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Review article

APPLICATION OF MOLECULAR MARKERS AND BIOINFORMATICS IN PLANT BREEDING

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In the past decade development of molecular genetics brought new dimension of a plant breeding. Molecular markers as universaly methods for all biological system virtualy effect on a success of directly examination of structure and function of genome and well as determination of genetic polymorphism of plant genomes. The potential applications of molecular markers in plant breeding are: analysis of molecular basis of evolution; germ plasm identification, classification and managment; assessing genetic diversity; identification of genes underlying agronomy important traits as yield, resistence to stress and dissease as well as heterosis. High density genetic linkage maps for a number of plant species as a basis for marker assisted selection of agronomically useful traits and isolation of these genes have been established. Important factor in the successes of the genetic improvement of crops was the development of faster and more reliable methods, which allowed easier analysis-of date as well as rapid information excange. The application of information tehnology and development of statistical techniques to analyze genomic information is know as "bioinformatics". By integrating genetics with informatics investigations of whole genomes aims to elucidate the structure, function and evolution of plant genomes are faster. Together these technologies as integral part of classical breeding programs con-

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tibuting significantlly to shorting of plant breeding process and cycles of selection.

Key words: molecular markers, bioinformatics

INTRODUCTION

Since Mendelian work with peas there have been five periods of gene marker evolution: early genetic - morphology and cytology markers; prerecombinant DNK period - protein and alozyme; pre-PCR age - Restriction fragment length polymorphism; PCR period - PCR based markers and post genomic era- DNK chips, DNK sequences. Many plant varieties and genotypes can be distinguished by characteristics that are visually different or that can be measured. Because of the scarcity of such markers often is impossible to discriminate between closely related lines that differ for the trait of interest. When it is not possible to distinguish plant materials based on morphological markers, molecular markers can be used. Even when a useful morphological marker is identified the process of using it can take long periods of time. A molecular marker is a sequence of DNA or protein that can be monitored and they range from highly conserved to hypervariable. Molecular markers are phenotypically neutral and that is a significant advantage compared to traditional phenotypic markers. The three most common types of markers used today are protein markers, RFLP and PCR-based markers. There are two major ways in which molecular markers are used: (1) for mapping and tagging traits of interest and (2) as indicator of genetic diversity. Twenty years ago all genetics maps consisted of only morphological markers and or isozymes. These maps consisted of no more than a few markers per chromosome. With the advance of DNA marker technologies and the availability of almost unlimited number of DNA markers most genetic maps now consist of several hundreds of markers.

Molecular markers. - Molecular markers were introducing in the 1960s and have proliferated even since. At that time naturally occurring genetic polymorphism at the protein level be recognized (GEBTS, 1990). There are two classes of protein markers seed proteins and isozymes. Protein marker assays measure the rate of protein migration through a gel in response to an electrical current. Isozymes are different molecular forms of an enzyme sharing a catalytic activity. The technique is based on the staining of proteins with identical function, but different electrophoretic mobilities. The weakness of isozyme markers is that each of the proteins that are being scored may not be expressed in the same tissue and at the same time in development as well as the number of loci that can be scored limited. DNA markers were introduced in the 1970s, with the appearance of DNA sequencing and restriction fragment analysis. DNA markers describe genotype and can sample both coding and noncoding regions of genome. RFLP - Restriction Fragment Length Polymorphism is identified as variation in the molecular size of DNA fragments, produced by different restriction endonucleases, which contain nucleotide sequences homologous to specific or random DNA probes. The marker is specific to a single clone/restriction enzyme combination. The high degree of

polymorphism of plant genomes enables the use of RFLP for investigation of the structure and function of the genome and its application in plant breeding (HELENTJARIS *et al.*, 1983; DRINIĆ *et al*, 1993). RFLP markers have several advantages: they are codominant and unaffected by the environment; anv source DNA can be used for the analysis; and many markers can be mapped in a population that is not stressed by the effects of phenotypic mutations.

The number of different molecular markers has increased since the late 1980s, with introduction of polymerase chain reaction. The polymerase chain reaction provides a simple, faster and less expensive means for genome analyses compared with RFLP. The polymerase chain reaction is a powerful by simple techniques for amplifying a tiny amount of target DNA sequences several million times. The PCR based markers will be favorable because of degree of polymorphism that revealed, their requirement for small amounts of DNA and speed of assay. Numerous PCR based markers have been developed and use in plant breeding.

SSR or simple sequence repeats are short nucleotide sequences, from 2 to 3 bases in length that are repeated in tandem arrays. The SSR loci can be amplified by PCR (SAIKI *et al.*, 1988) using primers complementary to the regions flanking the repeats. They are highly polimorphic, codominant genetic marker with Mendelian inheritance. SSR compared with to other marker systems revealed the highest level of polymorphism and the highest level of information per single marker. AFLP Amplified Fragment Length Polymorphism is generated by a combination of restriction digestion and PCR amplification and following restriction enzyme digestion of DNA, a subset of fragments representing many loci is selected for PCR amplification. AFLP have several advantages over other marker systems including: a 10-fold increase in the number of information; its ability to give high reproducible banding patterns and no prior sequence information of DNA is necessary, AFLP detect the highest number of polymorphism in a single assay. RAPD - Randomly Amplified Polymorphic DNA is based on the differential PCR amplification of a sample of DNAs from short olgonucleotide sequences. Important features of those markers are: nothing is known about the identity of amplification products, simplicity, relatively inexpensive. But those markers are dominant and have problem with reproducibility.

An ideal marker system would have to meet a number of criteria as: high level of polymorphism; even distribution throughout the genome; selectively neutral behavior; easy and fast assay; codominant inheritance; high reproducibility and resonable cost. Unfortunately there is no single molecular markers which meets all of these criteria so one can be choosing from variety of marker systems according to their properties (Table 1).

Table 1. Comparative assessment of some of the characteristic of different molecular markers

	Abundance	Level of polimorphism	codominance of alleles	Reproducibility	Cost *per assay	Quantity of DNA	Automation possible
allozyms	low	low	yes	high	low	-	no
RFLP	high	medium	yes	high	high	high	no
RAPD	high	medium	no	low	low	low	yes
SSR	high	high	yes	high	low	low	yes
AFLP	high	medium	no/ yes	high	medium	medium	yes

When choosing a markers for particular study few considerations have to be taken: what information is required?; at which level is discrimination sought?; how many loci are required?; reproducibility of results; cost; speed; DNA ability and mode of inheritance (KRESOVICH and MCFERSON, 1992).

Potential uses of molecular markers in plant breeding are: plant genetic resources; strain identification and plant variety protection; genetic diversity; heterosis; mapping and monitoring qualitatively inherited traits and marker assisted selection. The utility of DNA markers is still rather limited and under development. There are few reasons for that: most of them is still expensive; the mostly anonymous markers have reliable and meanful information associated with them in order to have utility in breeding program.

Plant genetic resource. - Plant genetic resources for agriculture include the reproductive or vegetatively propagated material of (i) cultivars in current use and newly developed varieties; (ii) farmers traditional cultivars and landraces (iii) wild relatives of cultivated species (iv) elite breeders line, aneuploids and mutants (FRANKEL *et al.*, 1995). The last few years' molecular marker technology applications are beginning to have significant impact on plant genetic resources conservation and use. Possible application of molecular markers in germplasm collections are: identification and verification of old and new collected genotypes; detection of duplicates; genetic purity analysis; genetic diversity analysis; construction of "core collection" and selection of interesting, gene resources; monitoring of viability and helth and genetic changes due to long-term storage at low temparature.

The most interest of plant breeding programs are knowladge of aviable germ plasm, genetic content of collections, distribution of genetic diversity among samples and relation of collections to elite germ plasm. Usualy genetic diversity in genebanks is characterized by description of variation for morphological traits but this approach have same limitation. Molecular markers techniques can be used as a complementary methods for the detection, characterisation and evalution of genetic diversity. Forty four rice accessions classified on morphological grounds as indica or japonica were analyzed by RAPD markers and cluster analysis group them into two major groups (VIRK *et al.*, 1995). All 31 accessions from first group has been clasified as indica as well as five from second group. The other eight accessions

from second group had been designed as japonica. The RAPD classification do not always correlate exactly with classification based on morphology but fit well with classification of rice based upon crossability and isozyme date. The new developed PCR-based markers has been use for fine-scale genetic characterizations. RONGWEN *et al.* (1995) find out that seven SSR loci cleary differentiated 96 soybean cultivars. Molecular genetic markers such as RFLPs, RAPDs, and SSRs have been used to characterize genetic diversity represented by elite inbred genotypes and cultivar races of sorghum and results have suggested that molecular assays are suitable for conservation of sorghum germplasm. Fifteen SSR markers provide substantial genetic resolution among 19 Orange sorghum accesions (DEAN *et al*, 1999). SCHUT *et al.* (1997) identified 31 barley lines with only eight AFLP primers combinations. Each primer was able to discriminate all of analysed barley lines.

Molecular markers can be used for assessing how much allelic diversity is present in a crop and for providing unique fingerprints for geneticaly distinct genotype. ALLARD (1992) found that *Hordeum spontaneum* have average 5.15 allele per locus for 20 isozyme and RFLP loci comparing with 2.75 alleles per locus in barley landraces from the same geographic area. The same author noted that barley cultivation from California possessed 1.4 alleles per locus while landraces from the Middle East have 2.75 alleles per locus. If ecogeographical variation existing within a given species need to be analyzed in hundreds of accessions it seem appropriate to be screened first for isozymes and subsequently with RFLP or PCR-based markers. This approach is also more cost-efficient and useful for identification of a core collection. Core collection, subsets of germplasm collection at large, has been established to represent the genetic diversity within entire collection of many species. BROWN, (1989) suggested that at least 70% of the alleles present in the entire collection would be represented in a core collection comprised of at least 10% of the accessions. The core collection approach has been taken for sorghum, wheat, barley, cassava, and *Phaseolus* (HODGKIN *et al.* 1995). Due to size of core collection molecular markers may be very useful in the exercise of adjusting the core for the elimination's of duplicates, or for the inclusion of variability that was absent in the original core. The identification of duplicates has had to rely on comparison of morphological date with passport date. At the Maize Research Institute "Zemun Polje" Gene bank duplicate accessions have been identified by comparison of embryo salt soluble proteins separated by PAA and complemented by morphological characters (DRINIĆ *et al.*, unpublish date). VIRK *et al.* (1995) indicate that in rice for very similar pair of accessions detecting a difference between them can be 99% confident with examination a total of 86 RAPD. They also suggested a novel procedure which would allow the level of certanity of identifying duplicate to be set before become part of collection.

Various methods have been developed for genotype identification and genetic purity assessment. Traditionaly morphological traits, measured in field conditions have been used for testing genetic identity and purity but they are often unreable. Along with the isozyme electrophoresis of salt soluble proteins (WANG *et al.*, 1994) can provide usefull alternative. KONSTANTINOV *et al.*, (1996) reported

that salt soluble proteins can be used as markers for genotype identification and determination of genetic purity of commercial hybrid seed. All analysed genotypes had unique protein pattern. The uniform protein representation was found in all individual samples in all studied hybrids, pointing to genetic purity of observed seed.

Genetic diversity - Knowledge of germplasm diversity and relationships among breeding materials is useful in parent selection, planning crosses for hybrid and line development, assigning lines to heterotic groups and in plant variety protection. It can be estimate indirectly by the coefficient of coancesary (MALECOT, 1948) based on pedigree information or directly by biochemical or DNA markers. Malecots coefficient of coancesary (*f*) estimate the probability that two random allels sampled from each of two individuals will be identical by descent. Numerous studies have been conducted to compare estimates of genetic divergence, diversity and discrimination based on coancestry and molecular marker date in different crops (MESSMER *et al.*, 1993; SMITH *et al.*, 1997; GRANET *et al.*, 1994; AHNERT *et al.*, 1996). In studies with maize inbred lines of various orgin *f* was tightly correlated with GD (genetic distance) based on molecular marker date but in study with barley as well as sorghum lines correlation of *f* with GD were moderate to low.

MESSMER *et al.* (1993) compared Malecots coancestry (*f*) with genetic symilarity (GS) based on RFLP date of 188 clone-enzyme combinations for their ability to quantify the degree of relatedness among inbreds. Based on linear regression of GS on *f*, coancestry explained 82 and 70% of variation in GS for related pairs of flint and dent lines, respectively. Authors concluded that both the coancestry and marker approach are well-suited to (i) measure the average level of relatedness and (ii) identify closely related lines. The potential use of molecular markers for study od genetic diversity between maize inbred lines has been disscussed by SMITH *et al.* (1991). According to that using of molecular markers cleary depend on four parameters: 1) the quality of the molecular markers; 2) ther choice of distrinct index which suits molecular date and specific plant material; 3) precision of the estimation of the genetic distance and 4) the relationship between genetic distance based on molecular date and distance based on morphological distance. Estimates of genetic similarity based on molecular markers in common between two individuals do not necessarly portray similarities based on common ancestry: the bands may merely reflect genes that are identical in state and not identical by descent (SMITH and SMITH, 1992).

Genetic variability revealed by molecular marker analysis is possible calculated by transformation of presence or absence of corresponded molecular markers to binary date. The genetic distance (GD) can be calculated from, binary data as JACCARD (1908), NEI (1978), or ROGERS distance (1972). The next step is the hierarchic cluster analysis according to their mutual genetic similarity or dissimilarity by UPGMA (Unweighted Pair Group method using Arithmetic averages) metode or other methode.

Molecular marker date can be useful for description of existing heterotic groups and identification of new heterotic groups as well as assigned inbreds of

unknow genetic orgin to establish heterotic group. Heterotic group has been defined as a set of lines that trace back to a common orgin and that display similar combining ability when crossed with lines from different genetic backgrounds. Isozymes have revealed associations of, inbreds which are consistent with pedigree information (SMITH and SMITH, 1988), but they cannot provide an accurate asessment of genetic background due to limited number of loci and small degree of polymorphism. The utility of embrio salt soluble proteins to characterize maize inbred lines, validate pedigree and show association among inbred lines was evaluted using a set of 15 inbred lines (DRINIĆ *et al.*, 1999). There is a major split between Stiff Stalk and nonStiff stalk pedigreed inbreeds (Fig. l). Associations among inbreds revealed by the present analysis generally agreed with the pedigrees of these lines. 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 they conclude that classification by molecular distance was convenient for identifying heterotic groups and for assigning origins to unknown or broadly based lines. Marker based classifications should prove useful in the choice of combinations of inbreeds to be evaluated in hybrid trails and in the choice of parents for breeding programs.

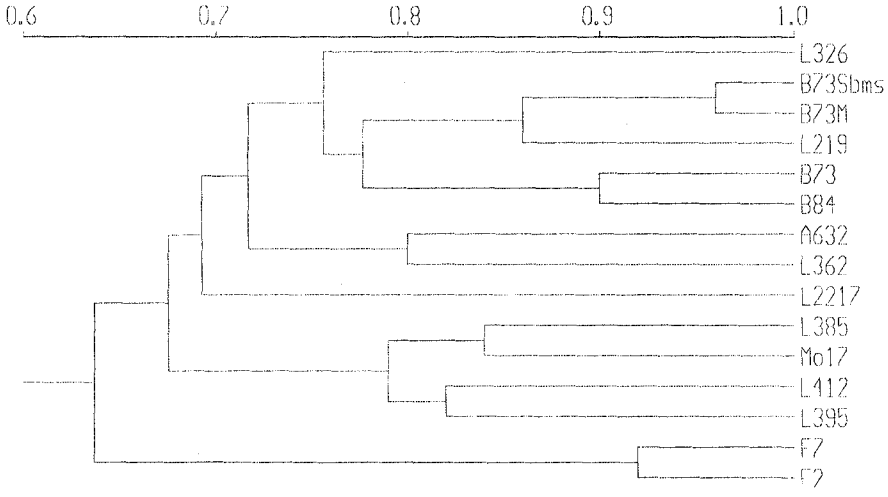

Fig.1 Dendrogram for fifteen maize inbred lines based on cluster analysis (UPGMA) of genetic distances from embryo salt soluble proteins date.

Comprehensive studies of genetic diversity based on RFLPs, AFLPs and SSR have been reported in maize (MESSMER *et al.*, 1992; MELCHINEGER *et al.*, 1991: AJMON MARSON *et al.*, 1998; PEJIĆ *et al.*, 1998; SMITH *et al.*, 1998), rice (ZHANG *et al.*, 1994) and barley (MELCHINEGER *et al.*, 1994). Regardless of the type of molecular markers employed and the material investigated, combination of genotypes from different germplasm group had on average significantly greater

mean GD than combination of lines from the same germplasm group. MELCHINEGER *et al.* (1991) arrived at the following conclusions (i) reliable classification of the line of unknown origin to established breeding pools requires determination of its GD to a large number of representative lines from each germ plasm group and (ii) GD estimates must be sufficient accuracy. RFLP molecular markers are the most widely used but PCR based marker techniques are playing an increasingly important role in these investigations. Many experimental studies demonstrated that with a sufficient number of molecular markers it is possible to reveal genetic relationships among germ plasm with any degree of precision required. Informativeness and applicability of different markers for the study of genetic diversity using a set of 33 maize inbred lines was compared by PEJIĆ *et al.* (1998). All marker systems (RFLP, AFLP, RAPD, SSR) indicated that lines of BSSS origin were more similar in comparison to inbred lines of other heterotic groups. It is known that SSR compared to other marker system have revealed the highest level of polymorphism (WU and TANKSLEY, 1993; POWELL *et al.*, 1996). SSRs on average carry two-fold more information than AFLPs and RAPDs and 40% more information than RFLPs, when the number of alleles per locus is the target. Ninety-four elite maize inbred lines were assayed for polymorphism at 70 SSR marker loci (SENIOR *et al.*, 1998). A unique fingerprint for each inbred line could be obtained from as few as five SSR loci and a cluster analysis placed the inbred lines in nine clusters according to pedigree. DRINIĆ *et al.* (2000) analysed a twelve maize inbred lines with a set of 21 SSR markers. Dendogram based on the polymorphism of SSR markers consist of three groups according to pedigree date. SMITH *et al.* (1998) concluded that SSR technology presents advantages of reliability, repetability, discrimination, genetic interpretation and cost effeetivness over RFLPs and other PCR based markers. These advantages promote the use of SSRs for identification and pedigree validation of maize genotypes. However, caution is necessery in comparing different marker systems due to the different nature of the information content; different base matherial and their instrict polymorphism; different degree of confidence regarding genome saturation and, awereness of the use of clustering methods (LANZA *et al.*, 1997).

The accurate description of new varieties is important to their protection through plant variety protection system. There is important difference between cultivar identification and de novo description of varieties. Cultivar identification is the determination of identity of a previously described variety. The new realised variety must be disctintly different from all previously realised varieties. Varietal identification requires a set of markers that can be distinguish between strains or varieties, while parentage identification requires a set of markers allowing positive parent identification in cases where control of mating opportunities is difficult (SOLLER and BECKMANN, 1983). Traditionaly morphological date have been used for plant variety protection and identification and distinction of cultivars and inbreds. But morphological traits do not reliably portray genetic relationships becouse of enviromental interaction (SMITH and SMITH, 1989). To complement morphological traits isozyme analysis has been used for identification for maize, wheat

and barley. Molecular markers has been used as aditional tools for varietal description (SMITH *et al.*, 1990; MESSMER *et al.*, 1991, MAILER *et al*, 1994).

Heterosis - While heterosis have been widely utilizing to improve crop productivity, the biological basis of heterosis are still unknown. Heterosis representing the superiority in one or more characteristics in F_1 plants relative to their parental lanes. Molecular markers have been used to explore several issues related to heterosis such as genetic diversity and heterotic groups, prediction methods and genetic mapping of QTL. As parental genetic divergence has been found to increase the potential for heterosis has been suggested the use of indirect measures of genetic diversity as possible predictor of heterosis (HALLAUER *et al.*, 1988). The association of genetic diversity based on molecular marker date with heterosis depend upon the type of crossess examined. In studies involving crosses between related lines as well as intra- and inter-group crosses, the correlations of genetic diversity of parental lines with heterosis were moderate to high (SMITH *et al.*, 1990; BOPPENMAIER, 1994; ZHANG *et al.*, 1995). Correlations in intra-group crosses were moderate to high in maize (AJMON MARSAN *et al.*, 1998) but not different from zero in rice (SAGAI MAROOF *et al.*, 1997). For intergroup crosses in maize (BOPPENMAIER er al., 1992) correlations of genetic diversity with heterosis were close to zero.

Many studies have been conducted to examine the relationship between RFLP based distance and heterosis as well prediction of heterosis (LEE *et al.*, 1989; MELCHINEGER *et al.*, 1990, 1991, 1992). In all these studies correlation of GD with heterosis were positive but small for prediction purposes due to small number of analysed lines. When, both parameters, the number of analysed genotypes and number of markers were increased the high positive correlation were found (SMITH *et al.*, 1990). ZHENG *et al.* (1994) analysed correlation of heterozygosity at all marker loci (117 RFLP markers and 10 microsatelites) as well heterozygosity calculated on the basis of positive markers with heterosis. When only date from the positive markers for each trait were used large correlation coeficient were obtained indicating that heterozygosity has contibuted an important component to heterosis in rice and it may be useful for prediction.

The maize inbred lines were surveyed for polymorphism with randomly chosen set of 58 SSR loci and 15 AFLP primers and corellation of marker based distance with heterosis (DRINIĆ *et al.*,. 1999). Genetic distance based on AFLP together with SSR marker date was correlated with both mid-parent heterosis and estimated heterosis for grain yield. Coefficients of correlation have been positive and significant, 0.628/0.608, respectively. The results indicated that genetic diversity among parental lines is certainly related to heterosis.

STUBER *et al.* (1992) mapped QTLs associated with seven major traits in a cross between two widely used elite maize inbred lines to explore heterosis and genotipe x enviroment interaction. The large QTL for grain yield is located near the marker *Amp* 3 on chromosome 5. Theoretical investigation (CHARCOSSET *et al.*, 1991) and computer modeling (BERNARDO, 1992) showed that with intra- and inter-group crossess the correlation of GD with heterosis is espected to decrease if (i)

QTL influencing heterosis are not closely linked to markers used for calculation of GD and vica versa, (ii) markers employed for calculation of GDs are not linked to QTL.

Molecular markers can substitute for kinship coefficient to predict heterosis but they provide no information for the genes and molecular mechanism involved in heterosis. Recently, efforts have been made at the molecular level and the results suggested that both the regulatory proteins and the mechanism of regulation of gene activity are significant in mechanism of heterosis. TSAFTARIS (1995) provide a review of molecular techniques used to study heterosis in plant looking at RNA or protein marker polymorphism and DNA methilation level. KONSTANTINOV *et al.* (1985) obtained the different level of genomic DNA methylation in inbred lines belonging to dent and flint kernal type as well as between parental components and hybrids. Perhaps part of heterotic effect could be explained as a consequence of DNA methylation, resulting in repression or derepression of the parental genome in particular crosses. Variability in gene expression can be assessed through the individual proteins amounts (PAP, LEONARDI *et al.*, 1991), polymorphism of individual RNA amounts (RAP, TSAFTARIS and POLIFOROS, 1993) and polymorphism of embryo salt soluble proteins (DRINIĆ *et al.*, 1997a, b).

The diversities in the embryo salt soluble protein complex in maize inbreds can be used for revealing links between hybrids and parental inbreds (DRINIĆ *et al.*, 1995). Hybrid specific protein fraction, not present in the parental componenets, along with protein fractions inherited from both or from just one parent were discovered in hybrids. A higher number of new fractions was detected in hybrid combinations with a higher- heterotic effect. This indicates that these, probably, de novo synthetised protein fractions can have a certain role in expression of the heterotic effect.

Genetic maps - The important step in genetic analysis is to produce genetic linkage maps which provide a framwork within which important genes can be located. There are two stages of mapping. First step is construct linkage map i.e. to arrange the markers in a linear sequence seperated by appropriate map distance (KEARSAY and POONI, 1996). The second is to relate map to particular chromosome. A linkage maps have been developed for many plant species as maize (HELENTJARIS *et al.*, 1986), rice (MCCOUCH *et al.*, 1988), sorghum (PEREIRA *et al.*, 1994). The construction of the linkage maps was based on the estimation of recombinant frequencies between genetic loci and on determination of the linear order of loci in the linkage group.This process involves collection and identification of markers; crossing of proper genetic sources; and detection of linear order of marker loci and the map distances between them. A range of software packages are aviable to estimate recombination frequencies, assign markers to the most likely order and space them in map units on these linkage groups, for example MAPMARKER (LANDER *et al.*, 1987) and JOINMAP (STAM, 1993). The choice of the population used for mapping can have cosequence on the efficiency and accessibility of the mapping information. Recombinant inbred lines offer certain ad-

vantages over other mapping populations for many species. The genetic maps of most crop species are based on analysis of RFLP but in the future most maps probably will be based on PCR-based markers.

The aviability of maps has made it possible to approach the dissection and manipulation of simple inherited as well as complex traits. The maps can be used for gene localization and isolation, marker-aided selection and evolutinary studies. High density genetic linkage maps of important crops provide a basis for marker asssissted selection of traits of interes, for pyramiding of resistance genes and the isolation of genes by map based cloning (TANKSLEY *et al.*, 1995).

Monitoring quantitative trait - One of the major problems in the plant breeding is the complex nature of most important agronomy traits. Quantitative traits is consider controlled by many genes and each of the genes has a small effect on the trait by traditional approach. The availability of high resolution genetic maps and statistical methods has greatly increased the ability to identify the number and chromosomal location of QTL (LANDER and BOTSTEIN, 1989).

There are essentialy two approaches for using genetics markers to locate and study QTL (KAERSEY and POONI, 1996). The first relies on the asociation between the quantative traits and marker loci in segregating populations. The second uses markers to engineer chromosomes of particular genetical constitution in order to confine genetical differences to defined chromosomal region. QTL mapping have three steps: detection of genetic factors that have effects on a trait and are segregating in population; location of QTL relative to marker loci and estimation of the QTL effects and their interaction. The two types of populations have been used to identify markers linked to QTL are F_2 populations and recombinant inbred lines. The main conditions for using genetic markers to identify loci controlling a particular trait are, accoding to GUFFY *et al.* (1989), definition of the actual number of marker loci, an equal distance between them, proper distribution of markers in the genome and linkage disequilabria.

Over the past decade there have been numerous reports on the use of DNA markers for the identification of quantitative traits loci (TANKSLEY, 1993; PATERSON, 1996). In the most of the studies the distribution is charactarized by one locus with major effects plus additional QTLs with lesser effect. Grain yield and plant height, affected by more than one gene and enviroment, have been studied in the most QTLs experiments in maize (BEAVIS *et al.*, 1991, 1994; EDWARDS *et al.*, 1992; STUBER *et al.*, 1992). For practical using of molecular markers in detection of QTL the issue is the degree to which estimates of QTL effects and localization may be transferred from one population to another. Several studies have reported few if any QTL in common between populations: plant height in maize (BEAVIS *et al.*, 1991), protein content in maize (SCHON *et al.*, 1994). DOEBLEY and STEC (1993) found that the largest QTL mapped to the same genomic site in two maize x teonsinte populations for six morphological traits. On the other hand BEAVIS *et al* (1991) compared the QTLs for plant height for four population and found that no QTL mapped to the same site in all four populations. The most likely explanation was that different sets of polymorphic alleles were segregating in the different ge-

netic backgrounds. QTL for yield identified using $F_{2:4}$ progeny from B73 x Mo17 cross (BEAVES *et al.* 1994) did not map to same genomic sites as QTL identified by STUBER *et al.* (1992). The both studies used the same date analysis tehniques and progeny of the same inbred lines crosses. A number of confounding factor such as different set of markers, source of parental lines, type of progeny, set of environment and particulary sampling of progeny have possible cause this discrepancy. AJMON-MARSAN *et al.* (1995) mapped QTL for grain yield, dry matter content and test weight in a F_2 population from cross of maize inbred lines B73 x A7 and test-crossed to two geneticaly different inbred. They found that QTL exibited by one tester may not be detected with second one and only loci with large effecs were consistent across testers.

RFLP have been used to estimate the genetic locations and effects of QTL for plant height across generation and enviroments (ANHERT *et al.*, 1995). The genetic location of the QTL mapped with F_2 plants coincidence with the locations of the QTL mapped in the same linkage groups in the $F_{2:3}$ population indicating that the same genomic regions affecting plant height across generations. AUSTIN and LEE (1995) compared QTL for plant heigh in $F_{2:3}$ and RI ($F_{6:7}$) lines of the same population grown at the same location in different years and despite the difference in generation and enviroments, most regions assocoated with plant heigh variation in the $F_{2:3}$ were also detected in the $F_{6:7}$.

Marker-assisted selection - Marker - asssissted selection (MAS) is the use of easily recognizable molecular markers to facilite or accelerate the selection of linked genes controlling useful traits. It is based upon the principle that if a gene(s) confering a trait of interest is linked to easily identifiable molecular marker, it may be efficient to select in a breeding program for the marker than for the trait itself. According to KEARSAY (1997) there are several reasons for using markers to improve selection as: earlier selection, more intense selection, difficulties in identifying trait genotype, nondestructive scoring and linkage drag. Traditionaly breeders have used backcrossing with selection at each generation to introduce a useful allelle. This offen results in linkage drag. The use of molecular markers can reduce size of unwanted associated region and accelerate the speed of return to the desired genotype of recurent parent.

MAS has been successfully used far quantitative traits in several crops (HOSPITAL *et al.*, 1993, STUBER, 1995) and the efficiancy of MAS has been examined by LANDE and THOMPSON (1990). They have shown that the potential selection efficiency by combination of molecular and phenotypic information depends on the heritability of the trait, the proportion of additive genetic variance associated with the marker loci and selection scheme. OPENSHAW *et al.* (1994) determined the population size and marker density required in background selection and reported that the number of BC generation could be reduced from seven to three by using a modest sample size (<100 backcross progeny) and number of markers (<80) for maize inbred. Results of FRISCH *et al.* (1999) show that marker assisted selection has the potential to reach the same level of recurent parent genotype in BC3 as reached in BC7 without use of markers.

Studies focused on the efficiency of MAS over several generation using computer simulation (ZHANG and SMITH, 1992; GIMELFARB and LANDE, 1995) showed that MAS could be more efficient that phenotypic selection in large population and for traits with low heritability. KNAPP (1998) reported that breeder using MAS have to test 1.0 to 1.6 time less progeny than by using only phenotypic selection to be assured of selecting superior genotype. According to LEE (1995) the efficiancies of MAS for improving quantitative traits may be more efficient than traditional selection under the following circumstances: the trait under selection has low heritability; tight linkage between QTL and markers with additional efficiency realized when coupling linkages predominante; in earlier generation of selection prior to fixation of alleles at or near marker loci; larger sample sizes for mapping and selecting QTL used to improve estimates of QTL effect.

Marker based breeding can be useful to expedite introgression of specific genetic material from a donor parent into a background of the elite variety, through backcrossing. The first steps are the identification and mapping of the genes targeted for transfer to desired line. When the gene is identified and associated with specific marker allels the next step is repeated backcrossing to recepient line with choosing in each cycle only progeny with linked marker allels. Results from the introgression of targeted segment from Tx303 into B73 and Oh43 into Mo17 demonstrated that marker facilitated backcrossing can be successfully employed to manipulate complexly inherited traits in maize (STUBER *et al.*, 1994). CHEN *et al.* (2000) obtained improved version of line of rice Minghni 63, using MAS and three generation of backcrossing folowed by one generation of selfing. Improved version contained only fragment of less than 3.8cM in lenght surrounding the Xa21 locus from a donor parent, with the rest of genome exactly the same as the recipient parent. They have the same level and spectrum of resistance as donor parent.

For marker-assisted backcrossing the key element are distances between the flaking markers and the target locus. FRISCH *et al.* (1999) conducted study to determine the optimum distances between the flanking markers and the target locus and minimum number of individuals required for obtaining with a certain probability a given number of individuals that carry the donor allele at a target locus and have a minimum proportion of donor genome on the carrier chromosome. Analitic solutions for relevant parameters required to obtain at least one desirable individual depend on the length of the carrier chromosome, the chromosomal position of the target locus, its distance to the flanking marker loci and the number of evaluated individuals.

DNA markers are of great utility in rapid backcross conversion of elite inbred lines for expression of novel genes introduced via transformation. By assuming acceptable level of transgene expression markers could help by identifying inserts within chromosome regions of elite parents. MAS was efficiently used for introgressing a transgene construct, containing Bt gene, from a transformed parent into an elite maize inbred (RAGOT *et al.*, 1995).

Limitation that may affect the potential utility of MAS in applied plant breeding programs are: i) the level of linkage disequilibria in the population, which

affected the number of marker loci needed; ii) sample size needed to detected QTL for traits with low heritability and iii) sampling errors in the estimation of relative weights in the selection indices (LANDE and THOMSON, 1990).

Bioinformatics - High-throughput genomic technologies such as genome sequencing and wholegenome expression analysis have introduced the biological sciences to the information age. Alongside the development of fast and reliable computers allowed easier management and analysis of date. The term bioinformatic is used to describe a spectrum of methods and activities from laboratory information management systems through date analysis, interpretation and integration, document preparation and electronic publishing by way of submitting sequence and mapping data to databases (BOGUSKI, 1994). It use the rules and principles of biological, chemical and physical science to extract useful information from raw molecular data (BOSTEIN and CHERRY, 1997).

Bioinformatics include: i) date analysis; ii) prediction of function and structure of new protein sequence; iii) prediction of genes in genome DNA; iv) prediction of 3D structure; v) biological databases. Until the late 1980s there were three way of accesing databases by Internet: electronic mail, TELENET and File transfer Protocol (ANDERSON and CARTINHOUR,1997). The introduction of GOPHER and WAIS increased database accession but they have been replaced by the World Wide Web. WWW has revolutinized the way in which we hold and access information, the most things can be done without the need to download local copies of software or databases. Information concerning molecular databases, plant genome databases, genetic stock resources, biotechnology resources and journals are distributed through the WWW. Plant genome datebases include information about loci, genetic maps, germline resources, DNA sequences, physical maps all interlinkated and searchable. Many plant genome specific databases are being developed as: Cotton DB, Maize Genetic DB, Grain Genetic DB, RiceGenes, SoyBase, SorghumDB, SolGenes. General sequence datebases are EMBL, SWISSPROT, dbEST and general structural databases is PDB. 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.

The performance of bioinformatics relies upon developments in computer hardware and software. After formation of protein and DNA databases, software became aviably to search that databases (GARDNER, 1999). PC/GENE software package enabling researcher to translate a nucleotide sequence into amino acid sequence and to predict protein structure (PERSIDIS, 1999). Different software can be used to reading nucleotide sequence from gels, identified primers for gene amplification, database searching, predicting encoded protein sequences and structure (SMITH, 1999). It is possible to predict the three-dimensional structure of a protein using algorithms that have been derived from our knowledge of physics, chemistry and most importantly, from the analysis of other proteins with similar amino acid sequences. The most pressing tasks in bioinformatics involve the analysis of sequence information and it involves the following: 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.

Future - As we enter the twenty first century many futurist predict that the doming century will be century of biology and informatics. Genome research, high, throughput molecular markers tehniques and high capacity computing will change conventional breeding during next decade. As individual gene function becomes better understood multigenic trait will also be better understood. Scientis will study not only individual genes but how circuits of interacting genes in different patways control the spectrum of genetic diversity in any crop species. Genetic marker assays have to incorporate methods to detect, describe, interpret and store DNA sequence information. Molecular tools are expected to be accurate, precise, low cost and automated. Bioinformatics is a field in flux and new tehniques continuosly being developed. In the future we need more powerful computer, more feature-laden softwares and faster networks. The application of advanced statistical tools will help breeders to use information from databases and development future breeding material.

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PRIMENA MOLEKULARNIH MARKERA I BIOINFORMATIKE U OPLEMENJIVANJU BILJAKA

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Izvod

Tokom posledenje decenije razvoj molekularne biologije uneo je novu dimenziju u oplemenjivanje biljaka. Molekularni markeri kao univerzalna metoda za sve sisteme je značajno uticala na uspeh direktnog ispitivanja strukture i funkcije genoma kao i ispitivanje genetičkog polimorfizma. Potencijalne primene molekularnih markera u oplemenjivanju biljaka su: ispitivanje molekularne osnove' evolucije; identifikacija, klasifikacija i održavanje germplazme; ispitivanje genetičke raznovrsnosti; identifikacija gena koji kontrolišu prinos, heterosis, stres ili bolesti. Formirane su visoko zgusnute genetičke mape za brojne biljne vrste kao osnova selekcije zasnovane na markerima za agronomski važne osobine kao i izolovanje gena. Važan faktor u uspehu genetičkog poboljšanja biljaka je razvoj brzih i pouzdanih metoda koji omogućavaju lakšu analizu podataka kao i brzu razmenu informacija. Primena informatike i razvoj statističkih metoda za analizu genetičkih informacija poznata je kao "bioinformatika". Integracijom genetike sa informatikom ispitivanja na nivou genoma sa ciljem da se rasvetli struktura, funkcija i evolucija biljnog genoma su ubrzana. Zajedno ove tehnologije kao sastavni deo konvencionalnih programa oplemenjivanja značajno doprinose skraćenju procesa selekcije.

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