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GENETIC CHARACTERIZATION OF EARLY MATURING MAIZE HYBRIDS (*ZEA MAYS* L.) OBTAINED BY PROTEIN AND RAPD MARKERS

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Knowledge of maize germplasm genetic diversity is important for planning breeding programmes, germplasm conservation *per se* etc. Genetic variability of maize hybrids grown in the fields is also very important because genetic uniformity implies risks of genetic vulnerability to stress factors and can cause great losts in yield. Early maturing maize hybrids are characterized by shorter vegetation period and they are grown in areas with shorter vegetation season. Because of different climatic conditions in these areas lines and hybrids are developed with different features in respect to drought resistance and disease resistance. The objective of our study was to characterize set of early maturing maize hybrids with protein and RAPD markers and to compare this clasification with their pedigree information. RAPD markers gave significantly higher rate of polymorphism than protein markers. Better corelation was found among pedigree information and protein markers.

Key words: maize, protein markers, RAPD

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INTRODUCTION

Knowledge of maize germplasm genetic diversity is important for planning breeding programmes, germplasm conservation *per se* etc. Genetic variability of maize hybrids grown in the fields is also very important because genetic uniformity implies risks of genetic vulnerability to stress factors and can cause great losts in yield. In spite of this, consumers are demanding greater product uniformity and the maize farmers also prefer uniform crop fields. Risks of environmental damage may be reduced by use of best performing unrelated hybrids for which are estimation of genetic diversity needed.

Three methods have been mostly used in assesing genetic diversity among maize germplasm: pedigree records, field trials and molecular markers. Molecular markers have several advantages over two other methods: results are obtained faster than in time and space consuming field trials and are more reliable than pedigree records. High discrimination power among similar genotypes is the main reason for use of molecular markers in studies of genetic diversity.

The advent of marker technology designed to detect naturally occuring polymorphisms at the DNA level has become an invaluable tool for both applied and basic diagnostic studies in plant, animal and humane genomes as well as for microorganisms. In past decade there has been an explosion of new DNA-based marker methods utilizing the PCR reaction such as RAPD, VNTR, SSR, AFLP, SNP etc. Other, more specific approaches exploit mitochondrial, chloroplast or ribosomal DNAs. Strategies based on the PCR have the highest potential for routine diagnosis.

Early maturing maize hybrids are characterized by shorter vegetation period and they are suitable for growth in areas with shorter vegetation season. Because of different climatic conditions in these areas lines and hybrids are developed with different features in respect to drought resistance and disease resistance. In colder regions with more rain, lines with flint kernel type are developed, while in wormer regions with arid climate lines with dent kernel type are developed. Maize germplasm of diverse sourses is used in breeding programs of hybrid maize in the Maize Research Institute „Zemun Polje".

The objective of our study was to: (1) characterize maize hybrids by protein and RAPD markers; (2) compare their clasification with their pedigre information.

MATERIALS AND METHODS

For this study we have chosen 11 early maturing hybrids from FAO groups 100-200 which were selected in Maize Research Institute „Zemun Polje": ZP TC 105, ZP TC 110, ZP TC 125, ZP TC 150, ZP TC 192, ZP TC 196, ZP TC 198, ZP TC 209, ZP TC 243, ZP TC 244, ZP TC 260. Genetic characterization and genetic diversity assessment were conducted using protein and RAPD markers. The extraction of salt solubile proteins from embryo tissue was performed (Wang *et al*., 1994). Proteins were separated by gel electrophoresis (SDS-PAGE)

(LEAMMLI, 1970). The genomic DNA was isolated from embryo tissue following the CTAB procedure (ROGERS and BENDICH, 1988). RAPD-PCR amplification was performed with 15 RAPD primers (Genosys Biotechnologies) (WILLIAMS, 1990). Primers which gave clear and reproducible patterns of amplification are shown in table 1.

RAPD fragments were separated on 1.4. % agarose gels in 0.5x TBE buffer. Presense/absense of protein fractions or DNA fragments was transformed to binary data (0,1). The computing of binary data including coefficients of similarities and UPGMA clustering was performed using NTSYS-pc software (ROHLF, 2000).

Table 1. RAPD primers used in this study which gave clear and reproducible bending patterns

RAPD primers	5'-3' sequence
GEN 2-80-3	ACCCGTCCCC
GEN 2-80-1	GCAGCAGCCG
GEN 2-80-7	GCAGGTCGCG
GEN 2-80-5	CGAGACGGGC
GEN 2-80-8	GGCCACAGCG
GEN 2-80-9	GCACGTGAGG
GEN 2-80-10	CGCGAACGGC
GEN 4-70-8	GAGAGGGAGG
GEN 4-80-4	GGACCGCTAG
GEN 2-80-2	CGACGGGTGC

RESULTS

The analysis of embryo salt solubile proteins showed that each studied genotype had a unique protein pattern (refers to number of protein fractions and their gel position). Totally 43 protein fractions of different molecular weight were observed which of 55.8 % were polymorphic. Quantitative differences in concentration of protein fractions were not further analised. Presense/absense of protein fractions was transformed to binary data and coefficient of similarity (JACCARD, 1908) was calculated. The Jaccard's coefficient of similarity was in range from 0.69 for hybrids ZP 105 and ZP 243 to 0.93 for hybrids ZP 192 and ZP 196. The protein based dendogram obtained from similarity matrix according to Jaccard is presented in figure 1. On the upper side of cluster there is a subcluster with four hybrids joined. Hybrids with very similar parental lines are joined together, as ZP TC 192 and ZP TC 196 which show higher genetic similarity, and ZP TC 198 and ZP TC 150 with two of three parental components incommon. Also, two hybrids with two of three parental components incommon ZP TC 110 and ZP TC125 which are very similar and from the same sourse form separate group. All hybrids are three way crosses which all have one parental line F2 in common accept ZP TC 243 which clustered separately and was loosely agregated with other hybrids.

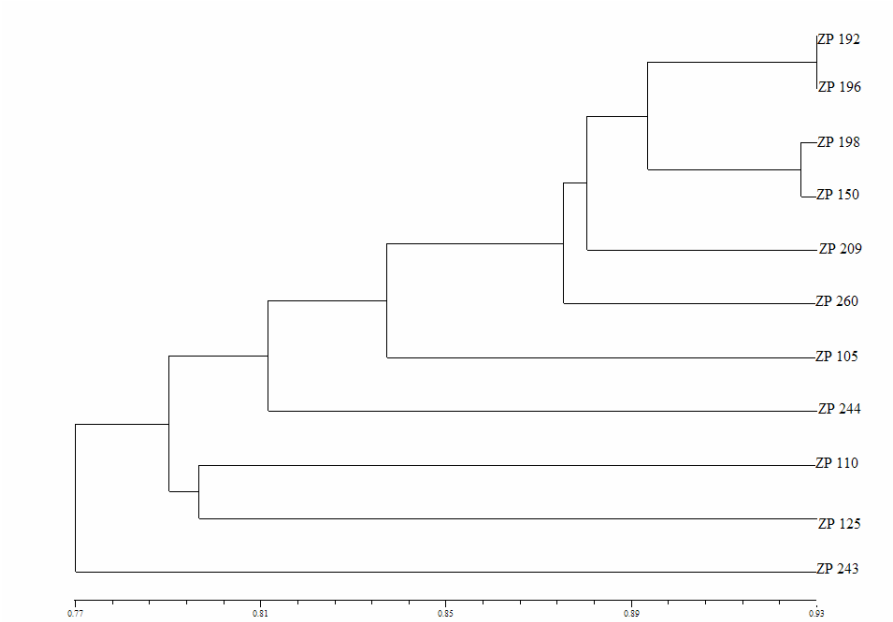

Fig. 1. Protein based dendogram of investigated early maturing hybrids obtained by UPGMA clustering

PCR amplification of genomic DNA was tested on 15 RAPD primers in two rounds of amplification which of 10 primers gave clear and reproducibile banding paterns. PCR amplification with 10 RAPD primers gave totally 69 RAPD fragments of different molecular weight, which of 78.2 % were polymorphic (in presence/absence of bands, while there were not significant diferences in intensity of bands). Number of fragments obtained with different primers was in range of 5-9, average number was 6.9.

Based on presense/absense of RAPD fragments coefficient of similarity was calculated (DICE, 1945). The genetic similarities based on Dice's coefficient were in range from 0.61 for ZP 192 and ZP 110 to 0.91 for two pairs of hybrids ZP 192 and ZP 198. The RAPD based dendogram obtained from similarity matrix is presented on Fig. 2. Cluster consists of three distinct subclusters which show good separation of hybrids. RAPD grouping shows moderate agrement with their pedigre data.

DISCUSSION

Genetic diversity of maize germplasm is important for planning breeding programmes, conservation of maize germplasm *per se* etc. It is also important that farmers have opportunity to choose among hybrids one that will give highest yields and be able to answer to environmental stess due to their existing genetic diversity (TROYER *et al*., 1983).

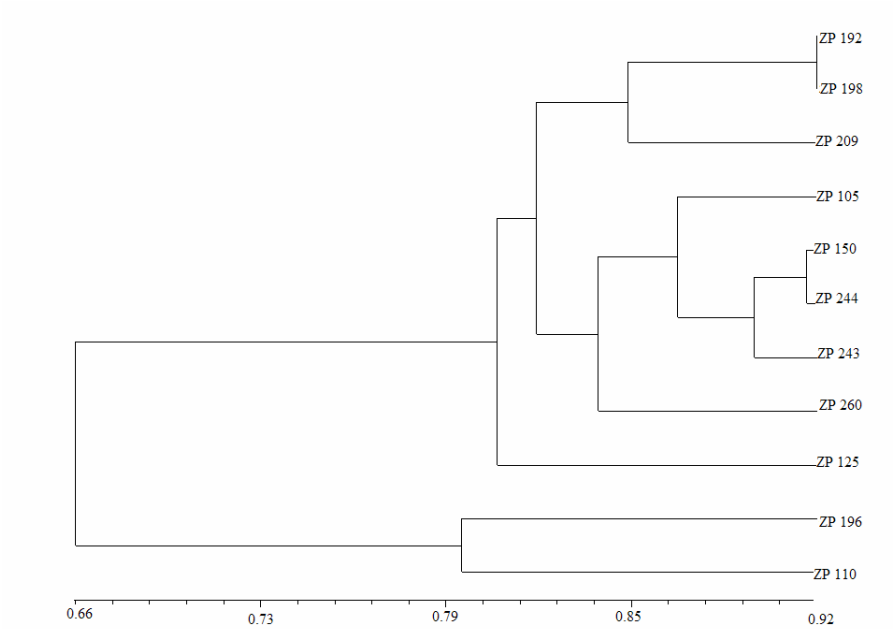

Fig. 2. RAPD based dendogram of investigated early maturing hybrids obtained by UPGMA clustering

In our study we used protein and RAPD markers for characterization of maize hybrids and evaluation of their genetic diversity. Maize embryo salt-solubile proteins are suitable for characterization of maize hybrids as shown previously (ERIĆ *et al*., 2003). All studied genotypes were differentiated from each other on the basis of protein fractions polymorphism. This is in agreement with results of several authors who used seed proteins for characterization of maize hybrids and inbred lines from different geografical regions as well as different cotton cultivars (WANG *et al*., 1994; ZHANG *et al*., 1998; GONZALES *et al*., 2001). Clustering of hybrids based on embryo salt-solubile protein markers showed good agreement with their pedigre data, because hybrids with similar parental components were joined together in smaller groups. In our study dendogram of hybrids based on embryo salt-solubile protein markers showed better agreement with their pedigre data than on RAPD data. One possible reason for this is a small number of RAPD markers used which doesnt neccesarily represent variability of the entire genome.

RAPD technology has been used successfully for measuring diversity of plants, and the patterns of variation observed have been shown to closely resemble those obtained using more classical characters (BRUMMER *et al*., 1995; BEEBE *et al*., 2000; CASLER *et al*., 2003; DIABY and CASLER., 2003). RAPD method was originaly developed for identification of clones (fingerprinting), and its use in genetic analysis of maize began somewhat later (AJMONE-MARSAN *et al*., 1993). The advantages of RAPD essay include ease and rapidity of analysis, the use of a general set of universal random primers for DNA amplification and their requirement

for minimal substrat DNA. RAPD can efficiently generate both randomly dispersed markers as well as markers linked to specific genes. Constrain of the tehnique is its reproducibity, which difficults possibility of interchanging results in and between laboratories (JONES *et al*., 1998). It is important to confirm reproducibility of results for every change of reaction conditions and primers. Problems related with reproducibility of RAPD can be resolved by rigorous attention to detail.

RAPD markers were suitable for genetic characterization of hybrids and for assessing relatedness among them, as documented previously (ERIC, 2004). RAPD analysis with 10 random primers gave totally 69 fragments which of 78.2 % were polymorphic. These results concerning rate of polymorphism are in agreement with results of several other authors. (STOJŠIN *et al*., 1996) compared 28 commercial corn hybrids grown in Canada and found that 80 % of RAPD fragments from embryo DNA were polymorphic. Also, study of 57 elite corn inbred lines which were used in production of hybrid corn in Central and Northwestern Europe with 54 RAPD primers (HAHN *et al*., 1995) showed that 84% RAPD fragments were polymorphic. Slight differencies in level of RAPD marker polymorphism in different studies can be atributed to different hybrids and lines chosen for the analysis and differnt regions of genome that were amplified by selected primers.

Embryo salt-solubile protein markers are not a marker sistem of choise for assesing genetic relatedness of maize hybrids (they have been used for their characterization and genetic purity assessment), because of a relatively low number of protein fractions obtained, low polymorphism and unknown mode of inheritance. For percise assessment of genetic relatedness among maize hybrids and lines it is well documented usage of DNA based markers (AJMONE–MARSAN *et al*., 1998; BERNARDO *et al*., 2000). RAPD markers have been used extensively for maize fingerprinting. Due to their reproducibility problem they have been replaced with SSR markers which are currently the system of choise for maize genetic diversity studies due to their high rate of polymorphism, reliability, cost and time effectivness (GETHI *et al*., 2002; LEE *et al*., 2002; REIF *et al*., 2003).

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GENETIČKA KARAKTERIZACIJA RANIH HIBRIDA KUKURUZA (*ZEA MAYS* L.) PRIMENOM PROTEINSKIH I RAPD MARKERA

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I z v o d

Poznavanje genetičke varijabilnosti germplazme kukuruza je važno zbog planiranja programa selekcije, konzervacije germplazme *per se*. Genetička uniformnost uključuje rizik od velikih gubitaka u prinosima zbog izuzetne osetljivosti ovakvih useva na stresne faktore sredine. Rani hibridi se odlikuju kraćim vegetacionim periodom i namenjeni su za gajenje u područjima sa kraćom vegetacionom sezonom. Zbog različitih klimatskih uslova u ovim područjima stvorene su linije i hibridi sa različitim osobinama u pogledu otpornosti na sušu i bolesti lista, stabla i klipa. Cilj rada je bila karakterizacija ranih hibrida različitog porekla primenom proteinskih i RAPD markera. U ovom radu RAPD markeri su pokazali znatno viši nivo polimorfizma, dok su proteinski markeri pokazali bolje slaganje sa pedigre podacima ispitivanih hibrida.

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