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Molecular Markers In Forest Management And Tree Breeding: A review

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Abstract: *Molecular Markers In Forest Management And Tree Breeding: A review*. Recently, extensive stress has been placed on the development of molecular markers to be used for the identification of genotypes, for applications such as: taxonomic studies and studies of phylogenetic relationships, biological studies, and genetic fingerprinting. Here we try to describe different molecular techniques, currently used in forest management and tree breeding.

Keywords: molecular markers, PCR, DNA, RFLP, RAPD, SSR, AFLP

INTRODUCTION

In the progressive degradation of the environment management of natural resources and protection of biodiversity is one of the major challenges that now stands before man. Worldwide, multiple institutions, that through creation of gene banks and establishment of *ex-situ* collections, are designed to protect genetic resources and provide a valuable source of genetic diversity essential for tree breeding. Currently, the greatest emphasis is on improving the quality of plant collections, increasing the efficiency of their conduct and to provide users with a genetic material. Effective resource management is possible only after an accurate characterization of plant material collected. In the past, characterization of accessions was mainly based on morphological and biochemical traits. A major breakthrough in the way of describing and evaluating plant materials was made after the introduction of molecular markers based on Polymerase Chain Reaction (PCR). Markers detecting DNA sequence polymorphisms have revolutionized both the speed, and precision of describing large plant populations.

Improvement in the efficiency of conventional plant breeding is another significant application of molecular markers. This can be obtained by using indirect selection through molecular markers linked to the traits of interest. Molecular markers are not influenced by the environment and can be scored at all stages of plant growth.

Although each marker system is associated with some advantages and disadvantages the choice of marker system is dictated to a large extent by the intended application convenience and the cost involved.

GENETIC MOLECULAR MARKERS

The genomes of all organisms are characterized by the natural variation of the nucleotide sequence - DNA polymorphisms. Polymorphism occurs both in the coding regions of DNA (exons) and non-coding (introns). Whereby, it is possible to detect much larger differences between the genomes of single species individuals, than might be obtained with phenotypic

characteristics. DNA markers in comparison to other have many advantages. First of all, the DNA sequence is fixed and not a subject to change under the influence of the environment (except for mutations, which are generally rare). Due to the fact, that there are no differences in the composition of DNA on a cellular level, any type of cells can be used, regardless of the tissue type and stage of development (Dzialuk and Burczyk, 2001). In order to identify the genetic variability, using the DNA markers, enzymes such as: *Taq* DNA polymerase, and T4-DNA ligase are used. It's also possible to combine various enzymatic reactions.

RFLP (Restriction Fragments Length Polymorphism) was widely used in research programs since the early 1980s. RFLPs have been previously well described in a number of reviews (e.g. Landry & Michelmore 1987, Tanksley *et al.* 1989, Nance & Nelson 1989, Neale *et al.* 1989)

The technique is based on restriction enzyme, that cuts the DNA at occurrences of a particular recognition sequence (usually 4 to 8 bases in length) throughout the strand. Several hundred of these enzymes, each with its own specificity in terms of recognition sequence, are known. The number and lengths of resulting fragments depends then on the number and distribution of recognition sites. Common sources of polymorphisms are (Nance & Nelson 1989): 1. Two DNA molecules may differ in the number of restriction sites for a particular enzyme, 2. Two DNA molecules may differ in the length of the sequence separating common restriction sites.

The first step is digestion of genomic DNA with one or two restriction enzymes and separation of the resulting fragments on agarose gels. The separated fragments are then transferred to a nylon membrane. Detection of individual fragments occurs by nucleic acid hybridization with, radioactive or fluorescent labeled, probe(s). To be useful, probes in the "probe library" must be homologous to single-copy, low-copy, or tandemly repeated sequences in the target DNA. Probes are prepared from the same (or closely related) genome under study. The major strength of RFLP markers are high reproducibility, codominant inheritance, good transferability between laboratories, no sequence information required, and relatively easy to score due to large size difference between fragments. There are, however, several limitations for RFLP analysis. It requires the presence of high quantity and quality of DNA (e.g., Potter and Jones, 1991; Roy et al., 1992; Young et al., 1992). It depends on the development of specific probe libraries for the species. The level of polymorphism is low, and few loci are detected per assay. It is time consuming, laborious, and expensive (Yu et al., 1993).

For forest tree species, RFLPs have demonstrated interpopulation differentiation in *Gliricidia sepium* (Lavin *et al.* 1991), *Pinus attenuata, P.muricata* and *P.radiata* (Strauss, Hong & Hipkins 1992), *Quercus robur* and *Q. petraea* (Kremer *et al.* 1991). Nuclear RFLPs have been used to distinguish *Populus tremuloides* and *P.grandidentata*. RFLPs have been also applied for fingerprinting. Calculations reported by Landry & Michelmore (1987) proved their effectiveness. Combined RFLP banding patterns of six probes could be used to individually identify each of 39 cultivars of peach examined (Ballard *et al.* 1992).

The breakthrough in studies of genetic diversity at the DNA level was the use of polymerase chain reaction (PCR) for the *in vitro* enzymatic synthesis of specific DNA fragments (Mullis and Falcoona, 1987). The source of polymorphism identified with this type of markers are point mutations, insertions, deletions, and chromosomal rearrangements, that

change the DNA-primer complementation pattern. In classical PCR two different oligonucleotide primers with specific sequences are used. Primers are complementary to the marginal sequence of the target genomic DNA fragment. Following amplification, a mixture of DNA fragments of different sizes is obtained. In PCR two major types of primers are used:

- Specific, used to amplify DNA fragments of, at least, partially known sequence, e.g.: particular alleles, microsatellite sequences;

-Random, usually from a few to several nucleotides in length, typically containing 50-80% C,G bases. These are used for DNA probes of unknown sequence. Amplification of DNA fragments may be followed by electrophoretic separation. The presence or absence of the amplified product, of a certain size, shows polymorphism of the marker molecule (Erlich et al., 1991). Marker techniques, that use single primers having random sequences are referred to as random techniques (MAAP-Muliple Arbitary Amplicon Profiling). Depending on the length of the primer there are three types of MAAP techniques: DAF-(Amplified DNA Fingerprinting) (Caetano-Anolles et. al, 1991), RAPD (Randomly Amplified Polimorphic DNA) (Williams et al., 1990), and AP-PCR (Arbitrary Primed PCR (Welsh i McClelland, 1990). MAAP markers were used in taxonomic studies, genetic maps construction, fingerprinting. Linkage between the important agronomic trait and this type of molecular marker can also be identified and further used for marker assisted selection.

One of the most widely applied systems is RAPD (Random Amplified Polymorphic DNA), that uses single-10 base oligonucleotides to amplify short inverted repeats, distributed throughout the genome being assayed. Such inverted repeats are highly polymorphic, and provide large numbers of readily accessible dominant markers. The major advantages of these marker systems is that they can be applied to organisms with complex eukaryotic genomes without any prior information. A typical survey of pine DNA with 100 different arbitrary primers will provide 100 to 200 polymorphic markers. Carefully chosen markers are reliable and repeatable. However, control of reaction parameters and quality of reagents is essential, because amplification is extremely sensitive to initial conditions. Furthermore, the small amounts of DNA required (ng/reaction) makes possible the application of RAPD methods to the conifer haploid megagametophyte (O'Malley et. al.,1996).

Species specific RAPD markers have been sought in *Quercus robur* and *Q.petraea*, in order to detect natural hybrids between the species (Moreau *et al.*1992). Many mapping projects were conducted using RAPDs e.g.:61 RAPD markers have been mapped for a single *Picea glauca* tree (Tulsieram *et al.* 1992). For the hybrid between *Pinus elliottii* and *P. caribaea,* a genetic map with an average resolution of 10cM was constructed in just five weeks (Dale, Gates *et al.* 1993. RAPD markers were also used by Yin et. al., 2002 for *Populus* genome mapping.

Microsatellites (Simple Sequence Repeats- SSRs) consist of segments of DNA containing numerous tandem repeats of a short "motif" sequence, usually of one to six bases (*e.g.* CACACACA...). They are amplified, by PCR reaction, using primers designed to match unique sequences flanking the tandem repeat. PCR fragments are usually separated on polyacrylamide gels in combination with AgNO3 staining, autoradiography or fluorescent detection systems. Multiple allelic length variants can be identified at most microsatellite loci. However, the establishment of microsatellite primers for a new species presents a considerable technical challenge. Advantages of microsatellites are their abundance, high degree of

polymorphism, multi-allelic and co-dominant nature, basis on PCR reactions (requiring only small amounts of DNA and no radioactive labels), and that they can be shared among laboratories by exchanging primer DNA sequences. Microsatellites are codominant markers (heterozygotes can be distinguished from homozygotes) and are therefore far more informative for genotyping individuals and for linkage mapping than dominant markers such as RAPDs. The utility of microsatellites is due to their high variability, together with the ability to semi-automate their analysis and scoring.

Microsatellites are a type of DNA marker which are coming into prominence for individual genotyping and studies of gene flow in forest trees. The first microsatellites developed in forest trees were in *Pinus radiata* (Smith and Devey, 1994). They have since been developed from the nuclear genomes of a range of temperate and tropical forest trees (e.g.: *Melaleuca alternifolia*, *Eucalyptus*, *Quercus, Picea*). Microsatellites from the chloroplast genome have also been isolated in several *Pinus* species (Powell *et al*. 1995; Cato and Richardson 1996; Vendramin *et al*. 1996) and *Abies alba* (Vendramin and Ziegenhagen 1997). The chloroplast genome is paternally inherited in most gymnosperms, offering opportunities for gene flow and paternity testing (Kent and Richardson, 1997).

A commonly used genetic fingerprinting technology is Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995). It combines advantages of RFLP and PCR methods. Selectively amplified whole genome restriction fragments are separated via electrophoresis on polyacrylamide gels. AFLPs are mostly dominant markers. It requires very small amount of DNA, and allows the analysis of many segregating loci on one autoradiogram. The number of bands produced can be manipulated by the number of selective nucleotides and the nucleotide motifs used. The applications of AFLP markers include biodiversity studies, analysis of germplasm collections, identification of closely linked DNA markers, construction of genetic DNA marker maps, construction of physical maps, gene mapping, and transcript profiling. Due to its great discrimination and reliability its often used for fingerprinting (Masojć, 2009).

Conclusions

Molecular markers have important applications in studies for advanced breeding programs with industrial species - mainly in relation to quality control, e.g. checking of clonal identification, orchard contamination and within-orchard mating patterns.

Molecular markers proved to be useful for supportive research for tropical hardwoods and non-industrial species, in particular for essential studies of mating systems. They can be useful for the quantification of genetic variation, although they must be used conservatively.

The major application of markers lies in strategic research - in the great contributions which marker studies are making to rapid advances in the understanding of basic genetic mechanisms and genome organization at the molecular level.

Comparison of the molecular marker methods described is shown in table 1. As concluded by Guo-Jang (2013) the advantages or disadvantages of a marker system are relevant to the purposes of research, available genetic resources or databases, equipment and facilities, funding and personnel resources, etc. The choice and use of DNA markers in research and breeding is still a challenge for plant breeders. A number of factors need to be considered when one or more molecular marker types are chosen (Semagn et al., 2006).

	RFLP	Microsatellite	RAPD	AFLP
Genomic abundance	high	medium	very high	very high
Part of genome analyzed	low copy coding regions	whole genome	whole genome	whole genome
Amount of DNA required	high	low	low	medium
Type of polymorphism	SNP[1], Ins[2], Del[3]	repeats length changes	SNP, Ins, Del	SNP, Ins, Del
Level of polymorphism	medium	high	high	very high
Effective multiplex ratio	low	medium	medium	high
Inheritance	codominant	codominant	dominant	dominant
Reproducibility (reliability)	high	high	medium	high
Type of probes/priers	low copy genomic DNA	cDNA clones	specific repeat DNA sequence	random nucleotides sequence
Radioactive detection	usually yes	no	no	yes/no
Development/start status	high	high	low	medium
Utility for genetic mapping	species specific	species specific	cross specific	cross specific
Proprietary rights status	no	some are licensed	licensed	licensed

Table1. Comparison of the five most widely used DNA markers in plants. (Modified after J. Perry Gustafson and R.B. Flavell (1996). [1-]single base changes, [2-] insertion, [3-] deletion

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Streszczenie: *Markery molekularne w aspekcie gospodarki i hodowli drzew leśnych.* Duży nacisk kładzie się na rozwój technik molekularnych, które znajdują wykorzystanie w genetyce populacji, badaniach taksonomicznych, identyfikacji genotypów, badaniu bioróżnorodności i hodowli roślin. W artykule przedstawiono różne techniki molekularne, obecnie wykorzystywane w gospodarce leśnej i hodowli drzew leśnych.

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