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APPLICATION OF DNA-BASED MOLECULAR GENETIC MARKERS FOR PLANT IDENTIFICATION

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Abstract. The article outlines the main DNA-based genetic molecular markers currently used to identify various plants, including fruit plants. It was noted that the choice of one or another marker depends on the execution technique and the characterized part of the genome. It also provides a brief description of DNA markers based on RFLP hybridization (RFLP), DNA markers based on PCR with primers that have multiple localization in the genome (multilocus markers) RAPD markers, AFLP - a method for obtaining molecular markers, DNA markers of unique SCAR sequences -markers, the principle of the CAPS technique, an important feature of micro-satellites based on SSR markers is noted.

Keywords: *genetic marker, genome, DNA, fruit plants, primers, sequencing.*

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1. Introduction

Genetic markers are DNA fragments corresponding to nucleotide sequences included directly in the structure of an agronomically important gene or linked to this gene. The choice of a specific type of markers for work depends on the objectives of the study, the capabilities of the laboratory, and the amount of genetic information available about the object and markers previously obtained for it. There are several groups of molecular markers: markers based on protein polymorphism (biochemical markers) and DNA (molecular genetic markers).

Plant identification is one of the main applications of DNA-based molecular genetic markers. The reliability of DNA analysis for fruit plants is associated with high levels of genetic variability between cultivars and the lack of variation within a cultivar (Romanova *et al*., 2007).

Currently, depending on the execution technique and the characterized part of the genome, a large number of various DNA markers are isolated: RFLP, AFLP, RAPD, CAPS, SSR, SCAR, SNP, etc. (Chesnokov, 2005).

More promising is the use of polymorphic nucleotide sequences of DNA as marker systems, which make it possible to test genetic polymorphism directly at the level of genes, and not at the level of gene products, as in the case of using the protein polymorphism method. DNA markers allow solving the problem of saturating the genome with markers and marking almost any DNA segments, including non-coding ones. In addition, this marker system makes it possible to use any tissues and organs for

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analysis, regardless of the stage of development of the organism, and has a number of advantages over other types of markers (Sulimova, 2004).

Repeated SINE and LINE sequences can also serve as genetic markers. They are retrotransposons or mobile genetic elements that are widely represented in the genomes of the entire eukaryotic kingdom and make up a huge part of genomic DNA (up to half of the plant genome) (Romanova *et al*., 2007).

The literature describes an easy-to-perform method for analyzing the polymorphism of retrotransposons of the R 173 family of repetitive DNA sequences that are specific to rye. It is about 15,000 copies per diploid rye genome, which are distributed over all 7 chromosomes in a dispersed manner.

2. DNA markers based on RFLP hybridization (RFLP)

One of the earliest methods for obtaining DNA markers is RFLP (Restriction Fragment Length Polymorphism), also called RFLP (Restriction Fragment Length Polymorphism). The principle of the method is based on the detection of specific nucleotide sequences in genomic DNA cleaved with restriction endonucleases using the so-called "blot hybridization" proposed by Southern in 1975 (Southern, 1975). This method of analysis is still in demand, but in the field of plant identification, it has been replaced by methods based on NDP amplification of DNA using randomly selected primers or primers complementary to known genome regions.

3. DNA markers based on PCR with primers having multiple localization in the genome (multilocus markers) RAPD markers

One of the most popular types of markers in plant genome mapping is RAPD (Random Amplified Polymorphic DNA). The method consists in the amplification of the object's genomic DNA during PCR with a random primer, usually 10-11 nucleotides long. The technique for detecting RAPD markers is technically simple. It is especially important that the successful application of RAPD does not require any knowledge of the genetic constitution of the object; therefore, studies on molecular mapping of the genomes of poorly studied species begin with this type of markers (Lodhi *et al*., 1995). When optimizing the protocol, the RAPD method is fast, convenient, and well suited for plant genome analysis. The method has been successfully used for mapping the genomes of many crops (Lodhi *et al*., 1995), for marking varieties, and for analyzing intra- and interspecific polymorphism. Particularly promising in fundamental and applied terms is the use of the RAPD method for mapping genes of quantitative plant traits (Chegamirza, 2004).

4. AFLP - a method for obtaining molecular markers

AFLP (Amplified Restriction Fragment Length Polymorphism) is a technology for obtaining random molecular markers using specific primers. In the AFLP method, DNA is treated with a combination of two restriction enzymes. Specific adapters are ligated to the "sticky" ends of the restriction fragments. These fragments are then amplified using primers that are complementary to the adapter sequence and the restriction site and carry one or more additional random bases at their 3' ends. The set of resulting fragments depends on restrictases and randomly selected nucleotides at the 3'

ends of the primers. Agarose or polyacrylamide gels are used to separate fragments (Vos *et al.*, 1995).

AFLP is a fairly cheap and reliable method that can quickly generate hundreds of highly reproducible markers randomly distributed throughout the genome. To create an AFLP map of the genome, knowledge of its sequence is not required (Konovalov, 2006).

5. DNA markers of unique sequences SCAR markers

SCAR (Sequence Characterized Amplified Region) or amplified by sequencing. These are PCR-based molecular markers derived from RAPD or ISSR fragments that overcome most of the shortcomings of RAPD markers and are applicable to a variety of studies (Zijlstra *et al*., 2000). According to numerous observations, the data obtained using RAPD analysis using random primers is rather relative due to the sensitivity of this method to reaction conditions. Therefore, in order to study polymorphic RAPD fragments and create specific DNA markers on their basis, it became necessary to switch to the classical PCR method using a pair of long primers complementary to a certain sequence. To this end, polymorphic RAPD fragments (or ISSR-) are excised from the gel, cloned and sequenced. After sequencing, SCAR primers, usually 20–25 bases long, are selected for the end portions of the fragments (Konovalov, 2006).

6. CAPS methodology

The CAPS (Cleaved Amplified Polymorphic Sequences) method, like SCAR, belongs to the STS (Sequence Tagged Site) group, since it is based on the amplification of strictly defined genome fragments with a known sequence. The principle of the method is as follows: genomic DNA is amplified using a pair of highly specific primers, then the resulting fragment is processed by a restriction endonuclease; differences between genomes appear as different numbers and lengths of restriction fragments on agarose gel electrophoresis.

7. SSR markers

Micro-satellites, also called simple repeating sequences (Simple Sequence Repeats, SSRs), are short tandem repeats of simple nucleotide motifs that contain from one to ten nucleotides per repeat unit. They occur in all eukaryotic genomes, with the most common repeats in plants being the (A)n, (AT)N, and (GA)n motifs, where n ranges between 10 and 80 (Hicks *et al*., 1998).

Micro-satellites are the first highly polymorphic markers for individual loci obtained using PCR, which are referred to as dispersed tandem repeat sequences. To create SSR markers, primers are selected for unique DNA sequences flanking the microsatellite repeat, which requires prior knowledge of their nucleotide sequence. The length of the polymorphic amplified fragment is then visualized by agarose or polyacrylamide gel electrophoresis. The polymorphism arises from a different number of tandem repeats, probably resulting from replication slippage and/or unequal recombinations.

An important feature of micro-satellites is that they evolve faster than the rest of DNA, undergoing "dynamic mutations" that result in alleles with varying numbers of

repeat units. As a consequence, microsatellites are highly polymorphic. Their high polymorphism, combined with their ubiquity and multi-allelism, makes them very promising as molecular markers (Nguyen *et al.*, 2005; Paniego *et al.*, 2005).

8. Markers Based on Single Nucleotide Polymorphism of the DNA Sequence (SNP Markers)

SNP markers represent a new, third generation of DNA markers (after RFLP and NDP markers). SNP - single nucleotide site polymorphism, or single nucleotide polymorphism, is most often represented by two allelic variants (substitutions) of a single nucleotide site. These markers detect polymorphism according to the "±" principle. Numerous single nucleotide substitutions are generated by point mutations, insertions and deletions in sections of nuclear and chloroplast DNA.

The total number of SNPs per genome of wheat and maize can be in the millions, which makes it possible to distinguish between individual organisms, justifying one of the definitions of DNA genotyping - "fingerprint method" (DNA fingerprinting). None of the previous analysis methods (RFLP-, RAPD-, SSR-, AFLP-analysis, etc.) can be compared with SNP-analysis in terms of informativeness and speed of analysis (Romanova *et al*., 2007).

9. Conclusion

Thus, molecular (or DNA-) markers are a new generation of genetic markers that differ from the previous ones in a large number and frequency of occurrence in eukaryotic genomes and based on universal, and therefore widely demanded and constantly developing methods of analysis. It turned out to be expedient and economically justified to use DNA markers in breeding, and their use in fundamental research made it possible to reach a new level of understanding the organization and evolution of the genomes of the studied objects.

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