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## IDENTIFICATION OF GENETIC CHARACTERISTICS OF MAIZE (*Zea mays* L.) USING GENETIC MARKERS

**ABSTRACT:** Different genetic markers are used for estimation of breeding material, its characteristics and potential for ultimate aim — heterosis of hybrids. They also point out to the qualitative seed traits at the level of linkage with genes responsible for desirable agronomic traits. This program encompasses testing methodologies for the new seed technology. Genetic analysis of breeding material during certain phases is comprised of isozymic gene expression and degrees of their variability, but it is continued (in order to be evaluated) until determination of presence or absence of some genes, existing or introduced for certain traits. Using combination of different molecular methods such as PCR, RAPD, and AFLP based on polymorphism of DNA fragments, the definite aim — identification of newly created products of improvement is achieved. Testing of traits of breeding material, its genetic variability and diversity is the first stage in analysis of the maize genome. It is also the condition for determination of presence of certain genes, used for obtaining the ultimate aim — attest of identity of the genotype.

**KEY WORDS:** Maize (*Zea mays* L.), biochemical-molecular markers, isozymes, PCR, RAPD, SSR, AFLP, RFLP, heterosis.

### INTRODUCTION

Genetic identification of corn is necessary in the process of improvement as well as in the estimation of its quality. The source breeding material (Gene-pool), and generations included in the improvement program (self-pollination, and development of homozygous lines), and finding of parent components and their crossing must be included in the above mentioned. The result of these processes of maize improvement is the final product with certain agronomic traits, which genetic characteristics should be identified.

Biochemical investigations are included in the improvement program and analysis of qualitative seed traits. The biochemical-molecular markers are isozymes, expression of genes, and DNA sequences leading to complete genome coverage giving the genetic basis of quantitative and qualitative traits of breeding products. The introduction of the necessary methods on which deter-

mination of genetic diversity and relationship between European maize inbreds are given in the results of H a h n et al. (1995), where lines were of different origin, tested by ranging of methods starting from isozymes, via molecular methods with polymerase chain reaction (PCR) of DNA as a basis. Identification of QTL<sub>s</sub> using molecular markers has a key approach for interpretation of genetic basis of these traits, and their improvement (S t u b e r et al., 1992). The chromosome location of 76 isozymes and RFLP markers linked with QTL<sub>s</sub> for grain yield was shown. The development of maize inbred lines and their origin can be interpreted using molecular markers and these results can be used for fundamental and applied studying of maize (L e e , 1994). Description of inbred lines is given on the basis molecular polymorphism of the nuclear genome of maize, including several methods, ranging from cytology, via components of storage and functional proteins-enzymes, to basic molecular methods (PCR) and those leaning on the PCR.

Some of the physiologically different quantitative traits concerning maize development and its yield are mapped using molecular markers. So, the putative associations of developmental genes generally coincide with the location of homeotic genes (K h a v k i n and C o e , 1997). Drought-stressed maize plants are tested using genetic map with different RFLP loci, on the level of leaf abscisic acid (ABA) concentration in maize (T u b e r o s a et al., 1998).

Biotechnology of corn includes biochemical-genetic-molecular markers at the level of protein, RNA, and DNA respectively. They are used for gene mapping for desired traits, and the ultimate goal is genetic identification of genotype being tested and developed using method of breeding.

Maize is an excellent representative of open-pollinated plant species with present polymorphism of enzyme systems, allelic variability of isoenzymic loci, which is the crucial parametar in identification of material in the final stage of plant improvement process. Applicative side of genetics comprises new technologies in plant breeding with genetic engineering included. The food production is primary goal besides improvement of maize plant production, development and usage of genetic potential. New biotechnology methods make possible to develop the plant genotypes with new traits at the level of recombinant DNA.

Usage of molecular markers is being introduced into a basis of genetic researches by which all components of breeding are connected and have a key role in genetic, biochemical, physiological, and molecular basis of heterosis (S m i t h and B e a v i s , 1996).

The aim of this review is to give the pathway of using biochemical-molecular markers in genetic identification of breeding products.

## MATERIAL AND METHODS

Maize, as an open-pollinated plant species plays special role at biotechnological and molecular level in determining the linkage genes for all physiological-genetic important agronomic traits. Biochemical-molecular markers based

on protein polymorphism, fragments of RNA and DNA have the advantage in determining this relationship.

PCR — based DNA markers are:

Random Amplified Polymorphic DNA (RAPD<sub>s</sub>)  
Simple Sequence Repeat (SSR) or Microsatellite  
Amplified Fragment Length Polymorphism (AFLP)

Various methods are used to characterize plant varieties, lines and their hybrids, they include pedigree, agro-morphological and cytogenetic data, calculations of genetic distance, which is the condition for heterosis. Recent molecular technologies provide a tool for „fingerprinting” germplasm. These data may be required to provide sufficient information for the classification of populations or groups of lines in order to study genetic structure and genetic diversity of genotypes.

Fingerprinting of genotypes is particularly applied especially in the case of crops, such as maize in which hybrid breeding is a lucrative business. It could provide ways of estimating genetic distances between lines, and so genetic diversity existing within and between breeding populations and lines. In the case of maize, fingerprinting profiles may determine the heterosigosty between lines in the process of improvement and their crossing, which is at the same time the information concerning their genetic background.

## RESULTS AND DISCUSSION

Genetic markers, isoenzymes, are components of protein, which are gene expression, and sequences of RNA and DNA can be used as a molecular markers. The complete coverage of genome is achieved by combination of these methods making real identification of the observed genotype possible. Components of storage and functional proteins are direct gene products freed from environmental factors, and as markers participate in genetic estimation of breeding and seed material (Zloková et al., 1996). Biochemical and molecular markers are involved in pedigree data as a valuable source of information about genetic relationships among maize inbreds, which are at the base of certain isoenzymes, such as the genetic variability of lines, originating from domestic populations, and their hybrids (Fig. 1, Tab. 1). Allelic variability of genes was used for identification of genetic distance and cluster analysis. Corn inbred lines (78) originating from domestic populations are very different. Genetic distances range from very small to very big, and according to them the observed lines are grouped i.e. separated. Mapped protein loci were unevenly and insufficiently distributed in all plant species, so the genome coverage was not complete. They should be accompanied by techniques at DNA level, such as PCR, RAPD, SSR, RFLP, and by some others which are complementary. Their combinations are given full taxonomic, genetic, and physiologic information of genotypes.

Tab. 1. — Enzyme systems used to characterize maize hybrids

Enzyme system	Locus	Chromosomal location
Aconitase	Aco 1	4
Acidphosphatase	Acp 1	9L
Alcohol dehydrogenase	Adh 1	1L
Arginine aminopeptidase	Amp 1	1
Diaphorase-glucosidase	Dia 1	2
Diaphorase-glucosidase	Dia 1	4
Glutamate oxalacetate transaminase	Got 1	3L
Glutamate oxaloac trans.	Got 1	5L
Hexokinase	Hex 2	6L
Isocitrate dehydrogenase	Idh 1	8L
Isocitrate dehydrogenase	Idh 2	6L
Malatedehydrogenase	Mdh 1	8L
Malatedehydrogenase	Mdh 2	6L
Malatedehydrogenase	Mdh 3	3L
Malatedehydrogenase	Mdh 5	5S
Glucosidase	Glu 1	10L
Phosphoglucomutase	Pgm 1	1L
Phosphoglucomutase	Pgm 2	5S
6-phosphogluconate dehydrogenase	Pgd 1	6L
6-phosphogluconate dehydrogenase	Pgd 2	3L
Phosphohexose isomerase	Phi 1	1L

The polymerase chain reaction (PCR) is one of the basic methods of molecular markers such as RAPD, SSR, AFLP, RFLP. PCR includes extraction, sequencing, and separation of amplified DNA fragments, and is related to usage of randomly selected, or specific oligo-nucleotides (primers), used for detection of variability of DNA fragments and their amplification. Maize seed was analyzed on the basis of 3 random primers A, B, C and one specific 35S primer (D), of which different amplification of DNA fragments between genotype groups, but not between analyzed genotypes for individual primers was achieved (Fig. 2). The identification of species can be initiated by PCR method which is the basis for new molecular markers such as SSR (Simple sequence report), AFLP (Amplified fragment length polymorphism), and indirectly for RFLP (Restriction fragment length polymorphism) used for marking most of the desirable genes for monogene and polygene traits such as quality, quantity, resistance etc.

Genetic distances between genotypes are highly correlated with the distances based on known pedigrees, and the distances based on isozymes and RFLP are also highly correlated with coefficient of parentage and with the distances between inbred lines. Genetic similarity among hybrids is related by pedigree (Smith and Smith, 1992).

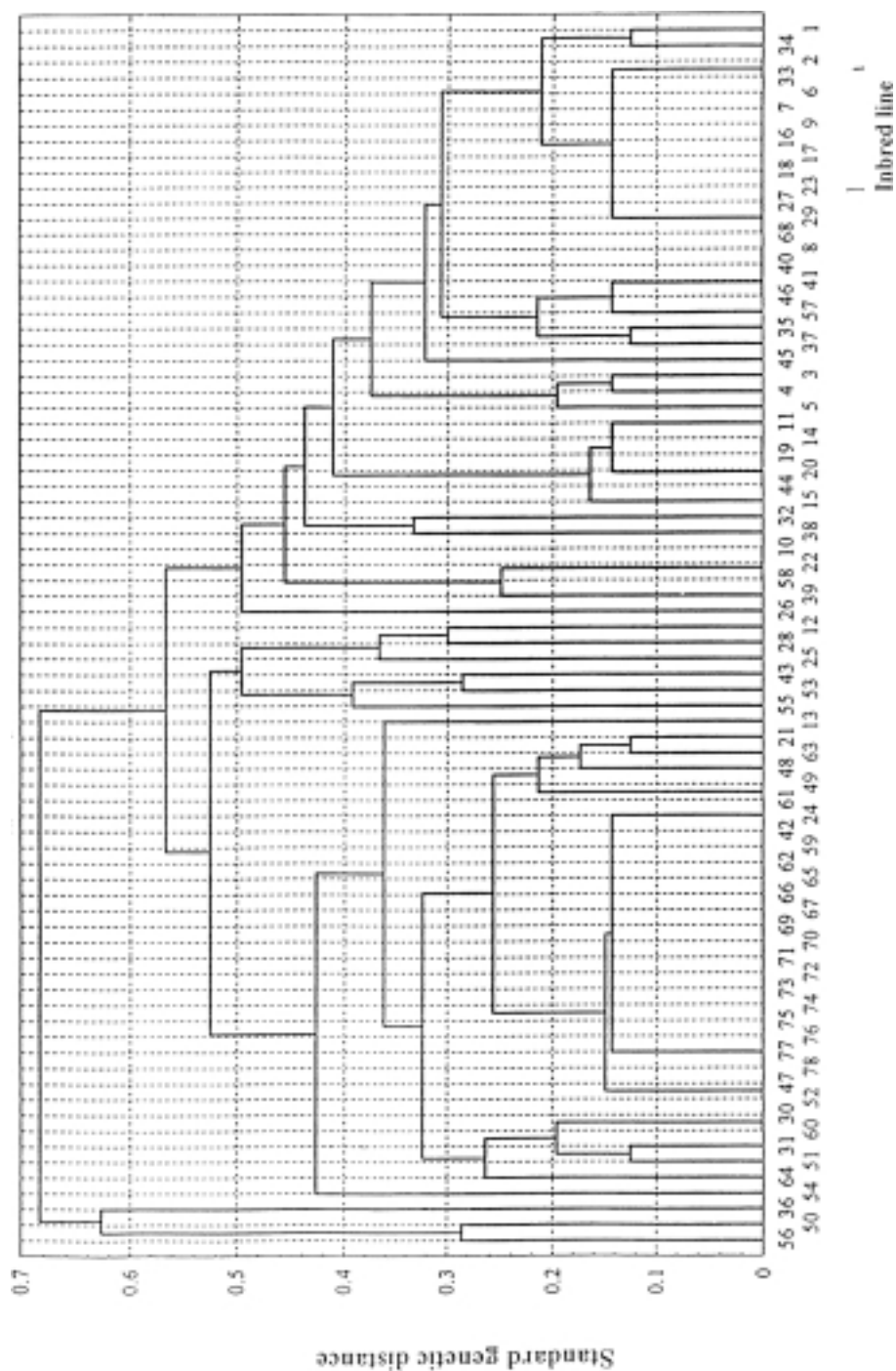


Fig. 1. — Cluster analysis of corn inbred lines (78) originating from domestic populations

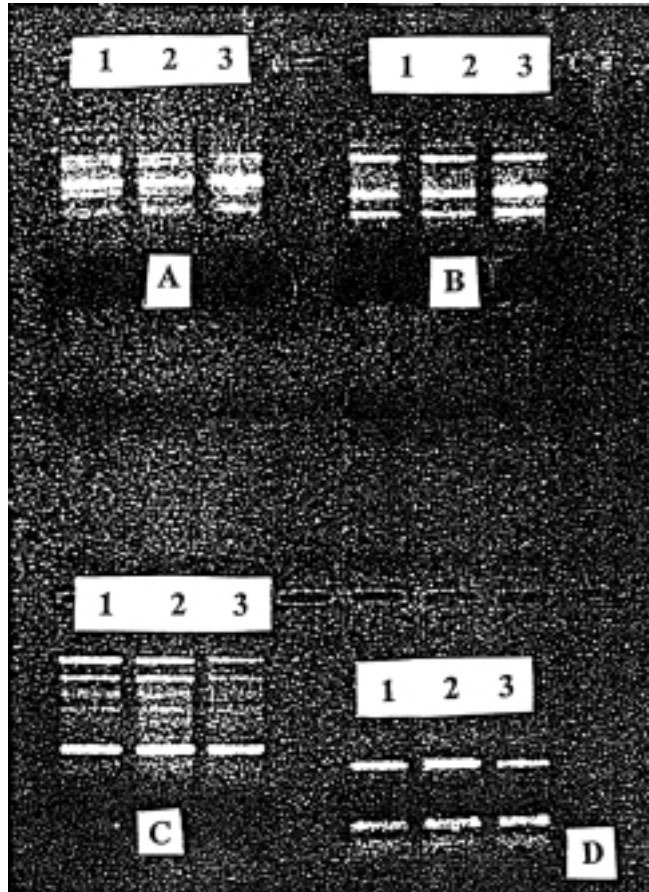


Fig. 2. — RAPD polymorphisms derived from whole corn seed using three random primers 1 (A), 3 (B) and 15 (C) as well as one specific primer for 35S promoter (D). From left to right: 1—178 genotype, 2—182 genotype, 3—186 genotype

Simple sequence repeats (SSR<sub>s</sub>), microsatellites are highly polymorphic markers and due to that are highly informative in plants. Their methodology approach is based on PCR, and they are codominant inherited. They are consisted of tandem repeated DNA sequences with the core sequence of 2—5 bp in eucaryotes genome, where they are uniformly distributed within genome (Ak-kaya et al. 1995, Rongwen et al. 1995). SSRs can show the difference between close relatives and they useful in determination of breeding material (Mudge et al., 1997). These markers have some advances such as large polymorphism, they are codominant, and fast according to PCR reaction, and equally distributed along the genome.

Combination of different molecular markers (isozymes, RFLP, SSR) is the best way to mark a trait of genotype, and they can be used for QTLs detection in segregating maize populations, derived from exotic germplasm (Kozumplik et al. 1996).

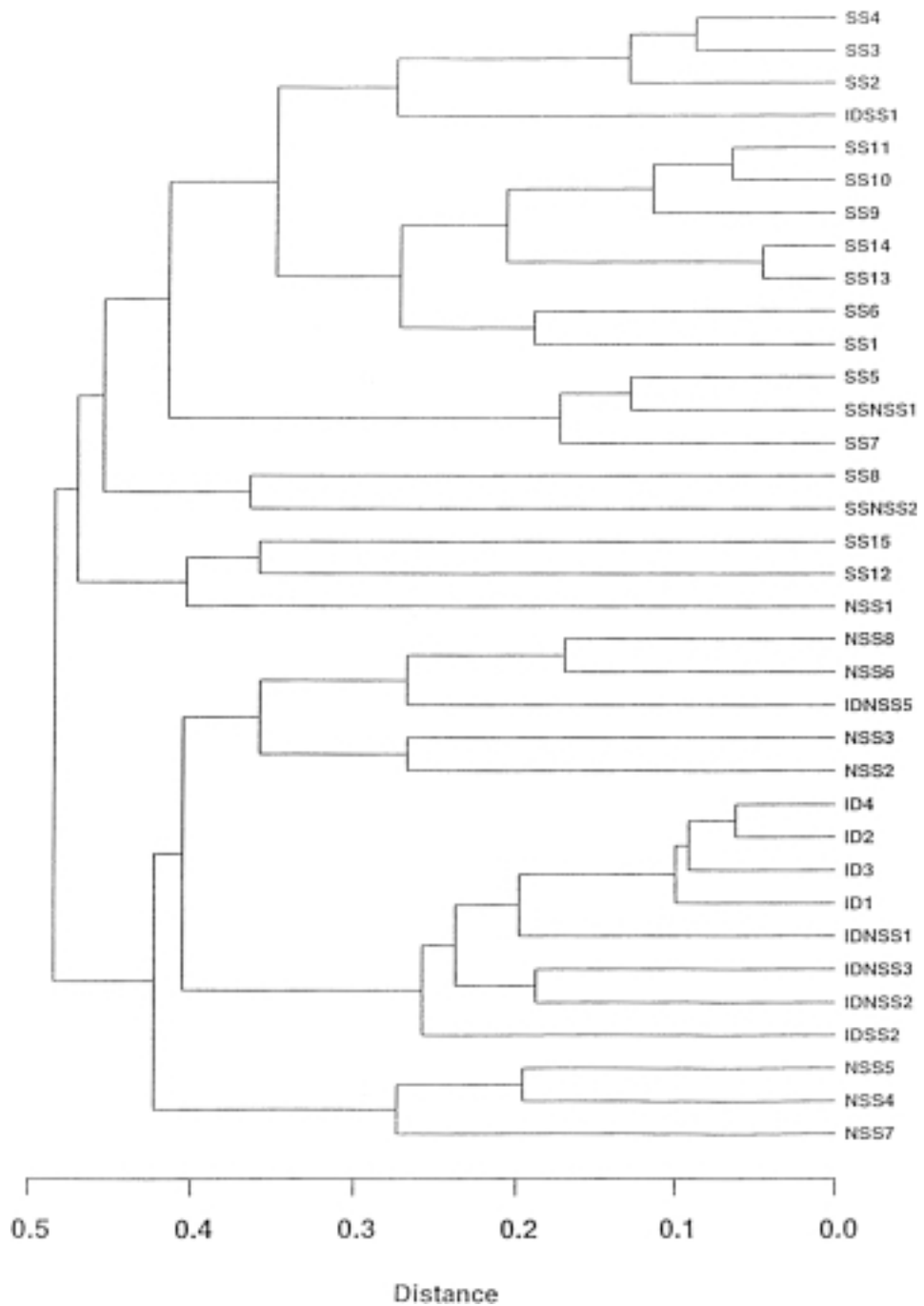


Fig. 3. — Cluster analysis of maize inbred lines in studying heterosis on the basis of AFLP

AFLP markers play significant role in the program of plant improvement and approval of the results. AFLP is based on selective amplification of restriction fragments from total genome DNA. Due to its technique of performance, AFLP technology is very efficient, the most reproductive, and it is the fastest way of maximum mapping of genes responsible for a series of desirable traits. According to many authors it is the ideal method for determination of identity of species. On the basis of electrophoresis — separation of DNA fragments, the differences between AFLP profiles are great, noticeable as presence or absence of fragments. These differences are inherited and thus marking the potential value of material being analyzed. On the basis of these markers obtained polymorphism is very high, being the most important trait in genotype identification, and in determination of diversity between second group of inbred lines, which is the basic condition for hybrid heterosis (fig. 3). Polymorphism of DNA fragments has the advantage in marking of polygene traits controlled by 20—100 loci. They are used for identification of individuals, populations, inbred lines, and hybrids, making the description of the species at the molecular level and its protection possible.

Restriction fragment length polymorphism (RFLP) analysis requires high level of DNA quality and quantity. These markers are based on variation of genome DNA sequence. Unique sequences of DNA are cloned from the nuclear genome to detect homologous sequences in plant DNA. Alleles are identified by differences in the size of the restriction fragments to which the probes hybridize.

Comparison of RAPD and RFLP marker-system for mapping F2 generation in maize was used (Beaumont et al., 1996). Higher possibility (80%) of RFLP marker in relation to RAPD (between 37% and 59%) was achieved. However it was pointed out to great advantage of using combination of these markers for construction of genetic linkage map.

PCR technique is in the basis of a great number of methods used as markers in detection of plant variability. It is used for identification of germplasm in plant breeding. This marker technology must be synchronized between laboratories (Jones et al., 1997). Standardization must be directed toward reproducibility of the results. The reproducibility of three popular molecular marker techniques (RAPD, AFLP, and SSR) was tested in this way in several European laboratories. Different results obtained in the compared laboratories, for example RAPD markers proved difficult to reproduce, AFLP<sub>s</sub> proved easy to reproduce, and SSR alleles were amplified by all laboratories, with differences determined.

The results of heterosis are followed by biochemical data related to isozyme variability, but the large number of restriction fragment length polymorphisms, has allowed the development of linkage maps, with high degree of resolution useful in locating and manipulating of quantitative trait loci (QTLs), according to Tsiftaris (1995).



## CONCLUSION

— Markers can be introduced for testing agronomic traits at the morphologic, biochemical and molecular level.

— Biochemical — molecular markers must be polymorphic in order to be used for linkage of qualitative and quantitative genes, and they must cover the genome, or the segments of interest.

— Genetic maps in plants are obtained using different DNA markers such as: RAPD, SSR, and RFLP.

— AFLP markers have the best possibilities. They are very efficient and reproducible, and they provide genetic maps 10—100 times denser.

## REFERENCES

- Akkaya, M. S., Shoemaker, R. C., Sprecht, J. E., Bhagwat, A. A. and Cregan, P. B. (1995): *Integration of simple sequence repeat DNA markers into soybean linkage map*, *Crop Sci.* 35: 1439—1445.
- Beaumont, V. H., Montel J., Rocheford J. R. (1996): *Comparison of RAPD and RFLP markers for mapping F2 Generation in maize (Zea mays L.)*, *Theor. Appl. Genet.* 93: 606—612.
- Hahn, V., Blaunkenhorn, K., Schwall, M., Melchinger, A. E. (1995): *Relationships among early European maize inbreds: III Genetic diversity revealed with APD markers and comparison with RFLP and pedigree data*, *Maydica* 40: 299—310.
- Jones C. J., Karp, A. (1997): *Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories*, *Molecular breeding* 3: 381—390.
- Khavkin E., Coe E. (1997): *Mapped genomic locations for developmental functions and QTL<sub>S</sub> reflect concerted groups in maize (Zea mays L.)* *TAG*, 95: 343—352.
- Kozumplik, V., Pejic, I., Senior, L., Pavlina, R., Graham, G., Stuber, C. W. (1996): *Use of molecular markers for QTL detection in segregating maize populations derived from exotic germplasm*, *Maydica* 41: 211—217.
- Lee Michael (1994): *Inbred lines of maize and their molecular markers. The handbook on maize*, Eds. by M. Freeling, v. Walbot, 423—432.
- Mudge, J., Cregan, P. B., Kenworthy, W. J., Orf, J. H., Young, N. D. (1997): *Two microsatellite markers that flank the major soybean cyst nematode resistance locus*, *Crop Sci.* 37: 1611—1615.
- Rongwen, J., Akkaya, M. S., Bhagwat, A. A., Lavi, U., Cregan, P. B. (1995): *The use of microsatellite DNA markers for soybean genotype identification*, *Theor. Appl. Genet.* 90: 43—48.
- Smith S., Beavis W. (1996): *Molecular markers assisted breeding in a company environment*, *The Impact of Plant Molecular Genetics BWS Sobral*, Ed. Boston, 259—272.
- Smith, O. S., Smith, J. S. C. (1992): *Measurement of genetic diversity among maize hybrids; A comparison of isozymic, RFLP, pedigree, and heterosis data*, *Maydica* 37: 53—60.

- Stuber C. W. (1992): *Biochemical and molecular markers in plant breeding*. P. 37–61. In J. Janic (ed.). *Plant Breed. Rev.* 9. Timber Press, Portland, OR.
- Stuber C. W., Lincoln S. E., Wolff D. W., Helentjaris T. and Lander E. S. (1992): *Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers*, *Genetics*, 132: 823–839.
- Tsaftaris, S. A. (1995): *Molecular aspects of heterosis in plants*, *Physiologia Plantarum* 94: 362–370.
- Tuberosa R., Sanguineti M. C., Landi P., Salvi S., Casarin E., Conti S. (1998): *RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (Zea mays L.)*, *TAG* 97: 744–755.
- Zlokolica M., Milosevic M., Hrustic M. (1996): *The role of genetic and molecular markers in corn breeding and production*, *Contemporary agriculture*, v. 45, No. 3–4, 5–14.

#### ИДЕНТИФИКАЦИЈА ГЕНЕТСКИХ ОСОБИНА КУКУРУЗА (*Zea mays L.*) КОРИШЋЕЊЕМ ГЕНЕТСКИХ МАРКЕРА

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#### Резиме

Различити генетски маркери се користе за тумачење селекционог материјала, његових особина и основе за крајњи циљ — хетерозис хибрида. Они су такође показатељи квалитета семена на нивоу везаних гена за одређена, тражена агрономска својства. Овај програм укључује нове методологије за тестирање селекционог и семенског материјала. Генетске анализе биљака у фазама оплемењивања обухватају изоензимску експресију гена, и степен њихове варијабилности, која се наставља до детерминације присуства или одсуства гена, одговорних за одређена својства. Примењује се комбинација различитих генетско-молекуларних метода, као што су изоензими, PCR, RAPD, AFLP, RFLP, базирани на полиморфизму протеина и секвенци ДНК, чији је крајњи циљ идентификација новостворених генотипова и њихово унапређење. Утврђивање особина селекционог материјала, његове генетске варијабилности и дивергентности је прва фаза у спознаји генома кукуруза. То је такође услов за детерминацију присуства одређених гена, потребну за постизање завршне фазе, потврде о идентитету генотипа.