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ANALYSIS OF THE ROLE OF MOLECULAR MARKER TECHNOLOGIES IN THE RESEARCH OF GENETIC DIVERSITY OF PLANTS

Abstract

Genetic diversity in plant selection refers to varieties and differences in the genetic composition of plant species. In this article, the types of molecular markers most commonly used in determining genetic diversity in plants are examined and their advantages and application in various research fields are covered. Also, future perspectives on this technology and innovations created by them in plant research will be discussed. The development of PCR-based molecular marker techniques plays an important role in the evolution of plant selection programs. It enables faster and more effective access to information about the genetic characteristics of particular economically valuable plant species. In plant selection, more commonly RAPD, ISSR, SRAP, and AFLP scar, SSR markers are used in assessing the genetic diversity of plants and have some positive properties according to the purpose of the study. The use of these marker techniques ensures a grade and reasonable improvement in the selection of plant species. These technologies help to make progress by distinguishing existing variations more efficiently and efficiently to identify the most desirable forms in selection programs. A significant step toward more effective selection processes, growth in crop cultivation, and protection of genetic diversity.

Keywords: *Molecular markers, PCR, RAPD, ISSR, SSR*

Introduction

Research at the molecular level of plants, along with the development of biotechnology, has made significant progress. Molecular markers in this field are widely applied to the study of the genetic diversity of plants, to the improvement of selection programs, and to the creation of disease-resistant varieties (Smith et al., 2020: 125). Molecular markers allow us to detect differences between the genotypes of plants, targeting very small portions of genetic information (Jones et al., 2018: 101).

The use of molecular markers is more accurate and effective than traditional selection methods. These markers, in particular DNA-based markers (SSR, SNP, AFLP, etc.), are widely used in various genetic analyses. The main purpose of DNA markers is to detect genetic differences between individuals, species, etc. These differences in a given region of the genome are called alleles. The advantage of detecting such differences at the DNA level is that any DNA chain can show allelic differences between two individuals (Gulshan & Mutlu, 2005: 29).

There are two DNA marker techniques available. These are RFLPs based on DNA hybridization, the other, and markers such as RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), sts (Sequence Tagged Site), SCAR (Sequence Characterized Amplified Region), SSR (Simple Sequence Repeat), and ISSR (Inter Simple Sequence Repeat), which are most commonly used in current cycles (Semagn et.al., 2006: 2542; Gulshan & Mutlu, 2005: 29).

With the use of molecular markers in plants, gene pyramids provide advantages such as creation, selection of recessive genes, gene transfer from wild gene sources, and early selection, thus accelerating the acquisition of new varieties. Molecular marker technology is considered to be complementary and supportive methods for classically breeding methods. Thanks to molecular marker technology and marker-assisted selection technique, successful and reliable results will be possible in a much shorter time compared to classical methods (Jealous et.al., 2015: 10).

1 Material and methods

As study material, the molecular marker techniques most commonly used in plant selection (RFLP, RAPD, AFLP, ISR, SNP, etc.) have been investigated.

1=1 Hybridization-based markers.

RFLP (Restriction Fragment Length Polymorphism) markers were first used to identify DNA sequence polymorphisms for genetic mapping of temperature-sensitive mutations of adenovirus serotypes (Grodzicker et al., 1975: 441). As the first generation of DNA markers, it is one of the most effective methods of mapping the plant genome and has a codominant property. RFLP is among the most commonly used molecular markers based on hybridization and is usually identified by radioactive agents. RFLP markers are molecular markers that work on the basis of cutting down DNA in certain regions of DNA by restriking enzymes and analyzing the lengths of fragments generated to detect genetic variations. This method allows the identification of genetic variations, polymorphisms, especially changes in restriction **.** RFLP markers are highly accurate and reliable. RFLP markers mainly detect polymorphisms in encoding regions, making them more useful for functional genomics research (Acquaah, 2012: 390).

1 - 2 PCR-based markers. Polymerase chain reaction (PCR) is a technology discovered in 1986 that directly amplifies a specific short segment of DNA without the use of a cloning method (Mullis & Faloona, 1986). One of the advanced markers, hybridization-based markers, is more reliable, and in the PCR-based molecular marker system, oligonucleotides called primers, which are 10-25 bp in length, are used (Acquaah, 2012: 390).

RAPD (Random Amplified Polymorphic DNA) markers are a technique that amplifies different regions of DNA using short and random primers. This technique allows to detect variations and polymorphisms in DNA (Williams et.al., 1990: 7214; Welsh & McClelland, 1990: 6532).

RAPD marker technology is distinguished by its economical efficiency, rapidity, and lack of upfront information about the nucleotide sequence of the genome. The RAPD method is used intensively in the study of genetic variation in plants. This is because it is cheaper, simpler, and requires less DNA than other molecular marker methods. At the same time, large quantities of samples are adequate for screening. Due to the use of random primers in RAPD technology, different regions of the genome are amplified and different DNA fragments are obtained (Jealous et. al., 2015: 10).

Regarding the negative characteristics of RAPD markers, it is possible to first mention the dominance characteristic (i.e., homozygous and heterozygous locus cannot be distinguished). Different lab conditions or different PCR machines may cause results to vary, with limited trust. By converting RAPD markers to scar markers, the reliability of genetic markers can be significantly enhanced, as shown in the study of Yu and his collaborators (2000: 413).

SCAR (Sequence Characterized Amplified Region) and sts (Sequence Tagged Site). It is possible to make longer primers (about 22-24 bp) by sequencing the ends of fragments obtained from PCR-based markers. These primers are used in the creation of SCAR markers and have a higher reproductivity than RAPD markers. SCAR markers are made by sequencing the ends of RAPD fragments, which means they are primers designed complementarily to specific DNA fragments obtained through the use of RAPD marker technology. At the same time, sts markers are obtained by sequencing the ends of RFLP fragments. SCAR markers are usually dominant markers, which makes them useful in specific genetic studies (Acquaah, 2012: 397).

The high repetition characteristic of SCAR markers, the codominant nature, as well as the fact that they require little and short time compared to RAPD primers, are some of their advantages. However, initial preparatory work and technical skills are required for their creation and use and to produce repeatable and accurate results (Acquaah, 2012: 397).

SNP (Single Nucleotide Polymorphism) markers are polymorphisms produced by changing one single nucleotide (A, T, C, G) in the genome. These changes are the simplest and most common form of genetic diversity and can affect many biological processes. SNP markers can be located in different parts of the genome and are widely used for genetic analysis at the individual, population,

and species levels. SNPs are the most abundant and widely used molecular markers in the genome. Their occurrence and distribution vary between species. More informative than multiallel markers such as RFLP and microsatellites (Acquaah, 2012: 398).

SNP markers, human genome mapping by various research groups, have been widely used to study genetic diversity (Lander et. al., 2001: 861).

SSR markers (Simple Sequence Repeats) are also known as microsatellites, are codominant, are high in polymorphism, and display multiple alleles, allowing high accuracy of genetic correspondence. It is widely distributed throughout the entire genome and can be applied to a variety of organisms. In genetic mapping research in plants, SSR techniques are commonly used because of their advantages. The results are very repeatable and do not vary from lab to lab. It also enhances its use by giving a codominant marker and making PCR easy (Röder et.al., 1995: 330; Acquaah, 2012: 398).

SSR markers are more accurate than RAPD markers in assessing genetic kinship. SSR markers can detect more polymorphisms among individuals. (Ravi et.al., 2003: 134).

In recent years, many molecular genetics laboratories around the world have successfully used SSR in a variety of plants. The most important drawbacks are that the mutation rate is high, markers are difficult to develop, it is an expensive process that requires a lot of labor, it does not require high-precision laboratory conditions. Alternative marker systems are used to address these challenges, for example the SNP marker can be used to address some of the disadvantages of SSRs. (Acquaah, 2012: 398; Jealous et.al., 2015: 10).

AFLPs (Amplified Fragment Length Polymorphism) are simply RFLPs visualized by selective PCR amplification of DNA restriction fragments (Acquaah, 2012: 390). AFLP markers are a marker system (Vos et.al., 1995: 4409) that works on the basis of DNA interrogation by restriking enzymes, binding of adaptors, amplification of PCR with select primers, and lastly, separation of fragments by electrophoresis. This technique allows us to detect polymorphisms in different regions of the genome and determine genetic diversity.

The AFLP technique has higher reproducibility and polymorphism levels than the RAPD method.

ISSR (Inter-Simple Sequence Repeat) markers are molecular markers generated by amplification of regions between microsatellites in the genome. Microsatellites are short tandem repeat sequences that are common in the genome. ISSR markers use the PCR technique through specific primers, targeting unique sequences around these microsatellites (Zietkiewicz et.al., 1994: 176).

Zietkiewicz and his collaborators (1994) first described the principles and effectiveness of ISSR markers and emphasized the sensitive and highly repeatable nature of this method.

ISSR markers are effective techniques that can be applied to many field crops in determining genetic diversity, phylogenetic research, designing genome maps, and evolutionary biology, making them easier to apply and more reliable because primers are longer (Reddy et al., 2002; Acquaah, 2012: 390).

ISSR is simple and quick to use. Combines the benefits of SSR, AFLP, and RAPD markers. The main disadvantage is that it is dominant (unable to distinguish heterozygous locale) and requires some technical expertise and equipment to perform ISSR analysis. (Acquaah 2012: 397).

Desirable properties of molecular markers:

The application of molecular markers in plant selection and biology is diverse and growing exponentially. Markers desire a variety of features to be useful.

1. High Polymorphism: Markers are required to have high polymorphic properties to distinguish between widely distinguished genotypes.

2. Codominant heredity: Heterozygous and homozygous individuals must have codominant properties to distinguish them.

3. Frequent occurrence and random distribution in the genome: Markers should be frequent in the genome and should be observed in all tissues.

4. Selective neutral behavior: Markers should behave selectively and show neutral genetic variants.

5. Easy access and low cost: markers should be easily accessible and the value spent on research goods should be low.

6. High yields and high reproduction: Markers must have a high degree of reproducibility and be capable of transferring between populations and species.

7. Easy and fast analysis: Markers should be suitable for automated processes and analyses should be carried out in a fast manner.

8. Low mutation rate.

(Semang et.al., 2006: 2540; Broadel, 2013: 11-12; Jealous et.al., 2015: 10; Acquaah, 2012: 390).

Conclusions and discussions

Based on the information gathered about the molecular markers, the table below is constructed and shows their pros and cons. However, researchers can select markers that align with their goals (the body they have, laboratory conditions, etc.) according to these characteristics.

Comparison of molecular markers Table 1.

Marker	Being PCR-based	Dominance	Positive aspects	Negative aspects
RFLP	-	Codominant	- Accuracy and reliability -In areas that encode, detects polymorphism - Required among populations transferable	-Very time-consuming -There is a high level of funding - Complicated procedure - Low polymorphism
RAPD	+	Dominant	-Low financial -Quick results and easy to use -Small amounts of DNA enough -High polymorphism	-Limited reliability -Low migration -Difficult to analyze -Low reproduction
AFLP	+	Dominant	-Trustworthy -High repeat-probability - High polymorphism	-Very time-consuming - Goodness high - Methodology compound - Large quantities requires DNA
SSR	+	Codominant	- High polymorphism - High repeatability and time requirement - Multiple alleles - Extensive in genome spread	- High volume of work - High mutation - High-financial
ISSR	+	Dominant	- High polymorphism - Easy to apply, need to use fast - Low financial-Trustworthy	- Technical experience - Difficulty of the primer design

SCAR	+	Codominant	- Specificity - Accuracy - Easy analysis	- Compound and time requirement - High quality DNA requirement - Difficulty of the primer design
SNP	+	Codominant	- High polymorphism - Rapid analysis - Low mutation rate - Stable and repeatable	- Time-consuming - High finance

Table 1 shows the advantages and disadvantages of markers in a comparative way, and as can be seen, while there is no optimal marker available that meets all the requirements, researchers can choose the markers that match their own.

Conclusion

Each type of marker has its own unique advantages and disadvantages, and the choice of a particular marker depends on the specific objectives of the study. In order to determine which molecular marker technique is best suited for researching genetic variation in plant species, molecular markers are widely used when compared, as SSR markers offer high polymorphism and reproductivity. ISSR markers, however, combine both the stability of SSRs and the simplicity of RAPD. The RAPD and ISSR are more financially sound. RAPD is alsodistinguished by a hearty and simple analysis process, requiring no special knowledge or equipment. AFLP can be used because of its high marker density and speed.

Consequently, the use of molecular markers in plants is of great importance for genetic studies and selection programs. These technologies play an important role in preserving and enhancing the genetic diversity of plants, improving productivity and disease resilience. Future research and developments, expanding the application of molecular markers, can create new opportunities for agricultural and biotechnology development. In future prospects, molecular markers are expected to be further improved and combined with new technologies. Thanks to modern DNA modification technologies, selectors can create new variations that were previously impossible to achieve. These technologies help to effectively and efficiently distinguish existing variations to identify the most desired forms in breeding programs, thereby aiding in achieving progress.DNA marker technology plays an important role in this process, allowing selectors to make more accurate selections.

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