grupama. Na osnovu ovoga može se zaključiti da je karakterizacija linija putem podataka dobijenih molekularnim markerima uglavnom u saglasnosti sa podacima o njihovom poreklu i da se na osnovu klastera može dobiti jasna i precizna slika o njihovom klasifikovanju u odgovarajuće hterotične grupe. Ovo je potvrđeno i vrednostima koeficijenta korelacije ranga koji su bili visoko značajni između oba metoda markera i heterozisa, a naročito između RAPD i heterozisa (0,876**).

Primljeno 18. IV 2007.
Odobreno 17. VIII 2007.