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Molecular Genetics / Molekularna genetika
Ratar. Povrt. / Field Veg. Crop Res. 47 (2010) 409-416
review article/ pregledni rad

Application of Genetic Markers in Seed Testing and Plant Breeding

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received / primljeno: 02.03.2010. accepted / prihvaćeno: 10.05.2010.

Summary: Genetic markers have been used at Institute of Field and Vegetable Crops in Novi Sad for a number of years, both for seed quality control and for research purposes. The Laboratory for Seed Testing was the first in the former Yugoslavia to use the method of control of hybrid seed genetic purity based on enzymatic polymorphism. This paper presents the application of protein markers, isozymes, seed storage proteins and DNA markers for evaluation of seed and breeding materials of various agricultural crops in Serbia.
Key words: DNA markers, isozymes, seed storage proteins

Protein Markers

Genetic marker is a gene or a part of DNA having a readily recognizable phenotypic expression, which is used for identification of the entity or cell that contains it or as a probe for nucleus, chromosome or locus marking (King & Stansfield 1990). It is typically defined as a characteristic that marks a part of a genome.

As specific gene products, proteins could indicate the genetic specificity of tested plant material, and therefore could be used as markers for characterization of varieties, for seed purity testing, or to resolve taxonomic relationships (Drinić-Mladenović & Konstantinov 2001, Nikolić et al. 2008).

Seed Storage Proteins

Seed storage proteins in cereals, such as gliadins in wheat, hordeins in barley, zeins in corn and avenins in oats are used for identification of species (Wrigley et al. 1985). Identification of species can also be performed indirectly by comparing the frequencies of protein components obtained by electrophoretic separation.

Gliadins are one of the major protein fractions, which are deposited in endosperm protein bodies of grain. Gliadins represent efficient and reliable genetic markers in wheat genetic study. Composition of gliadin components for each cultivar was specific (Đukić et al. 2005). Analysis of 10 Kragujevac wheat cultivars identified only 3 alleles (a, b, k) at the Gli-A1 locus, while 5 alleles were identified in cultivars from Novi Sad (Kneževi ć 1992). The pronounced polymorphism of the reserve proteins in the cereal species from the family *Gramineae* may be used for the study of species identity, heterogeneity and origin as well as in the breeding of new cultivars (Nikolić et al. 2007a) (Fig. 1).

Composition of high-molecular-weight glutenin subunits (HMW) affects bread making quality. Eighteen bread wheat cultivars developed at Institute of Field and Vegetable Crops in Novi Sad were analysed for two main x-type alleles which code for HMW glutenins 2 and 5, and two main type alleles which code for 10 and 12 HMW glutenin subunits, present at Glu-D1 locus. Among the analysed cultivars, 55.6% expressed the presence of 1Dx5 and 1Dy10 alleles at Glu—D1 locus, which are typically associated with high dough strength and good bread making quality (Vapa et al. 1995, 2003).

Together with glutenins, gliadins play an important role for bread making quality. Five gliadin blocks encoded by different alleles at Gli-D1 locus and four gliadin blocks different at Gli-A1 locus were clearly expressed and identified. Variability of determined block components

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This research results from project 20081: Stvaranje genotipova uljane repice (*Brassica napus* L.) za ishranu i industrijsku preradu (2008-2010) financed by Ministry of Science and Technological Development of the Republic of Serbia

indicates the existing polymorphisms of gliadins alleles (Knežević et al. 2007).

The cultivars developed in the first 20 years of intensive wheat breeding in Serbia showed high variability at the Glu-B1 locus, similar frequencies of different GSs (N, 1, 2*) at the Glu-A1 locus and the prevalence of GS 2 + 12 over GS 5 + 10 at the Glu-D1 locus. Significant changes occurred in the combinations of HMW GSs as a consequence of 40 years of breeding. The increase in gluten structure stability and appropriate combinations of high molecular weight glutenin subunits have contributed to the improvement of other quality indicators (Hristov et al. 2010).

Seed storage proteins are reliable markers in studying domestication and dispersal of bean cultivars as well as in analysing phylogenetic relationships among species in the genus *Phaseolus*. Most of the bean cultivars grown in our country have the S type phaseolin, which indicates that Central American germplasm has been used for the development of new cultivars (Nikolić et al. 2007b) (Fig. 2). Considering the similarities in climatic conditions between Serbia and Bulgaria, i.e. high temperatures and irregular rainfall, it seems reasonable to accept the explanation that the cultivars with S type phaseolin are well adapted to the prevailing environmental conditions (Genčev et al. 2002).

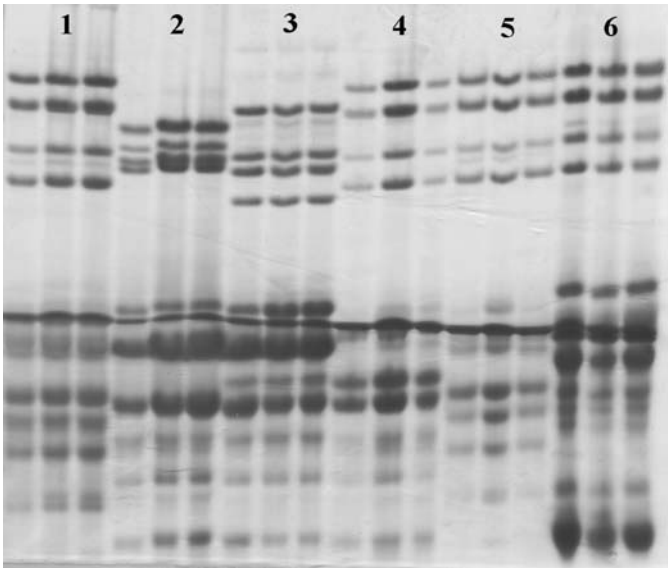

Figure 1. Identification of barley cultivars (samples 1-6) based on seed storage protein polymorphism (Nikolić et al. 2007a)
Slika 1. Identifikacija sorti ječma (uzorci 1-6) na osnovu polimorfizma rezervnih proteina semena

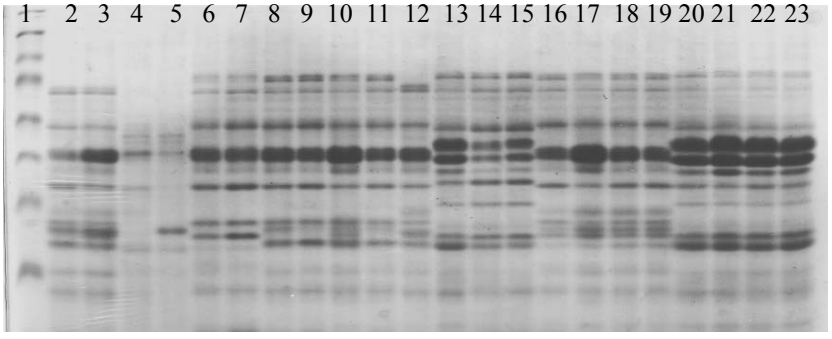

Figure 2. Different types of phazeolin obtained by SDS PAGE electrophoresis:1 - protein marker (170-11kDa), 2-3 - S type phazeolin control, 4-5 - C type control, 6,7 S type control, 8-11 - C-20, 12-15 - Aster, 16-19 - Ludogorje, 20-23 - Jovandeka (Nikolić et al. 2007b)
Slika 2. SDS PAGE elektroforetska analiza fazeolina: 1- proteinski marker (170-11kDa), 2-3 –S tip fazeolina (kontrola), 4-5 –C tip fazeolina (kontrola), 6,7 S tip fazeolina (kontrola), 8-11 - C-20, 12-15 - Aster, 16-19 - Ludogorje, 20-23 - Jovandeka

Protein seed analysis confirmed low level of genetic diversity in soybean genotypes. In the plant with a narrow genetic base in their pool, such as soybean, protein markers may not be sufficient for characterization and study of genetic diversity (Nikolić et al. 2005).

The utility of embrio salt soluble proteins to characterize maize inbred lines, validate pedigree and show association among inbred lines, was evaluated using a set of inbred lines (Drinić et al. 1996). Clustering of hybrids based on embryo salt-soluble protein markers showed good agreement with their pedigree data, because hybrids with similar parental components were joined together in smaller groups. Embryo salt-soluble protein markers are not a marker system of choice for assessing genetic relatedness of maize hybrids because of a relatively low number of protein fractions obtained, low polymorphism and unknown mode of inheritance (Erić et al. 2003).

Based on SDS – PAGE electrophoresis of different pepper varieties (*Capsicum annuum* L.) it was found that the seed protein profile could be useful in the study of phylogenetic relationships. The analysis of soluble seed proteins showed that all studied genotypes had a specific protein pattern (Zečević et al. 2002).

Isozymes

Izozymes are reliable indicators of genetic purity of various plant species. They are especially suitable for solving problems in seed production and processing, such as inadvertent mixing of seed of hybrids and lines, incomplete pollination in corn, etc. (Gerić et al. 1989).

Corn is the plant species with the largest number of genetic markers on the protein level. Isozymes have been used long in various corn studies: assessment of diversity and variability of breeding material (Zlokolica et al. 1995, Nikolić et al. 1999, Nikolić et al. 2004), identification of combining ability potential in corn lines (Zlokolica et al. 1997), etc.

Isozyme polymorphism has been found to be suitable for assessment of genetic uniformity and/or variability of sunflower inbred lines (Zlokolica et al. 1996a) and for identification and registration of lines and hybrids (Carrera & Poverene 1995). Long-term studies of sunflower breeding materials have shown that the following enzymic systems are polymorphic in most lines and hybrids: phosphohexose isomerase (PHI), phosphogluco dehydrogenase (PGD), malate dehydrogenase (MDH) and phosphoglucomutase (PGM) (Fig. 3). The electrophoretic method allows extraction of storage proteins from sunflower seeds and making of helianthin electrophoregrams.

The comparative analysis of genetic purity level of the sunflower hybrids have shown that the methods of electrophoresis of isozymes and seed storage proteins were in agreement, but due to low polymorphism levels seed storage proteins and isozymes cannot be used for genetic identification of sunflower hybrids (Nikolić et al. 2008).

An analysis of sugar beet haploids obtained by gynogenesis and dihaploid lines obtained by colchicine treatment has indicated that the haploid genome from an unfertilized egg cell is stable. Changes in the loci for phosphoglucomutase

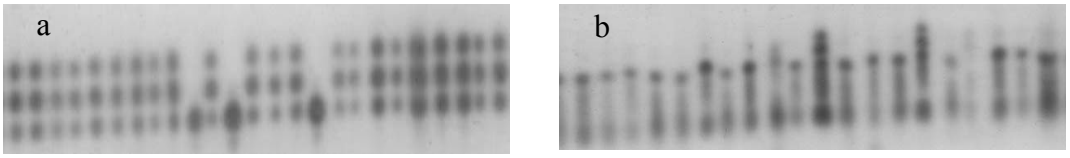

Figure 3. PHI (a) and PGD (b) polymorphisms detected in the sunflower cultivar Monarch (Nikolić et al. 2008)
Slika 3. Polimorfizam PHI (a) i PGD (b) nađen u hibridu suncokreta Monarch

In seed production, maintenance of genetic purity requires continual monitoring of seed quality. Here it is important to add that each crop (corn, soybean, sunflower, etc.) has its specific features and requirements with regards to field history, purity standards and production technology. In the course of time, a cultivar may lose its identity due to various reasons, such as mechanical mixing with other seeds, natural crossing with other species, genetic mutations, genetic drift, selection pressure, etc.

(*Pgm2)* and malate dehydrogenase (*Mdh1)* occurred in response to colchicine application (Zlokolica et al. 1994) or in response to Ti plasmid transformation of sugar beet calli (Zlokolica et al. 1996b).

High degrees of isozymic polymorphism have been found during identification and genetic characterization of vegetable crops such as cabbage, cauliflower, cucumber, carrot, tomato

and pepper (Zlokolica et al. 1996c). Different phenotypic profiles within a cultivar are an indication of seed non-uniformity, i.e. of genetic impurity. An analysis of cabbage breeding material has indicated uniformity in some lines and presence of different phenotypic profiles in others (Nikolić et al. 2007c) (Fig. 4 and Fig. 5).

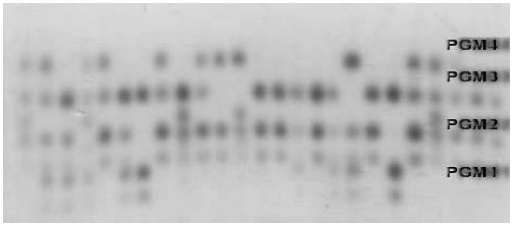

Figure 4. PGM zymogram of the cultivar NS-14 (Nikolić et al. 2007c)
Slika 4. PGM zimogram sorte kupusa NS 14

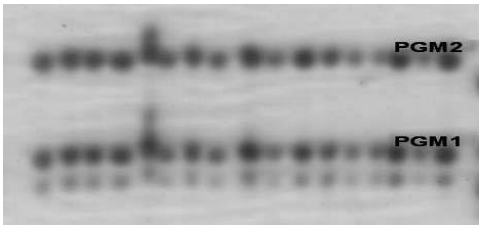

Figure 5. PGM zymogram of the cabbage line (Nikolić et al. 2007c)
Slika 5. PGM zimogram linije kupusa

The expression of isozymic loci is codominant and it is generally independent of environmental factors. Most isozymes come to expression in seed or in early stages of germination, which is a major advantage of these markers over field or greenhouse tests which require a considerably longer time.

DNA Markers Used in Agricultural Research

The numerous shortcomings of the morphological and biochemical markers found in studies of various plant species encouraged the development of molecular markers. Molecular markers have many advantages compared with morphological markers, robustness to environmental change, nearly unlimited number and relative ease and rapidity of data collection (Lombard et al. 2000).

Molecular markers are not influenced by environmental factors and are also fast, efficient and more sensitive than field testing for detection of large numbers of distinct differences between genotypes at DNA level (Smith & Smith 1992). They are applicable in the process of identification and approval of cultivars, determination of genomic differences between cultivars and genetic diversity assessment. During registration of cultivars, molecular markers provide additional information which helps distinguish between similar cultivars. Each cultivar or hybrid should have an ID card that would include descriptors of morphological traits and specific combinations of polymorphic molecular markers (Nikolić 2002) (Fig. 6).

The marker assisted selection (MAS) permits simultaneous improvement and acceleration of breeding process. Implementation of MAS in conventional breeding programs could allow assessment of the genetic potential of specific genotypes prior to their phenotypic evaluation.

The random amplified polymorphic DNA (RAPD) marker system has been used in many different applications involving the detection of DNA sequence polymorphism, but most often in construction of linkage maps and in bulk segregant analysis for identification of markers linked to genes of interest, such as gene(s) for resistance to sugar beet cyst nematode (*Heterodera schachtii* Schmidt) (Nagl et al. 2007).

The study of actual rapeseed breeding material of Southeastern Europe has shown that PCR-based techniques such as RAPD can be successfully used for detecting genetic variability in rapeseed. The simplicity of the technique makes it particularly suitable for breeding programs in which a large number of lines need to be analyzed (Marjanović-Jeromela et al. 2009).

Genetic diversity studies using DNA finger printing techniques have become simple and efficient in detection of sufficient polymorphisms in various crop species including maize (Pejic et al 1998). The main goal of the study conducted by Bauer et al. (2007) was to assess temporal changes in genetic diversity over the past four decades among ZP maize hybrids within the largest planting area in Serbia. Patterns of RAPD markers were unique for each studied genotype, but there was no significant change in genetic variability of hybrids throughout the periods. Genetic distances of ten maize inbred lines of different origin, based on protein and RAPD markers, were similar and in concurrence with the date on the origin of maize inbred lines and also with grain yield heterosis of their crosses (Srdić et al. 2007).

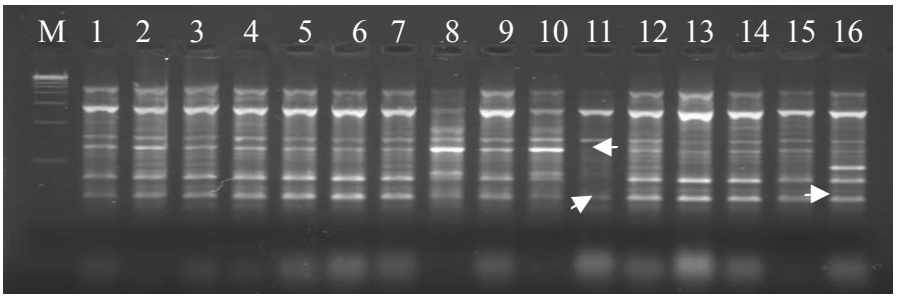

Figure 6. RAPD polymorphism in 16 soybean genotypes obtained with primer no.12 (Nikolić 2002)
Slika 6. RAPD polimorfizam 16 genotipova soje sa prajmerom broj 12

Drinić et al. (2002) reported that simple sequence repeats (SSR) markers provide an effective method for predicting hybrid performance and heterosis. SSR analyses have been used in order to develop genetic fingerprints for their characterization, identification and classification, as well as for estimation of their genetic diversity of local populations from Maize Research Institute gene bank collection. Restriction Fragment Length Polymorphisms (RFLP) and RAPD analysis of some of the local populations for identification of variability and duplicate accessions were performed and the results between different marker techniques were consistent (Ignjatović-Micić et al. 2003, Ignjatović-Micić et al. 2008).

lar markers can be successfully applied to investigate diversity of sunflower inbred lines, wild sunflower species, as well as to identify interspecies hybrids, for some important agronomic traits such as drought tolerance and resistance to downy mildew (Saftić-Panković 2007). The results on genetic variability between 15 populations of each *H. giganteus* and *H. maximiliani*, obtained with SSR markers, confirm clustering pattern between examined populations obtained after analysis of 30 morphological traits (Saftić-Panković et al. 2004).

The inheritance of the reaction of sunflower to downy mildew race 730 was investigated by Panković et al. (2007). The mapping data indicate that several dominant markers and two CAPS

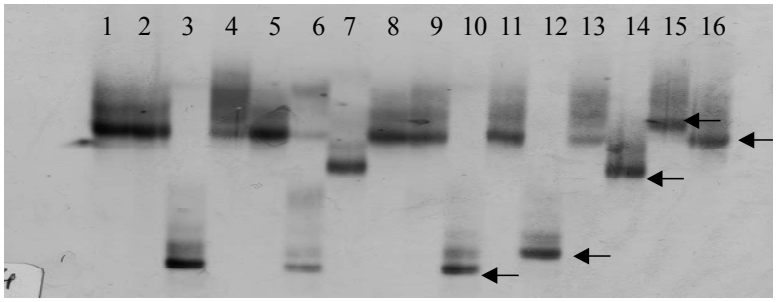

Figure 7. SSR polymorphism detected by primer Satt 534 in 16 soybean genotypes (Nikolić et al. 2006)
Slika 7. SSR polimorfizam 16 genotipova soje sa prajmerom Satt 534

For genotypes with a relatively narrow genetic basis, such as soybean, DNA markers offer possibilities to differentiate related genotypes as well as to identify those possessing specific germplasm. In the case of incomplete data on origin, molecular markers may provide data on mutual relationships between genotypes (Nikolić et al. 1998, Nikolić et al. 2006) (Fig. 7).

In the fields of plant breeding, molecular markers are powerful tools in search for new sources of variation and the analysis of genetic factors that control quantitative traits. Molecu-

markers, developed, completely co-segregate with the Pl6 gene conferring resistance to race 730, the most abundant race in this region of north Serbia. CAPS markers will facilitate efficient marker-assisted selection for sunflower resistance to downy mildew race 730.

Correlation between SSR based genetic distance of new sunflower inbred lines and heterosis for six agronomic traits indicated that better results are obtained if hybrid combinations for each tester and each trait are analyzed separately (Gvozdenović et al. 2009).

Potential uses of microsatellite markers in molecular evaluation of bread making quality were tested in wheat genotypes that were analysed with 3 microsatellites linked to previously mapped QTLs for loaf volume and Hagberg falling number on chromosome 3A. A significant association was found of a specific allele at the GWM674 locus with Hagberg falling number in wheat (Obreht et al. 2006).

The microsatellites are very effective molecular markers for the assessment of genetic diversity in wheat. Combined with multiplex PCR, microsatellite markers permit the fast and high-throughput fingerprinting of large number of genotypes (Kondić-Špika et al. 2008). They are easy to use and automate, mapped to specific genomic location and relatively inexpensive. Using STSs and SSR markers, the most important major height reducing genes, Rht-B1b, Rht-D1b and Rht8 were evaluated in wheat genetic core collection (Kobiljski et al. 2006, Tošović-Marić et al. 2008).

Conclusions

Genetic markers can be used routinely in crop breeding programs, for genetic diversity analysis, cultivar identification, phylogenetic analysis, characterization of genetic resources and association with agronomic traits.

Identification of genes and molecular markers underlying these agronomic traits will help accelerate the breeding process and lead to varieties with improved quality, yield, tolerance to unfavourable environmental conditions and resistance to diseases.

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Primena genetičkih markera u kontroli kvaliteta semena i oplemenjivanju biljaka

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Izvod: Genetički markeri se dugi niz godina koriste u Institutu za ratarstvo i povrtarstvo u Novom Sadu u kontroli kvaliteta semena i u istraživačke svrhe. Laboratorija za ispitivanje semena je prva u bivšoj Jugoslaviji uvela metod kontrole genetičke čistoće semena zasnovan na polimorfizmu enzima. U radu je dat pregled primene proteinskih markera, izoenzima i rezervnih proteina semena, kao i DNK markera u oceni kvaliteta semena i materijala za oplemenjivanje različitih biljnih vrsta u Srbiji.

Ključne reči*:* DNK markeri, izoenzimi, rezervni proteini semena