DNA Analyses and their Applications in Plant Breeding

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Abstract: In recent years, molecular markers have been developed based on the more detailed knowledge of genome structure. Considerable emphasis has been laid on the use of molecular markers in practical breeding and genotype identification. This review attempts to give an account of different molecular markers currently available for genome mapping and for tagging different traits – restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites. Other markers, expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs) are also mentioned. The importance of structural, functional genomic and comparative mapping is also discussed.

Keywords: DNA; marker; breeding; genetic resources; genomics; RFLP; AFLP; RAPD; SSR

Man has carried out selection and development of desirable plant genotypes since the dawn of mankind. Man has applied basic principles of plant science throughout history. However, the possibilities of improving plants expanded only in the last century as a result of Mendel's investigations into hereditary traits in peas and subsequent discoveries of the genetic basis of inheritance. Among them chromosomal theory, explanation of mutations and also the discovery of DNA structure and function played an important role.

Induced mutagenesis, i.e. changes in the genetic basis of the plant using chemical compounds or radioactivity, was employed after World War Two. So-called "*Green Revolution*" in the 50ies involved the simultaneous development of new varieties of crop plants and altered agricultural practices that greatly increased crop yields. Once the genetic basis of heredity was understood, plants with different desirable traits were systematically selected and crossed in order to produce new varieties that combined better characteristics of the donor material. The end-use quality of crops has also been improved with respect to e.g. protein or oil contents.

The demands of the market have called for speeding up the breeding process and developing cultivars with high and stable yield, superior or altered qualities. Two methods and their combinations appeared at the end of the last century – genetic transformation and marker assisted selection. Whereas the first approach offers a rapid method combining genetic materials from different species, e.g. transmission of a bacterial gene conferring herbicide tolerance into several plant species, the other intends to use the information on the structure and function of plant genome to efficiently assort parental material and speed up selection of the best progenies using molecular markers.

What molecular markers are

Molecular markers are molecules that could be used to trace a desired gene(s) in examined genotypes. In fact a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evaluation of morphological traits (STAUB *et al*. 1996), isozymes (STUBER & KHANNA 1991), storage proteins like glutenins, gliadins, hordeins, etc. (VAPA & RADOVIC 1998; METAKOVSKY 1991; SHARIFLOU *et al.* 2001; KRAIC *et al.*1995; ČERNY & ŠAŠEK 1996a, b). However, DNA markers seem to be the best candidates for efficient evaluation

and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective.

Development of molecular markers

Before molecular markers can be used, they have to be developed using DNA technologies, appropriate plant material and suitable algorithms. Selection of suitable plant population is as important as selection of suitable DNA technique(s) (BERLOO 2000).

There are two basic categories of molecular markers: (1) Markers segregating and determining the presence of a single, dominant or recessive, gene and (2) QTL (**Q**uantitative **T**rait **L**oci) associated markers. It is much easier and cheaper to develop marker(s) for a single gene inherited trait than QTLs. Generally, development of DNA markers consists of several steps. First it is necessary to analyse the nature of studied trait(s) e.g. by genetic analysis. After appropriate mapping the population has to be developed. A much larger population (e.g. a set of DH lines, advanced backcross population) is required for QTL mapping in comparison with a single gene mapping. The mapping population has to be evaluated in the field and/or in a laboratory. It means that each individual line must be tested for a selected trait value.

The same lines are analysed by DNA techniques. Based on field/laboratory analysis and DNA tests putative marker(s) are identified: mapping is usually done by establishing a statistical association between molecular marker genotype and phenotype. Most statistical approaches require a continuous distribution of the response variable for QTL development, but odds utilisation is also possible (SPYRIDES *et al*. 2000). Finally marker(s) have to be validated using additional plant material (BARR *et al*. 2000; PERRETANT *et al*. 2000).

How molecular markers and dna technologies are applied

The breeding process consists of several steps. First, parental material must be carefully selected. Lines are usually chosen from the currently available gene pool of contemporary varieties. Besides that, in some cases wide relatives or exotic germplasm are used to introduce a new trait, mostly disease resistance (CENCI *et al*. 1999; KELLER *et al*. 1999; SEYFAHRT *et al*. 1999; MARTIN *et al*. 2000). **To test genetic resources** for their productivity, quality parameters and stress tolerances field trials and chemical/physical/biochemical tests are employed. Field trials are usually time consuming, there-

fore molecular markers and DNA technology are used to assess diversity in the gene pool, to identify genes of interest and to develop a set of markers for the screening of progenies (KARP *et al*. 1998).

Parental lines that are used for crossing **are always carefully selected**. According to the breeding plan and breeding aim more or less related lines are used. There are several ways of estimating genetic similarity of cultivars. Basically they can be divided into morphology-based, pedigree-based and until now less frequently used marker-based methods. The value of some marker systems, such as isozymes, is questionable. DNA based technologies are more suitable. However, the estimated levels of polymorphism of the varieties widely varied with techniques used. MILBOURNE *et al*. (1998) found that SSRs consistently demonstrated the highest level of polymorphism (100% in barley and 90.8% in potato). AFLPs exhibited the lowest level of polymorphism in data sets (46.8% in barley and 41.7% in potato). RAPDs were intermediate (66.3% in barley and 65.8% in potato). Many authors found a low correlation between variabilities evaluated by morphological data, by pedigree and by DNA analysis (BARRETT *et al*. 1998; CHAVARRIAGA *et al*. 1999; DAVILA *et al*. 1999). VAN HINTUM (1994) explained the relatively low correlation by linkage to genes that are under selection pressure or by low reliability of observations of the marker system.

Much has been expected from DNA technology when hybrid breeding is considered. Until now, progeny testing has been a predominant method for the identification of the combining ability of genotypes (PANTER & ALLEL 1995) that is most important for parental line selection. This approach is costly and time consuming. Determination of genetic distances between parents was expected to predict the future hybrid performance. Several studies were conducted in maize (*Zeya maya* L.), rape (*Brassica napus* L.), soybean (*Glycine maximum* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and other crops showing that the simple detection of relative genetic distance is not sufficient for such a purpose (DIERS *et al*. 1996; GOPAL & MINOCHA 1997; BURKHAMER *et al*. 1998; GUMBER *et al*. 1999). Some genes were mapped controlling hybrid breakdown in rice (FU 1999) and deleterious genes with synergistic interactions decreasing progeny fitness were described in wheat (FU 1999). Their presence/absence in different genotypes might affect hybrid performance as well. Experiments were carried out in *Arabidopsis* to produce lines with better combining ability using recurrent selection (SILLS & NIENHUIS 1998). The research continues especially in corn.

Evaluation of the progeny follows. Whenever progeny is available, molecular markers can be used to help **to select the best lines**. The earliest investigations of

MAS (**M**arker **A**ssisted **S**election) effectiveness on multiple loci selection were conducted in corn (e.g. BERNARDO 1998) using multiple markers and the results were very promising. It has been shown until now that selection for traits encoded by a single gene is highly effective in several species using marker systems, especially when traditional evaluations are difficult, time consuming or expensive to run (e.g. ORDON *et al.* 1999; NACHIT *et al.* 2000; OVESNÁ *et al.* 2001). Marker-assisted selection can also accelerate the recovery of recurrent parent genome in backcross breeding (FRISCH *et al.* 1999) and identify the plants with a higher relative proportion of recurrent parent genome for further backcrossing (PENNER *et al.* 1998). Introgression of alien chromosome segments and effective chromosome markers can assist selection during the segregating backcross generations (JOUVE *et al.* 1998). QTLs that determine important traits like quality, yield components and resistance to various stresses are expensive to develop and their practical application is rather limited at the moment. Usually it is not possible to use QTLs to evaluate other non-related crosses (BERNARDO 1998). Transfer of QTLs is also easier in backcrossing breeding programmes (BARR *et al.* 2000). The efficiency of marker-assisted selection for quantitative traits depends on the power of QTL detection and unbiased estimation of QTL effects (MELCHINGER *et al.* 1998). It has been shown that the accuracy of QTL location greatly affects selection efficiency (CHARMET *et al.* 1999). Another important question of QTL mapping is the optimal choice of marker density (CHARMET 2000; UTZ *et al.* 2000).

Whenever reliable markers are available, MAS can be used to pyramid several resistance genes into a single host genotype and to estimate the presence of quality and agronomically important gene blocks (CHARMET *et al.* 1999). At the moment, of course, it is necessary to combine conventional analyses of plant material with marker-assisted selection techniques. It is proposed that the use of marker data together with phenotypic evaluations provides instruments suitable for more effective breeding (GRANER *et al.* 2000; STUBER *et al.* 1999; RIBAUT & BETRAN 2000; SHARIFLOU *et al.* 2001).

In case advanced lines are submitted for the state trials and approved for marketing, it is necessary to manage tools **to identify the cultivars precisely** – in seed lots, in products and processed food. At the moment, seed storage protein and isozyme analysis are recognised by international organisations (UPOV, ISTA) as a regular tool for identification of plant varieties. Etalons were developed and published in special catalogues (METAKOVSKY 1991; ČERNÝ & ŠAŠEK 1996a, b). However, there are several advantages of DNA fingerprinting over protein analysis. DNA analysis is currently more expensive than protein analysis, but it is possible to run it at any developmental stage of the plant and also in processed food (PECCHIONI *et al.* 1996; MAR-TYNKOVÁ *et al.* 1997) and it covers the whole genome variability. It has been proved that molecular markers represent a fast and efficient tool to evaluate cultivar authenticity and purity (LAW *et al.* 1998). The techniques are still under validation.

CURRENTLY USED TECHNIQUES

After the DNA structure and function were discovered, methods of DNA analysis expanded. From laborious and time consuming procedures used in the 70ies and 80ies laboratory protocols changed into pre-made semi-automated systems. The basic methodologies for marker development and application have also evolved.

RFLP (Restriction Fragment Length Polymorphism)

The method is based on the restriction endonuclease digestion of DNA and the transfer of DNA fragments to a filter where they can be hybridised by a labelled DNA fragment (SOUTHERN 1975). Restriction endonucleases cut specific nucleotide motifs in a DNA sequence. The fragments have to be separated according to size in the gel by electrophoresis and the fragments of interest are identified by hybridisation to labelled probes (NEUHAUS & NEUHAUS 1993).

A polymorphism in a restriction pattern occurs thanks to mutations such as single base-pair loss or gain mutations or mutations based on insertion/deletion, etc. RFLP patterns of nuclear DNA behave like classical co-dominant genetic markers and can be used to create RFLP linkage maps (BRETTSCHNEIDER 1998). The limitations of this method are that it is very labour intensive and expensive.

RFLP analysis is a well accepted method in plant breeding and is used for many different purposes (BACKES *et al.* 1995; BURR *et al.* 1983; HELENTJARIS *et al.* 1985): e.g. the selection of traits of agronomic importance linked to RFLP markers, quality testing of seeds and segregation analysis of progenies, evaluation of diversity in a germplasm collection. Molecular linkage maps based on RFLP markers were developed for major crop species including e.g. potato (BONIERBALE *et al.* 1988), maize (HELENTJARIS 1987) and barley (GRANER *et al.* 1990). RFLP was also used as a tool to describe the genetic variability of crop species (BECKMANN & SOLLER 1983).

PCR (Polymerase Chain Reaction)

The development of PCR technique is a milestone in genome analysis (SAIKI *et al.* 1988; SCHUTZBANK *et*

al. 1993; WHITE *et al.* 1992). The basic concept was tested for the first time with Klenow polymerase but the real breakthrough came when a thermostable DNA polymerase, *Taq* polymerase (MULLIS & FALLONA 1987), was isolated and purified. PCR was originally conceived as a technique for detection base changes in the genome, as a tool for DNA diagnosis of genetic diseases. In the last few years, several assays to reveal DNA polymorphism at multialellic loci have been developed in the field of PCR. Considerable advantages of PCR-based methods are e.g. simplicity, speed, and specificity.

RAPD (Random Amplified Polymorphic DNA)

Arbitrarily Primed PCR (AP-PCR) and Randomly Amplified Polymorphic DNAs (RAPDs) are essentially the same technique. Most molecular biologists use more frequently the acronym RAPDs.

RAPD technique requires only the presence of a single 'randomly chosen' oligonucleotide. Individual RAPD primers are able to hybridise to several hundred sites within the target DNA, however, not all of these hybridisations lead to the production of PCR fragments. The ability of RAPDs to produce multiple bands using a single primer means that a relatively small number of primers can be used to generate a very large number of fragments. These fragments are usually generated from different regions of the genome and hence multiple loci may be examined very quickly (EDWARDS 1998).

The power of RAPD is that it is a fast technique, easy to perform and comparatively cheap. It is immediately applicable to the analysis of most organisms because universal sets of primers are used without any need for prior sequence information (HALLDEN *et al.* 1996). This marker system was used in many different applications involving the detection of DNA sequence polymorphisms, mapping in different types of populations (CARLSON *et al.* 1991; REITER *et al.* 1992), isolation of markers linked to various traits or specific targeted intervals (GIOVANNONI *et al.* 1991; MICHELMORE *et al.* 1991) and applications such as variety identification and analysis of parentage (TINKER *et al.* 1993; MAILER *et al.* 1994).

The RAPD technology, however, has some limitations. RAPD markers are in general dominant, thereby they have a lower information content than codominant markers in the linkage analysis of F_2 populations (WILLIAMS *et al.* 1990). PENNER *et al.* (1993) reported on difficulties in obtaining identical band patterns from the same set of primers and materials among different laboratories. In their study the type of thermocycler used for RAPD analysis seemed to be a key determinant of the reproducibility of band patterns. Another type of problem that has been reported is the occurrence of RAPD bands in progeny but not in their parental DNAs, a phe-

nomenon explained as heteroduplex formation (RIEDY *et al.* 1992; HUNT & PAGE 1992; AYLIFFE *et al.* 1994). It has been suggested that the outcome of RAPD reaction is in part determined by a competition for priming sites in the genome (WILLIAMS *et al.* 1993). In several mapping projects non-Mendelian inheritance for a significant fraction of all polymorphic bands was detected, possibly indicating problems with reproducibility and with competition (REITER *et al.* 1992; ECHT *et al.* 1992; GIESE *et al.* 1994). On the other hand, OBARA-OKEYO and KAKO (1998) reported that the amplifications were generally reproducible and examples of successful application of the methods are known.

VNTR (Variable Number of Tandem Repeat Loci)

The existence of microsatellite loci in eukaryotic genomes has been known since the 1970s. TAUTZ *et al.* (1986) showed that many of the simple sequences occurring in eukaryotes were 5 to 10 times more frequent than equivalent-sized random motifs, and that high numbers of 'cryptic' repeats or scrambled arrangements of repetitive sequences also occurred (TAUTZ *et al.* 1986). JEFFREYS *et al.* (1985) discovered hypervariable tandem repeats in the human genome having a longer repeat unit (minisatellites). Minisatellites as well as microsatellites vary in the number of tandemly repeated elements, hence the general designation for both is a variable number of tandem repeat loci (VNTRs).

VNTR analysis utilised the PCR, however only a limited subset of variations could be analysed by PCR due to the generally large sizes of minisatellite alleles (CHENG *et al.* 1994). Microsatellites have the advantage of minisatellites because the allele sizes are smaller than 500 bp and the variation is over a narrow size range. Microsatellites have become the most important class of markers for linkage mapping in diverse organisms.

SSR (Simple Sequence Repeat) or STR (Short Tandem Repeat)

Microsatellites consist of tandemly repeated units, each between one and 10 base-pairs in length, such as $(TG)_n$ or $(AAT)_n$ (BRUFORD & WAYNE 1993). They are widely dispersed through eukaryotic genomes and are often highly polymorphic. These markers are one of the molecular tools of choice for biodiversity studies because of their high information content (MORIN & WOODRUFF 1996).

PCR amplification protocols used for microsatellites employ either unlabelled primer pairs or primer pairs with one of the primers being radiolabelled or fluorolabelled. Electrophoresis of unlabelled PCR products can

be carried out on smaller vertical polyacrylamide gels or on horizontal agarose gels. However, this approach is not precise enough (FRANCISCO *et al.* 1996). Automated systems are also available. A major advantage of automated systems is the availability of dyes with different wavelengths (e.g. 6-FAM, HEX and TET, Applied Biosystems), so it is possible to use simultaneous capillary electrophoresis (using ABI PRISM system, Applied Biosystems) of several loci with overlapping allele size ranges (ZIEGLE *et al.* 1992; POLÁKOVÁ *et al.* 2001).

Scoring microsatellite gels or autoradiograms is usually a relatively simple process because the used electrophoresis systems have a high resolution (to a single base-pair) and because the alleles differ in a very predictable way (multiples of the microsatellite repeat unit, e.g. two base-pairs). The automated systems using fluorolabelled PCR products separated by capillary electrophoresis (e.g. ABI PRISM 310, Applied Biosystems) allowed to analyse these products using the software such as Genescan™ and Genotyper^R (Applied Biosystems/ABI). These analysis programs provide algorithms that separate native alleles automatically from slippage products.

Microsatellites are co-dominant markers and the data generated are similar to those of allozymes, except that the number of alleles and heterozygosity revealed is almost always higher. Population genetic, parentage relatedness analysis can then be carried out. SLATKIN (1995) and GOLDSTEIN *et al.* (1995) took advantage of our knowledge of the predominant mode of microsatellite evolution (i.e. stepwise mutation) to derive the measures of population subdivision and average genetic distance (ASD).

AFLP (Amplified Fragment Length Polymorphism)

The principle of AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (MATTHES *et al.* 1998) or by capillary electrophoresis.

The technique involves four steps: (1) restriction of DNA and ligation of oligonucletide adapters; (2) preselective amplification; (3) selective amplification; (4) gel analysis of amplified fragments. Genomic DNA of an organism is digested with two different restriction enzymes, of which one has a 4-bp and the other a 6-bp recognition sequence (MATTHES *et al.* 1998). DNA isolated at first is digested with a pair of restriction enzymes usually recognising four and six nucleotide sequences, and adapters specific to the chosen restric-

tion sites are ligated. Amplification of restriction products follows. The selective amplification is achieved by the use of primers that extend into restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. One type of primer can be labelled, e.g. by fluorescent colour if the capillary electrophoresis system is used, or radioactive labelling can be used e.g. [γ-^{33}P] in the case of polyacrylamide electrophoresis. This method enables to visualise sets of restriction fragments by PCR without knowledge of nucleotide sequence (VOS *et al.* 1995).

The AFLP technology is a powerful tool for the detection and evaluation of genetic variation in germplasm collections and in the screening of biodiversity as well as for fingerprinting studies (WERNER *et al.* 2000).

Using the tools listed above many molecular markers have been developed throughout the world. They can be successfully used for marker-assisted selection. Only a few examples are listed here to document the usefulness of the approach: e.g. molecular markers linked to the Rfo restorer gene used for the Ogu-INRA cytoplasmic male-sterility system in rape (DELOURME *et al.* 1998), markers developed for linolenic acid content in rape (HO *et al.* 1999), markers allowing selection for BaYMV resistance in barley (ORDON *et al.* 1999), markers used to identify quantitative trait loci for grain yield and grain-related traits in maize (AJMONE-MARSAN *et al.* 1996), markers closely linked to the Rph7.g resistance gene of barley (GRANNER *et al.* 2000), DNA markers allowing marker-assisted breeding for Fusarium head blight resistance (LIN *et al.* 2000), DNA markers discriminating mutant and normal alleles at the Wx-D1 locus in wheat (SHARIFLOW *et al.* 2001) and some others. The application of the markers helps to speed up the breeding process and change some paradigms in plant breeding (for review see GUPTA *et al.* 1999; RANADE *et al.* 2001; KOORNNEEF & STAM 2001).

The above-mentioned techniques have been used to develop linkage maps of many plant species and DNA makers (e.g. KLEIN *et al.* 2000; LI-WEI MING *et al.* 2000). However, the technical background makes it possible to employ still more effective approaches to genome characterisation (CAI *et al.* 2001).

GENOMICS

It has been proved that DNA markers could be useful for characterisation of genetic resources, selection of parents and subsequently for easier screening of the best progeny or for identification of the genotypes to protect consumers or breeders in the market. However, only the precise knowledge of genome structure and function can lead to a better understanding of the genetic basis of superior genotypes and development of required culti-

vars. Therefore a new discipline called "genomics" started to develop. Genomics is a new field in biology that is concerned with the whole genome analysis, from sequence to function and derived information. The systematic analysis of plant genome function provides information on plant biology that will revolutionise plant and crop production. The crop species have been extensively studied, however, their genomes are quite large. More information is currently available about the model plant *Arabidopsis thaliana*, which is not of economic importance.

Arabidopsis thaliana, a crucifer, represents an important model system in plant molecular genetics. Due to its small genome, short generation time and high number of progeny, the plant is extremely suitable for genetic and mutation analyses. Likewise, the plant is ideally suited for molecular studies because with its content approximately 130 Mbp it is one of the smallest genomes known among higher plants (SCHMIDT 2001). The genome is characterised by a low content of repetitive sequences. Large collections of partial cDNA sequences (ESTs, expressed sequence tags) are also available and the entire sequence of the nuclear genome was deciphered (WAMBUTT *et al*. 2000; WIXON *et al*. 2001). All these materials and information are accessible through databases (http://genome-www.stanford.edu/Arabidopsis/) as well as DNA and seed stock centres (http://aims.cps.msu.edu/aims/, http://nasc.nott.ac.uk/).

Once the genome structure is recognised, the genome function is investigated. There are several approaches of studying the function of plant genome – (1) Gene expression analysis following an exposure of plants to stresses and comparative analysis is employed to identify functional isoforms of genes (BOHNERT *et al*. 2001), (2) Study of mutants – stock of hundreds of mutant lines is available and new ones are being developed using T-DNA tagging (YOUNG *et al*. 2001). A new system for insertional mutagenesis based on the maize Enhancer/Suppressor-mutator (En/Spm) element was also introduced into *Arabidopsis thaliana* (TISSIER *et al*. 1999; SPEULMAN *et al*. 2000). Recent progress in large-scale insertional mutagenesis opens new possibilities of functional genomics in Arabidopsis. The number of T-DNA and transposon insertion lines from different laboratories will soon represent insertions into most Arabidopsis genes. Vast resources of gene knockouts are becoming available that can be subjected to different types of reverse genetics screens to deduce the functions of sequenced genes (PARINOV & SUNDARESAN 2000). With rapid progress in the genome projects of different plants, large-scale transposon mutagenesis has become an important component of functional genomics, permitting assignment of functions to sequenced genes through reverse genetics (SRINIVASHAN *et al*. 2001). Knockouts of genes encoding enzymes of primary metabolism can produce mutants with clear and sometimes unexpected phenotypes (THORNEYCROFT 2001). Genome data have to be converted into knowledge to be useful to biologists. Many valuable computational tools have already been developed to help annotation of plant genome sequences, and they may be improved in future (ROUZE *et al*. 1999).

Comparative genomics is another possibility of gene discoveries. The genomic structure of *Arabidopsis thaliana* is compared with animal and microbial genomes to sense the function of some of the genomic regions (MARTIENSSEN & MCCOMBIE 2001). In fact the information on Arabidopsis structure can lead to discovery of genes of other plant species. The first homoeologous segments identified in the genomes of a dicot and monocot demonstrate that the fine-scale conservation of genome structure exists and is detectable across the angiosperms. Comparative sequencing studies reveal higher degrees of diversity at the microstructural (less than 1 million base pairs) level than predicted at the genetic map level and suggest that genes are densely packed in gene-rich regions (JASIENIUK & MAXWELL 2000). The conserved framework of identified genes is interspersed with non-conserved genes, which however indicates that the mechanisms beyond segmental inversions and translocations need to be invoked to fully explain the plant genome evolution, and that the benefits of comparative genomics over such large taxonomic distances may be limited (DODEWEER *et al*. 1999). Besides comparative DNA studies metabolite profiling can also be a new tool for a comparative display of gene function. It has the potential not only to provide deeper insight into complex regulatory processes but also to determine the phenotype directly (FIEHN *et al*. 2000).

Studies of the model plant *Arabidopsis* provide knowledge of the function of plant genes with unprecedented clarity and quantity. Comparative genetic mapping experiments established colinearity of genomes for the species of the Brassicaceae (SCHMIDT *et al*. 2001). While *Arabidopsis thaliana* is a model for dicotyledons, rice has been selected as a model plant for monocotyledonous families because of its relatively small genome, conservative genome organisation among the cereals and global use of rice (PEREIRA 1999; GOFF 1999). Sequence comparisons between *Arabidopsis* and rice can also define some potential functional relationships, and the information can be used to ascribe functions to genes in many cereals (BEVAN & MURPHY 1999). A major challenge now is to apply this new information to the improvement of crop plants in a systematic manner. Similar techniques are used for rice study including T-DNA mutagenesis (JEON-JONG SEONG *et al*. 2000).

Grasses are the most important plant family in agriculture. Comparative genetic mapping has revealed the conserved gene order (colinearity) between many grass species. It was demonstrated however that the micro-

colinearity of genes is less conserved: small-scale rearrangements and deletions complicate the microcolinearity between closely related species. Therefore studies of rice have to be complemented by more intensive genetic work on grass species with large genomes (maize, Triticale) (KELLER & FEUILLET 2000; YUAN-QIAO PING *et al.* 2001). The large genome of barley and wheat functional genomic approaches, focused on the expressed portion of the genome, have recently led to an exponential growth of expressed sequence tagged (EST) databases of cereals. Assigning gene function to these ESTs is now one of the major challenges in wheat genomics (LAGUDAH *et al.* 2001).

Important events in Arabidopsis genomics

2001 – Development of functional and comparative genomics
2000 – Genome sequencing finished
1999 – First DNA chips available
1999 – Chromosomes II and IV sequenced
1997 – Physical map available
1995 – Construction of BAC libraries
1994 – cDNA sequencing began
1993 – Efficient Arabidopsis transformation developed
1991 – Stock centres and database established
1990 – Arabidopsis genomic study began
1989 – First mutant clone by the use of T-DNA tagging developed
1988 – First RFLP map
1987 – Third International Arabidopsis Conference
1986 – First Arabidopsis DNA sequence published

It is apparent that plant scientists have an increasing collection of important plant genes at their disposal. More information on their function and allelic variants is still needed so that they can use them to improve plant production, drug production, and to solve environmental problems by combining well characterised plant genotypes and fast selection of the best progeny.

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Abstrakt

OVESNÁ J., POLÁKOVÁ K., LEIŠOVÁ L. (2002): **Analýza DNA a její aplikace v genetice rostlin**. Czech J. Genet. Plant Breed., **38**: 29–40.

V posledních letech byla získána řada nových poznatků o struktuře rostlinného genomu. Na jejich základě byla odvozeny molekulární markery. Značné úsilí bylo věnováno snaze využít tyto markery ve šlechtění rostlin a pro identifikaci genotypů. Pokusili jsme se podat přehled dostupných molekulárních markerů pro mapování genomu a přípravu sond identifikujících geny významných znaků – jedná se o RFLP (délkový polymorfismus polymorfních fragmentů), RAPDs (náhodně

amplifikovaná polymorfní DNA). AFLPs (délkový polymorfismus amplifikačních fragmentů) a mikrosatelity. Jsou zmíněny i další markery jako ESTs (značky exprimovaných sekvenovaných úseků genů) nebo SNPs (polymorfismus bodových mutací). Rovněž je diskutován význam strukturální a funkční genomiky a komparativního mapování.

Klíčová slova: DNA; marker; šlechtění; genové zdroje; genomika; RFLP; AFLP; RAPD; SSR

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