UDC 577.21
Review article

BIOTECHNOLOGY IN MAIZE BREEDING

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Mladenović Drinić S., D. Ignjatović Micić, I. Erić, V. Anđelković, D. Jelovac, K. Konstantinov (2004): *Biotechnology in maize breeding*. – Genetika, Vol. 36, No. 2, 93-109.

Maize is one of the most important economic crops and the best studied and most tractable genetic system among monocots. The development of biotechnology has led to a great increase in our knowledge of maize genetics and understanding of the structure and behaviour of maize genomes. Conventional breeding practices can now be complemented by a number of new and powerful techniques. Some of these often referred to as molecular methods, enable scientists to see the layout of the entire genome of any organism and to select plants with preferred characteristics by "reading" at the molecular level, saving precious time and resources. DNA markers have provided valuable tools in various analyses ranging from phylogenetic analysis to the positional cloning of genes. Application of molecular markers for genetic studies of maize include: assessment of genetic variability and characterization of germ plasm, identification and fingerprinting of genotypes, estimation of genetic distance, detection of monogamic and quantitative trait loci, marker assisted selection, identification of sequence of useful candidate genes, etc. The development of high-density molecular maps which has been facilitated by PCR-based markers, have made the mapping and tagging of almost any trait possible and serve as bases for marker assisted selection. Sequencing of maize genomes would help to elucidate gene

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function, gene regulation and their expression. Modern biotechnology also includes an array of tools for introducing or deleting a particular gene or genes to produce plants with novel traits. Development of informatics and biotechnology are resulted in bioinformatic as well as in expansion of microarrey technic. Modern biotechnologies could complement and improve the efficiency of traditional selection and breeding techniques to enhance agricultural productivity.

Key words: maize, molecular markers, transformation, genomics, bioinformatics

INTRODUCTION

Plant improvment was placed on a scientific basis and accelerate in the 20th century with rediscovery Mendel's laws, the application of statistic and ability to introduce mutation and select valued crop from populations and genetic crosses. The revolution in crop improvment was discovery of DNA as chemical supstance of genes. WATSON and CRICKS (1953) finding of double stranded structure of DNA provided insight into how this molecule can encode genetic information and can provide the blueprint under which each organism operates.

The same year (1953) have been consided as the beginning of modern maize breeding in Yugoslavia, connected with specialization of first experts from our contry in the USA. Knowledge and experiences gained in the USA had crucial influence on the futher development of maize breeding programs (IVANOVIĆ *et al*., 1995). Five cycles have determined breeding and growing of hybrid maize in Yugoslavia. Each period has been charactarized by introduction of the new significantly yielding hybrids. The first cycle of selection is presented by first local double cross hybrids, expelling formerly grown American double cross hybrids. They were introduced into the broader production at the end of 50´s and the beginning of 60´s. During 60´s remarkable progress was achieved during the second cycle, when first single cross hybrids were introduced. They had increased genetic potential and uniformity which lead to their predominant use and production during 70´s. The hybrids of the third cycle were introduced at the end of 70´s. Fourth cycle hybrids were introduced at the end of 80´s and had increased drought tolerance and increased tolerance to stalk lodging. The newly developed hybrids (fifth cycle) respond better to a higher level of cropping practices and achieve maximum genetic yield potential at greater plant densities (DRINIĆ *et al*., 2001). A total of 486 hybrids, derived at the Maize Research Institute, Zemun Polje, Yugoslavia, have been released or authorised to be included into production by the Federal Commision for the Variety Releasing since 1964.

Modern biotechnology application in maize improvment can be divided into two major categories: molecular genetics and genetic engineering. Molecular genetics focuses on the use of molecular markers to identify the presence of specific genes already present in organism and which govern traits of interest. Genetic engineering involves the insertion of native or foreing gene into maize. Scientists can utilize genes derived from various sources identified through genetic

mapping and functional genomics (HOISINGTON, 2000). Through the application of biotechnology coupled with convetional breeding these genes can be incorporated into modern maize varieties. Functional genomics is aimed at understanding the function of all genes in an organism, and it will become important tool in biotechnology.

MOLECULAR MARKERS IN MAIZE BREEDING

Genetic markers are specific locations on a chromosome which serve as landmarks for genome analysis. Genetic markers are basically of two types: morphological and molecular markers. Markers that reveal polymorphisms at the protein level are known as biochemical markers, while DNA markers reveal polymorphisms at the DNA level. Biochemical markers are proteins produced as a result of gene expression which can be separated by electrophoresis to identify the alleles. The most commonly used protein markers are isozymes which are variant forms of the same enzyme. Protein markers reveal differences in the gene sequence and function as co-dominant markers. However, their use is limited due to their limited number in any crop species and also because they are subject to post-translational modifications. DNA markers can be classified into two categories depending upon how the polymorphism is revealed: hybridization-based polymorphisms and PCR-based polymorphisms. Given the myriad of different molecular markers and the wide diversity of applications they can be used for the main question is how to choose the most appropriate molecular marker for a specific investigation. There are a large number of factors involved in this question, some of which concern the technology itself, others of which relate more to aspects of the problem under investigation and to circumstances of the investigator. Mode of action, level of polymorfism, informativeness, developmental cost, number of sample that could be run, level of skill, reliability are important considerations when selecting markers for specific applications.

The use of molecualar markers has been investigated in maize mainly for characterization of germplasm; verificaton of pedigre records; asigning inbreds to heterotic groups; understanding the basis and prediction of heterosis; identification and localization of gene; marker assisted selection.

Considerable effort has been spent in collecting and perseving varieties of maize in order to maintain the genetic diversity necessary for breeding (PATERNIANI and GOODMAN, 1978). *Zea mays* L. contains more than 200 races divided into three groups: ancient commercial races, the recent commercial races and indigenous races. Although indigenous races have no commercial value, they have important characteristics which can be incorporated into maize breeding programs. GIMENES and LOPES (2000) was assayed genetic variability in 15 population from four indigenous races of maize from Brazil and five indigenous cultivars, using five isoenzymatic systems. The analyses revealed a low level of variability among the samples studied. Overall, 64.3% of the loci analyzed being polymorphic in comparation with 95.3% and 86.5% polymorphic loci in Mexican

(DOEBLEY *et al*., 1985) and Bolivian germplasm (GOODMAN and STUBER, 1983), respectivelly.

Maize germplasm, accumulated through many decades, is stored in genbanks all around the world. Two percent of the total world collection is being conserved in Yugoslavia (FAO, 1996). Maize Research Institute genebank maintains the collection of 2178 local population of maize. MICIĆ IGNJATOVIĆ *et al*. (2003) analysed 13 local population with RAPD as well as RFLP markers. Both methods showed a high degree of discrimination between population, so can be used for genetic distance estimation among maize populations and screening duplicate in maize gene bank.

Maize breeders are mainly concerned with the genetic diversity among and within breeding population and elite germplasm, becouse it largely determine the future prospects of success in breeding programs. Compresive studies of genetic diversity based on molecular markers has been reported in maize (MESSMER *et al*., 1992; MELCHINEGER *et al*., 1991; LIVINI *et al*., 1992; AJMONE MARSAN *et al*., 1998; DUBREUIL *et al*., 1996). Irrespective of the type of markers employed and the matherials investigated combination of genotypes from different germplasm groups had on average significantlly greater genetic distance than combination of lines from the same germplasm group. Cluster analyses of 148 US maize inbred lines (MUMM and DUDLEY, 1994) partioned lines in accorditance with their origin and pedigree information. One hundred sixteen inbred lines of maize from different heterotic groups and miscellaneous origin were assayed for RFLP analysis (DUBREUIL *et al*., 1996). Based on the obtained results autors conclude that classification by molecular markers was convenient for identifying heterotic groups and for assigning origins to unknow lines. PEJIĆ *et al*. (1998) compared different DNA markers and their applicability for study of genetic diversity using a set of 33 maize inbred lines. All marker systems indicated that lines of BSSS origin were more similar in comparision to inbred lines of other heterotic groups. To addressess the issue of genetic relatedness and variability between maize inbred lines the protein analysis was performed on the series of 96 inbred lines (DRINIĆ *et al*., 2000). Reference inbred lines, encompassing the major heterotic groups were included in the analyses in order to maximize genetic variability across the data set. The analysis of proteins marker shows that all studied genotypes have a specific protein pattern. The UPGMA clustering algorithm grouped inbreds into nine clusters. Three main groups were distinguishable: a group of BSSS lines, a group of Lancester lines, and a set of lines with European background. Grouping of inbreds generally agreed with the pedigress of these lines and the clusters were representative of heterotic groups. The utility of protein and RAPD markers to characterize maize hybrids, validate pedigrees, and show association among hybrids was evaluated using a set of 30 ZP maize hybrids from 5 different selection periods. Genetic distances obtained from protein and RAPD date show low correllation, and cluster analyses and PCA show different grouping of hybrids. Grouping of 30 ZP hybrids from different cycles of selection, based on protein markers, generally agreed with the pedigrees but some

discrepanciens in forming subclusters within major group were noted (ERIĆ *et al*., 2003). The one discrepancy was that two double cross hybrids ZP1/4 and ZP1/3 from the first cycle of selection, having incommon three of four parental inbred lines were in different subclasters. The better agreement with pedigree date have been obtained with RAPD markers. According RAPD markers hybrids from each period of selection have been grouped in separate clusters (ERIĆ, 2004).

Information on the genetic diversity is useful for description of existing heterotic groups and identification new heterotic groups; selection of parental strains and in the prediction of hybrid performance especially in crops such as maize in which hybrids are commercially important. The various steps involved in hybrid breeding programs such as making several crosses and screening the combinations for superior performance and heterosis are very costly, laborious, and time consuming. Hence if heterosis can be predicted before making the crosses, then the number of crosses to be performed and the progeny to be screened can be considerably reduced. Various investigators are trying to correlate genetic diversity, as quantified by molecular markers, to predict hybrid performance, in various hybrid breeding programs because the level of genetic diversity between the parents has been proposed as a possible predictor of heterosis (HALAUER *et al*., 1988). SMITH *et al*. (1990) obtained very high correlation of genetic distances based on RFLP markers and heterosis in the research that encompassed crosses of inbreds from both the same and different heterotic groups. MELCHINEGER *et al*. (1992) conclude that the relation between genetic distance based on RFLP markers and heterosis depends on the type of crosses that were analysed. BETRAN *et al*. (1997) studied germplasm of tropical white maize by RFLP markers and obtained low correlation between genetic distance and SCA i.e. heterosis, respectively. SSR markers have been used to analyse the genetic relationship among twelve maize inbred lines and to predict heterosis in their crosses (DRINIĆ *et al*., 2002). The corellation coeficient between midparent heterosis for grain yield and genetic distances based on SSR markers are positive and mainly significant, while their magnitude is not large enough to be beneficary in prediction of heterosis. Low and negative correlation of RAPD-based genetic distances for a set of 18 inbred lines and heterosis for yield has been obtained by LANZA *et al*. (1997), whereby considerating the classification of inbreds into heterotic groups based on markers did not increase the correlation. SMITH *et al*. (1990) proposed that, when the analyses is carried out on a large number of inbreds and, most importantly, when a large number of markers are used, there will be positive correlation between parental genetic distance and hybrid performance. When higer number of markers has been applied (58 SSR and 15 AFLP) a significant correlation was obtained between genetic distance and heterosis (DRINIĆ *et al*., 1998).

The type of gene actions involved in hybrid performance and their relative contribution to the expression of heterosis is of particular importance in the establishment of the appropriate breeding methodology. One of the hypothesis for the explanation of heterosis is the overdominance. STUBER *et al*. (1992) was identified of large QTL for grain yield near the marker Amp3 on chromosome 5.

That QTL was significantly associated with grain yield in both backcrosses and in both cases the heterozygote marker class was superior to either homozygote indicating that overdominance is bases of heterosis. In same another study for identifying quantitative trait loci (QTL) in a maize population generated from the cross B73 and Mo17, major effect on grain yield was also detected on chromosom 5. GRAHAM *et al*. (1997) further characterized a major QTL on chromosome 5 affecting grain yield in maize. They dissected the region encompassing this QTL into two significant areas. The regions near NP1449 and NRZ5 show two QTL each with dominant effects. These genetic factors are in repulsion phase linkage and their effects support the dominance theory of heterosis. LU *et al*. (2003) analyzed F2 population of single cross LH200xLH216, random mated for three generations with 160 SSR markers. A total of 28 QTLs were identified for grain yield and 24 QTL of them showed overdominance. They gave two possible explanation of results: (1) QTL for grain yield exibit true overdominance or (2) QTLs for grain yield show partial to complete dominance, but they are so tightly linked such that three generation of random mating failed to separate their individual effects. The analyses of gene action for imformative (SSR) markers in study of MOHAMMADI *et al*. (2002) revealed the important of overdominance gene action in maize for the expression of heterosis for yield and its components.

Beside grain yield, majority of agronomically important traits such as quality, maturity, and resistance to several biotic and abiotic stresses are complex and quantitative in nature, influeced by many genes. With molecular markers it is possible to assign chromosomal positions to individual QTLs, to determine the types and magnitude of gene effects of individual QTLs, and also to determine which parent possesses the positive allele at each QTL. The ability to find an association between a QTL and a molecular marker depends upon the magnitude of the QTL's effect on the trait, the size of the population being studied, and the reombination frequency between the marker and the QTL.

Segregating population (F2, backcross, recombinant inbred lines, or double haploids) from the crosses between parents differing in expression of a particular traits could be use for determination number and location of genes of large effect regulating the particular trait of interest. Quantitative trait locus analyses is usually associated with a maping population of plants, each of which has to be genotyped with all the markers selected to cover the genome and phenotyped for the traits of interest. However, plants from such segregating populations can also be grouped according to phenotypic expression of a trait and tested for differences in allele frequency between population bulks: bulk segregant analysis (BSA). Individuals homozygous for parental alleles across a target interval are selected from the segregating population based on the genotype of the markers spanning that interval. The DNA from selected individuals is pooled into two bulks-one homozygous for one parental alleles and the other homozygous for the second parental alleles across a target interval. A large number of individuals in each pool increases the probability of a marker revealing polymorphism between the bulks, to be linked to the target interval. The exact position of the marker can

then be determined by segregation analysis. Once a tight linkage is found between a molecular marker and a gene of interest, the inheritance of the gene can be traced in breeding programs. QUARRIE *et al*. (1999) used BSA with molecular markers to locate QTLs associated with yield under severe drought. The identification of QTLs affecting agronomically important traits could be useful in planning breeding programs for the improvment of the charachteristic of interest and efficient marker assissed selection.

Marker-assisted selection (MAS) is based on the concept that it is possible to infer the presence of a gene from the presence of a marker that is tightly linked to the gene. If the marker and the gene are located far apart then the possibility that they will be transmitted together to the progeny individuals will be reduced due to double crossover recombination events. Hence a prerequisite to using markers in such selection is that they should be tightly linked to the gene of interest. For this purpose, saturation of regions (encompassing the locus of interest) on the genetic linkage map, is necessary. The ultimate utility of QTL mapping to a breeding program is in transferring specific QTLs via MAS. Application of markers to introgression programs can result in a reduction in the number of breeding cycles by improving selection efficiency, particulary at the early stage. Many of the published paper conclude with a statement that MAS will be useful, but the results of successful MAS effort have not yet be publish. Encougaging results have been obtained at CIMMYT in MAS for resistance to maize streak virus and drough tolerance (RIBAUT *et al*., 2001). Availability of tightly linked genetic markers for resistence genes will help in identifying plants carrying these genes simultaneously without subjecting them to the pathogen attack in early generations. The breeder need little amount of DNA from each individual plants without destroying plants and a set of primers for PCR. Individual plant for resistance or susceptibility could be directly identified by the presence or absence of the marker. Only material in advance generations would be tested in disease and insect nurseries. With MAS it is possible for the breeder to conduct many rounds of selection in a year without depending on the natural occurence of the pathogen. Of course, the presence of different races complicates the development and application of molecular marker assisted selection. Maize streak virus appears to be controlled by a single major gene located on chromosome 1 (KYETERE, 1995). Although this has led to the easy development of a MAS strategy for MSV, a question about durability of resistance is raised, because all sources of resistance is derived from alleles present at the same locus. Cultivar diversification, cultivar mixtures, pyramiding of resistance genes have been used to overcome that problem and in all these approachs MAS for resistance gene can be useful. Three QTLs conferring resistance to SCMV were identified on chromosomes 3, 5, and 10 at four developmental stages (seedlings, elongation, anthesis, and grain filing) while QTL on chromosome 6 was found at elongation stages (ZHANG *et al*., 2003). Authors recommended that MAS for SCMV resistance employ three QTL on chromosome 3, 5 and 10 will allow pyramiding QTL alleles into one line.

Also molecular markers are of great utility in rapid backcross conversion of elite inbred lines for expression of novel genes introduced via transformation. MAS could be use for line conversion i.e., transfer of elite alleles at target QTLs from donor to recipient lines; where phenotypic screening is expensive and difficult, including breeding program involving recesive genes, multiple genes, late expresion of the trait of interes; pyramiding resistance genes; selection in early segregating population and at early stage of plant development. The essential requirements for MAS in the plant breeding are: (i) marker should co-segregate or be closely linked with the desired traits; (ii) an efficient means of screening large populations for the molecular markers should be available; (iii) the screening technique should have high reproducibility acroos laboratories, be economical and use-friendly. One difficulty is that, for each new set of parental materials, QTLs must be located before attempting MAS. Another limitation has been the available marker systems, a marker developed for a gene in one cross may not be useful in other crosses. Also limitation are inadequate experimental design, high cost and complexity of qualitaitve traits. Although the cost of MAS is high for most applications, the precision of selection offered by DNA markers and the development of newer markers could make MAS more economical and the method of choice for breeding programs in the future.

TRANSFORMATION

Unlike traditional plant breeding, which involves the crossing of hundreds or thousands of genes, plant biotechnology allows for the transfer of only one or a few desirable genes. This more precise science allows plant breeders to develop crops with specific beneficial traits and without undesirable traits. Through traditional breeding methods, genes have been transferred from one individual to another with the aim of producing individuals which clearly exhibit particular desirable traits. These crossings are usually between individuals of the same, or closely related, species. The gene pool available for use, in traditional crossing, is thus limited to those genes present in individuals which can be induced to breed using natural crossing methods. The use of recombinant DNA technologies enables the movement of a single or a few genes within or across species boundaries to produce plants with new traits, genetic modified plants. Also, it is possible to get rid of an undesirable trait by shutting down the ability of the cell to make the product specified by the gene.

The first succesfull maize transformation was reported in 1988 (RHODES et. al., 1988), but it was two year later when the first fertile maize transformants were recorded (GORDON-KAMM *et al*., 1990; FROM *et al*., 1988). The first transgenic maize event was deregulated in the USA in 1995 and a year later transgenic maize was first time planted. During the eight –year period 1996 to 2003, global area of transgenic crops increased 40 fold, from 1,7 mil ha in 1996 to 67.7 mil ha in 2003 (JAMES, 2003). Genetically modifed maize was planted on 15.5 mil ha (23% of global GM area) or 11% of total area planted with maize (140 mil ha) in 2003.

Genetically modified maize have alredy been produced with herbicide tolerance and insect resistance (USDA, 1999). The main genetically modified herbicide tolerant maize varieties aviable present tolerance to the herbicides glyphosate or glufosinate ammonium. Roundup Ready maize plants are modified to express tolerance to glyphosate. That herbicide acts via inhibition of the protein 5-enolpyruvyl-shikimate-3-phosphate synthase, important in the production of essential aromatic amino acids. Genetically modified maize containes a form of the enzyme isolated from the CP4 strain of *Agrobacterium tumefaciens*. Maize plants have also been genetically modified for resistance to herbicide glufosinate ammonium. Gene encoded a protein phosphinothricin acetyl transferase from bacteria is transfered in maize plant. So, maize can make PAT enzyme and breakdown herbicide. Cry genes from strains of *Bacillus thuringiensis*, encode delta endotoxin that have been expressed in maize to protect against pests such as the European corn borer. Bt proteins expressed in genetically modified maize act by selective binding to specific receptors localised in mid gut of insects. New Bt products are alredy been launched including Cry3Bb gene for rootworm control in USA in 2003. One of the major animal feed crops is maize, but maize itself does not provide a fully balanced diet for animals. It is deficient to lysine and have a lower than ideal level of total proteins. Now, genetically modified maize with both increased protein level and higer percentages of lysine have been made (ABEUROPE, 2003). Also, high phytase maize, produce a very high level of own phytase, enzyme which breaks down phytates and releases the phosphorius, is in late stage of development (MARTINO-CATT, 1999). Work is under way to produce maize with tolerance to drought and other stresses.

At Maize Research Institute "Zemun Polje" marker gene controlling activity of neomycin phosphotransferase (NPTII) was used for maize transformation by the application of three different methods: microinjection into archerosporial tissue, cocultivation of dry seed in the solution of plasmid DNA and use of pollen grain as a carrier of plasmid DNA (KONSTANTINOV *et al*., 1993). Introduced marker gene induced changes in activity of particular loci and different mutants have been made. Now we use that mutants as model sistem to study effect of foreign gene on maize genome expression (KONSTANTINOV *et al*., 2000).

Genetically modified plants are subject to federal regulations and rules pertaining to their containment, movement and realise into the enviroment. Since May 2001 Federal Republic of Yugoslavia adopt regulation about genetically modified organisms according directives of European Union. According to that procedure, detection methods for GMO as or in products were established. The presence of GM can be evaluted by detecting the transgenic DNA or by detecting proteins derivated from this DNA. At the Maize Research Institute qualitative PCR-based test are used. PCR based methods generally utilize specific transgene sequence, promotor sequence and terminator sequence. For general GMO screening commonly used primers are designed from CaMV35S promoter.

GENOMICS

Advances in biotechnology and the equipment available for research in this field have allowed the rapid sequencing of large portions of the genomes of several species. The sequencing of several bacterial genome, as well as some eukaryotic genomes, such as those of the yeast *Saccharomyces cerevisae*, *Drosophyla melanogaster*, *Caenohabditis elengans*, *A. thaliana* have been completed. For species with more complex genomes, such as maize, sequencing of the entire genome still is very formidable challenge. Maize is middleweight with respect to genome size, about the size of human genome. Genetic variation in maize has been analyzed by sequencing 502 different loci in eight different accessions, covering about 90% of the genetic variation found in maize germplasm today (BHATTRAMAKKI *et al*., 2002). Comparative mapping of maize, wheat, rice and other grass species with common DNA probes has revealed conservation of gene content and gene order during evolution of *Poaceae* over the past 50-60 million years (AHN *et al*., 1993; DEVOS *et al*., 2000). However, as many as 15,000 local rearrangments differentiate the maize and rice genome.

Sequencing of numerious maize genes, BAC clones and thousends of random genomic clones would give picture of the genomic organization of maize. The complexity of the maize genome makes direct genome sequencing for gene discovery very difficult. Estimates of gene number for maize range from 50,000 to 80,000 (GAI *et al*., 2000). One of the major uses of a genome sequence is for efficient map-based cloning of genes and to associate candidate genes with important biological or agronomic traits. A physical map based on the isolation and fingerpriting of large numbers of BAC clones and sequencing BAC ends has been generated (BENNETZEN *et al*., 2001). That map is neccesary to enable the sequencing of the maize genome or its gene-rich parts and that can be accessed by assigning expressed maize sequences to their appropriate genomic position. ESTs provide large-scale information on the gene complement of maize. More then 160,000 EST sequences are deposited in GenBank and about 155,000 ESTs in ZmDB. With the advent of large collections of ESTs for maize, it is possible to "mine" these databases for corn homologs to genes characterized first in other species where some level of functional understanding has been established.

Information derived from DNA/RNA analysis have some limitation. DNA sequence analysis does not predict if a protein is in active form and RNA analyses does not always reflect corresponding protein levels.These analysis cannot predict amouth of a gene product that is made, if and when gene will be translated, event such as aging, stress response, disease response involving multiple genes. This has given rise to the development of proteomics, the study of all proteins produced by organisms. Proteomics involves the identification of proteins and the determination of their role in physiological function.

To detect changes in gene expression, a variety of techniques, including RNA blots, differential display, ribonuclease protection assays, and reverse transcription – PCR have been developed. Although these methods are effective, their dependence on gel electrophoresis places limit on the number of samples that

can be simultaneously analyzed. Microarray has provide the important advantage of allowing parallel quantification of the expression of tens of thousends of genes at a time (SCHENA *et al*., 1995). The basic concept behind all microarrays is the precise positioning of DNA fragments at high density on a solid support so they can act as molecular detectors. Two types of microarray, differ primaly in the length, are commonly used: DNA microarrays – probe cDNA (500-5,000bp long) is printed on memebranes or glass; oligobased microarrays (DNA chip) have array elements comprising short (~ 20bp) synthetic DNA molecules (LEMMING, 2002). Over 100,000 ESTs have been sequnced from cDNA libraries constructed from different maize tissue type and used for microarrays production (ELUMALAI *et al*., 2002). Analysis and annotation of ESTs from NSF Maize Gene Discovery Project enable identification of 22,000 tentative unique genes which are being used for the generation and application of microarrays (http://www.zmdb.iastate.edu/zmdb/microarray/).

There are two major application for the DNA microarray technology: identification of gene and determination of expression level of genes. The simplest way to identify genes of potentional interst is to search for those that are up- or downregulated in response to experimental condition. Becouse genes that are highly regulated in response to for examle some stress are likely to have important roles in stress response, this approach can be useful in correlating function with genes. Identifying patterns of gene expression and grouping genes into expression clases might provide much greater insight into their biological function. ANĐELKOVIC *et al*. (2003) used microarray technology to study gene expression profiling in response to heat and water stress in maize kernal. Beside grouping together genes with similar patterns by hierarchical clustering, genes are represented by different intesity of red, e.g. green color due to their up- or down regulation in stress condition. Advances in genomic and proteomic technologies have resulted in to a unprecedented amounth of date. This deluge of date has, in turn, led to an absolute requirement for computerized databases to store, organize and index the data, and for specialized tools to view and analyze the data (HOLLOWAY *et al*.,2002).

BIOINFORMATICS

Bioinformatics can be defined as the storage, manipulation and analysis of biological information via computer science. That involve computational management of all kinds of biological information, whether it may be about genes and their products, whole organisms or even ecological systems. As a consequence of the large amount of data produced in the genomic and proteomic, most of the current bioinformatics projects deal with structural and functional aspects of genes and proteins. First, the data are collected and organized in databases specialized for particular subjects. In the next step, computational tools are needed to analyse the collected data in the most efficient manner. For example, many bioinformaticists are working on the prediction of the biological functions of genes and proteins (or parts of them) based on structural data.

The simplest tasks used in bioinformatics concern the creation and maintenance of databases of biological information. A biological database is a large, organized body of persistent data, usually associated with computerized software designed to update, query, and retrieve components of the data stored within the system. Most biological databases consist of long strings of nucleotides and/or amino acids. Each sequence of nucleotides or amino acids represents a particular gene or protein (or section thereof), respectively. Sequences are represented in shorthand, using single letter designations. This decreases the space necessary to store information and increases processing speed for analysis. For researchers to benefit from all this information, however, two additional things were required: 1) ready access to the collected pool of sequence information and 2) a way to extract from this pool only those sequences of interest to a given researcher.

Some of molecular databases are: EMBL - nuclear acid sequences, SWISSPROT, PIR - protein sequence databases, PDB - protein data bank, dbEST, GSDS - genome sequence databases, Gene expression databases, Plant geneome databases. While most biological databases contain nucleotide and protein sequence information, there are also databases which include taxonomic information such as the structural and biochemical characteristics of organisms. For maize there are: MaizeGDB-maize genetics and genomic databases consist of the complete data from MaizeDB, as well as a current snapshot of ZmDB; MaizeDB - maize genome database include information about genetic maps, recombination and map score data, probes and availability, genetic stocks and variations, stock pedigrees, gene function information, quantitative trait loci, bibliographic references, indexed to genetic objects and addresses of maize researchers; ZmDB - maize genome databases that collect all maize genome information from GenBank.

The most pressing tasks in bioinformatics involve the analysis of sequence information. The actual process of analyzing and interpreting data is referred to as computational biology. Computational biology involves: finding the genes in the DNA sequences of various organisms; developing methods to predict the structure and/or function of newly discovered proteins and structural RNA sequences, clustering protein sequences into families of related sequences and the development of protein models, aligning similar proteins and generating phylogenetic trees to examine evolutionary relationships.

Databases of existing sequencing data can be used to identify homologues of new molecules that have been amplified and sequenced in the lab. They can be compared, usually by aligning corresponding segments and looking for matching and mismatching letters in their sequences. Genes or proteins that are sufficiently similar are likely to be related and are therefore said to be "homologous" to each other. If a a related molecule exists, then a newly discovered protein may be modelled-that is the three dimensional structure of the gene product can be predicted without doing laboratory experiments.

CONCLUSIONS

Undoubtedly, biotechnology will revolutionize the way in which plant breeding is undertaken in the future. Developments in marker technology together with marker assisted selection provide new solution for selecting desirable genotype. PCR-based markers would make DNA marker technology more efficient and cost effective by striking a balance between cost and informativeness. With the development of saturated linkage maps it is possible to map and tag almost any trait. Tagging of major genes is important from the practical point of view, especially for those traits that are difficult or laborious to score. DNA markers have facilitated the dissection of the genetic basis of complex traits and have helped in understanding their mode of action and how their functioning is modulated by the environment. As DNA markers can give a precise estimate of germplasm relationships, breeders can use this knowledge to systematically sample the germplasm and use the most productive materials in their crossing programs. Recent completion of human, animal and plant genome sequences have demonstrated that genomic sequencing is the most comprehensive route to gene discovery and first step toward identifying the function of every gene. Maize gene sequencing and functional analyses will help elucidate the molecular basis of agronomically important traits and thereby facilitate improvments in maize. New marker technologies as microarreys are offering the opportunity to understand the presence and expression of thousends of genes within a plant. This knowlage will making germplasm improvment faster, cheaper, and more effective. Genetic engeenering techniques are providing breeders with the capability to create novel plants by combining genetic materials from a wide array of sources.

Genes and genes products do not function independently, but participate in complex, interconnected pathways and molecular systems that, taken together, give rise to the workings of organisms. Understanding biological pathways will require information from several levels, genetic level, gene product level, metabolic pathways. Certainly, functional genomics as a growing science become an important tool in biotechnology. Although biotechnology is becoming increasingly important in agriculture, the fact that over 50% of the agricultural productivity in the world has been achieved through traditional plant breeding should not be ignored. While DNA marker technology cannot replace plant breeding, it will certainly augment the efforts of plant breeders by providing new tools to ease the many problems faced by breeders.

Received April 28th, 2004
Accepted May 28th, 2004

REFERENCES

ABEurope, 2003. Future developments in crop biotechnology. Issue paper 6. www.ABEurope.info

AHN S., J.E. ANDERSON, M.E. SORRELLS, and S.D. TANKSLEY (1993): Homoelogous relationships of rice, wheat, and maize chromosomes. Mol.Gen.Genet., *241*, 483-490.

ANĐELKOVIĆ V., S. DRINIĆ MLADENOVIĆ, M. BABIĆ, N. DELIĆ, and G. STANKOVIĆ (2003): Gene expression profiling in response to heat and water stress in maize kernal. Genetika, *35* (3), 139-147.

AJMON MARSAN P., P. CASTIGLIONI, F. FUSARI, M. KUIPER, and M. MOTTO (1998): Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor.Appl.Genet. *96*, 219-227.

BENNETZEN J.L., V.L. CHANDLER, and P. SCHNABLE (2001): National science foundation-sponsored workshop report. Maize genome sequencing projekt. Plant Physiol., *127*, 1572-1578.

BETRAN F., J. RIBAUT, D. BECK, and D. GONZALES DE LEON (1997): Correlation between molecular marker data and hybrid performance in tropical maize.International Sympozium on Heterosis in Crops "Genetics and exploitation of heterosis in crops" 17-22 August, Mexico City, Mexico.

BHATTRAMAKKI D., M. DOLAN, R. HANAFEY, R. WINELAND, J.C. VASKE, J.C. REGISTER, S.V. TINGEY, and A. RAFALSKI (2002): Insertion-deletion polymorphism in 3' regions of meize genes occur frequently and can be use as highly informative genetic markers. Plant Mol.Biol., *48*, 539-547.

DEVOS K.M., T.S. PITTAWAY, A. REYNOLDS, and M.D. GALE (2000): Comparative mapping reveales a complex relationship between pearl millet genome and those of foxtail millet and rice. TAG *100*, 190-198.

DRINIĆ MLADENOVIĆ S. and K. KONSTANTINOV (1998): Genetic diversity and its relationship to heterosis in maize as revealed by PCR- based markers. Genetika, *30* (3), 227-237.

DRINIĆ G., S. DRINIĆ MLADENOVIĆ, and N. BARIŠIĆ (2000): Genetic relationship among maize inbred lines revealed by protein molecular markers. XVIII International conference on maize and sorghum geentics and breeding at the end of the 20th century, Maize and sorghum Eucarpia, Belgrade, june 4-9.

DRINIĆ G. and S. DRINIĆ MLADENOVIĆ (2001): Genetika i oplemenjivanje kukuruza na početku 21. veka. Tematski okrugli sto stručnjaka, TOSS 12. "Dorada semena i semenarstvo između prošlosti i budućnosti". 13.12.2001. Beograd. Temaski zbornik radova. Str. 1-8.

DRINIĆ MLADENOVIČ S., S. TRIFUNOVIĆ, G. DRINIĆ, and K. KONSTANTINOV (2002): Genetic divergence and its correlation to heterosis in maize as revealed by SSR-based markers. Maydicam, *47*, 1-8.

DOUBLEY J.F., M.M. GOODMAN, and C.W. STUBER (1985): Isozyme variation in the races of maize from Mexico.Am.J.Bot., *72*, 629-639.

DUBREUIL P., P. DUFOUR, E. KREJCI, M. CAUSSE, D. DE VIENNE, A. GALLAIS, and A. CHARCOSSET (1996): Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci., *36*, 790-799.

ELUMALAI R., M. GCHACHU, E. PIERSON, V. CHANDLER and D.W. GALBRAITH (2002): Maize cDNA Microarrays. MNL, *76*, 56.

ERIĆ I., S. DRINIĆ MLADENOVIĆ, K. KONSTANTINOV, and G. STANKOVIC (2003): Genetic diversity among ZP maize hybrids from different selection cycles obtained by protein markers. MGNL, *77*, 7-8.

ERIĆ I. (2004):. Genetička karakterizacija ZP hibrida kukuruza iz različitih ciklusa selekcije primenom molekualrnih markera. Magistarski rad. Biološki fakultet, Univerzitet u Beogradu.

FAO, 1996. The State of the World's Plant Genetic Resources for Food and agriculture. Background documentation prepared for the international technical conference on plant geentic resources. Leipzig, germany, 17-23 june, Rome, Italy.

FROMM M.E., F. MORRISH, C. ARMSTRONG, R. WILLIAMS, J. THOMAS, and T.M. KLEIN (1990): Inheritance and expression of chimeric genes in the progeny of transgenic maize plants. Bio/technology, *8*, 833-839.

GAI X., S. LAL, L. XING, V. BRENDEL, and V. WALBOT (2000): Gene discovery using the maize genome datebase ZmDB. Nucl.Acids Res., *28*, 94-96.

GIMENES APERICADO M. and C. LOPES ROMERO (2000): Isoenzymatic variation in the germplasm of Brasilian races of maize (*Zea mays* L.). Genetics and Molecular Biology, *23* (2), 375-380.

GOODMAN M.M. and C.W. STUBER (1983): Races of maize. VI. Isozyme variation among races of maize in Bolivia. Maydica, *28*, 169-187.

GORDON-KAMM W.J., T.M. SPENCER, M.L. MANGANO, R.J. ADAMS, N.G. WILLETS, T.B. RICE, C.J. MACKERY, R.W. KRUEGER, A.P. KAUSH, and P.G. LEMAUX (1990): Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell, *2*, 603-618.

GRAHAM I.G., D. WOLFF, and C. STUBER (1997): Characterization f a yield quantitative trait locus on chromosome five of maize by fine mapping. Crop Sci., *37*, 1601-1610.

HALLAUER A.R., W.A. RUSSEL, and K.R. LAMKAY (1988): Corn breeding. *In:* Sprague GF., Dudley JW.(eds). Corm and corn improvment 3rd. Am.Soc.Agron.Madison WI, pp. 463-564.

HOISINGTON A.D. (2000): Maize and biotechnology –The importance in Africa. Paper presented at the african biotechnology stakeholders forum: Mass media and polcimakers workshop on biotechnology. Feb 29-March 3, 2000. Kenya.

HOLLOWAY A.J., VAN LAAR, R. TOTHILL, and D.OWTELL (2002): Option available from start to finish for obtaining data from DNA microarrays II. Nature genetics supplement, *32*, 481-489.

IVANOVIĆ M., R. PETROVIĆ, G. DRINIĆ, V. TRIFUNOVIĆ, L. KOJIĆ, M. VUKOVIĆ, M. MIŠOVIĆ, G. RADOVIĆ, D. RISTANOVIĆ, Z. PAJIĆ, B. TRIFUNOVIĆ i D. JELOVAC, (1995): Pedeset godina selekcije ZP hibrida kukuruza. Oplemenjivanje, proizvodnja i iskorišćavanje kukuruza, 50 godina Instituta za kukuruz "Zemun Polje". 3-17, Beograd, 28-29. IX.1995.

JAMES C. (2003): Global status of commercialized transgenic crops: 2003. ISAAA Briefs 2003; No. 24: Preview. ISAAA:Ithaca, NY.

KONSTANTINOV K., S. MLADENOVIĆ, G.SARATLIĆ, S.GOŠIĆ, B.TADIĆ, V. ŠUKALOVIĆ, and J.DUMANOVIĆ (1993): Transgenic maize: distrubance of host geneome expression by bacterial gene integration. In « Breeding and molecular biology: accomplishment and future promises». Proc. Maize and sorghum EUCARPIA, june 6-9, Bergamo, Italy.

KONSTANTINOV K. and S. MLADENOVIĆ DRINIĆ (2000): reliable level of maize genome polymorphism: genome structure or expresion. Genetika, *32* (3), 431-447.

KYETER D.T., 1995. Genetic basis of tolerance in maize to maize streak virus using molecular markers. Ph.D.Thesis, Ohio State University, Ohio, 169p.

LANZA L.B., C.L. JR DE SOUZA, L.M.M. OTTOBONI, M.L.C. VIEIRA, and A.P. DE SOUZA (1997): Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theor. Appl. Genet., *94*, 1023–30.

LEMING S.H.I. (2002): DNA microarray. www.gene-chips.com

MELCHINGER A.E., M.M. MESSMER, and M.MOTTO (1992): Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theor. Appl. Genet., *84*, 17–25.

LU H., J. ROMERO-SEVERSON, and R. BERNARDO (2003): Geentic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. Theor. Appl. Genet., *107* (3), 494-502.

MARTINO-CATT S.J., H. WANG, L.R. BEACH, B.A. BOWEN, and X. WANG (1999): Genes controlling phytate metabolism in plants and uses thereof. WO patent 99/05298.

MELCHINEGER E.A. (1999) Genetic diversity and heterosis. The genetics and exploitation of heterosis in crops.99-119. American Society of Agronomy.

MELCHINGER A.E., M.M. MESSMER, M. LEE, W.L. WOODMAN, and K.R. LAMKEY (1991): Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci., *31*, 669–78.

MELCHINGER A.E., J. BOPPENMAIER, B.S. DHILLON, W.G. POLLMER, and R.G. HERRMANN (1992): Genetic diversity for RFLPs in European maize inbreds, II. Relation to performance of hybrids within versus between heterotic groups for forage traits. Theor. Appl. Genet., *84*, 672–81.

MESSMER M.M, E.A. MELCHINEGER, J. BOPPENMAIER, E. BRUNKLAUS-JUNG, and R.G. HERRMAN (1992): relationship among early European maize (*Zea mays* L.) inbred lines: I:Genetic diversity among flint and dent lines revealed by RFLPs. Crop Sci., *32*, 1301-1309.

MICIĆ IGNJATOVIĆ D., T. ČORIĆ, D. KOVAČEVIĆ, K. MARKOVIĆ, and V. LAZIĆ-JANČIĆ (2003): RFLP and RAPD analysis of maize local populations for identification of variability and duplicate accessions. Maydica, *48* (2), 153-161.

MOHAMMADI A.S., M. B. PRASANA, C. SUDAN, and N.N. SINGH (2002): A microsatellite marker based study of chromosomal regions and geen effects on yield and yield components in maize. Cellular and molecular biology letters, *7*, 599-606.

MUMM R.H. and J.W. DUDLEY (1994): A clasiffication of 148 US maize inbreds: I Cluster analyses based on RFLPs. Crop Sci., *34*, 842-851.

QUARRIE S., V. LAZIĆ-JANČIĆ, D. KOVAČEVIĆ, A.STEED, and S.PEKIĆ (1999): Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. Journal of Experimental Botany, *50*, 1299-1306.

PEJIĆ I., P. AJMON MARSAN, M. MORGANTE, V. KOZUMPLICK, P. CASTOGLIONI, G. TARAMINI, and M. MOTTO (1998): Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSR and AFLPs. Theor. Appl. Genet., *97*, 1248-1255.

RHODES C.A., D.A. PIERCE, I.J. METTLER, D. MASCARENHAS, and J.J. DETMER (1988): Genetically transformed maize plants from protoplasts. Science, *240*, 204-207.

RIBAUT J.M., J. BETRAN, K. DREHER, K. PIXLEY, and D. HOISINGTON (2001): Marker-assisted seelction in maize: strategies, examples and costs. Plant and animal genome IX conference. 13-13.01.2001. California.

SHENA M., D. SHALON, R.W. DAVIS, and P.O. BROWN (1995): Quantitive monitoring of gene expression patterns with complementary DNA microarray. Science, *270*, 467-470.

SMITH O.S., J.S.C. SMITH, S.L. BOWEN, R.A. TENBORG, and S.J. WALL (1990): Similarities among a group of elite maize inbreds as measured by pedigree, F1 grain yield, grain yield heterosis and RFLPs. Theor. Appl. Genet., *80*, 833–40

STUBER C., S.E. LINCON, D.W. WOLFF, T. HELENTJARIS, and E.S. LANDER (1992): Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics, *132*, 823-839.

USDA ERS: Genetically engineered crops for pest maangment. U.S. Dept. of agriculture, economic research service, Washington, DC. 1999. http://www.ers.usda.gov/whatnew/issues/biotech

WATSON J.D. and F.H.C. CRICK (1953): Nature, *171*, 737.

ZHANG S.H., X.H. LI, Z.H. WANG, M.L. GEORGE, D. JEFFERS, F.G. WANG, X.D. LIU, M.S. LI, and L.X.YUAN (2003): QTL mapping for resistance to SCMV in Chinese maize germplasm. Maydica, *48*, 307-312.

BIOTEHNOLOGIJA U OPLEMENJIVANJU KUKURUZA

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I z v o d

Kukuruz je jedan od ekonomski najznačajnijih useva i model sistem za genetička ispitivanja kod monokotila. Razvoj biotehnologije je omogućio bolje razumevanje strukture i funkcije genoma kukuruza a konvencionalno oplemenjivanje je dopunjeno novim i moćnim tehnikama. Neke od njih omogućavaju naučnicima da sagledaju strukturu celog genoma i odaberu biljke s poželjnim svojstvima na molekularnom nivou, štedeći vreme i resurse. Primena molekularnih markera uključuje ispitivanje genetičke varijabilnosti i karakterizaciju germplazme; identifikaciju gena koji kontrolišu agronomski važne osobine; selekciju pomoću markera. Sekvencioniranje genoma kukuruza pomaže rasvetljavanju funkcije, regulacije i ekspresije gena. Moderna biotehnologija uključuje seriju tehnika koje omogućavaju prenos gena iz drugih organizama ili deaktivaciju postojećih gena i stvaranje genotipova sa novim osobinama. Razvoj informatike i biotehnologije rezultirao je u stvaranju bioinformatike i omogućio je širu primenu mikroarrey tehnike. Moderna biotehnologija može da dopuni i poboljša efikasnost klasičnog oplemenjivanja u cilju stvaranja visokorodnih genotipova kukuruza otpornih na bolseti i stres.

Primljeno 28. IV 2004.
Odobreno 28. V 2004.