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Review: DNA Fingerprinting and its Applications in Crop Improvement

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ABSTRACT

Plant breeding is increasingly using crop DNA fingerprinting due to its applications in forensic science research, dispute resolution, and variety protection. Before the advent of proteomic and genomic technology, the varieties were identified using morphological markers. In the middle of the 20th century, protein-based markers were discovered and used for genetic diversity analysis in crops. In the genomic era, DNA markers are primarily used for crop fingerprinting. Crop fingerprinting using DNA markers started with RFLPs (non-PCR based markers), then moved on to PCR based markers such as RAPDs (Randomly Amplified Polymorphic DNA), SSRs (Simple Sequence Repeat), AFLPs (Amplified fragment Length Polymorphisms), ISSRs (Inter Simple Sequence Repeats), SNPs (Single Nucleotide Polymorphism), and DAr (Diversity Array Technology). The future of crop fingerprinting depends on the creation of whole genome sequencing methods that are affordable. With such technology, it might be possible to differentiate between very similar varieties, mutations, specific clones, and vegetatively propagated crops. This review article provides information on the many DNA fingerprinting markers utilized as well as their uses in crop development.

Keywords DNA fingerprinting, DUS test, Genotyping, Morphological markers, Heterosis.

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INTRODUCTION

During breeding programs, seed production, trade, and product inspection, variety identification, classification, and sustainability are crucial. A crucial component of the preservation and upkeep of biodiversity and food security is the investigation of genetic variation and relatedness (Nybom *et al.* 2014). Historically, distinctness, uniformity, and stability (DUS) was used to identify species and variations based on morphological descriptions (Tiwari *et al*. 2013). Due to their multigene nature, these morphological descriptors were inconsistent, time-consuming, and less useful. Additionally, environmental changes, inadequate sampling methods, and unidentified genetic controls reduce the usefulness of morphological features for variety identification. In order to identify, distinguish, purify, research, and understand the genetic variability among the cultivars in order to facilitate breeding programs, molecular biology techniques have made significant progress. DNA markers offer a trustworthy and economical method for differentiating plant genotypes and quickly illuminating genetic

variety and variability among species and variants. The most promising techniques for identifying plant genotypes use molecular techniques, particularly DNA fingerprinting (Nybom *et al.* 2014). Paul Hebert created the phrase "DNA fingerprinting" in 2003 as an addition to the more traditional morphological-based taxonomy. It is now a commonly used technique for determining genetic relatedness and differences (Hebert *et al*. 2003). DNA fingerprinting makes use of DNA markers to classify breeding lines into different heterotic groupings and to identify different varieties. Additionally, it is least impacted by how the environment interacts with genes and by changes in gene expression through time and space. Restriction Fragment Length Polymorphisms (RFLPs) are one type of DNA marker technique that is not PCR-based; however, PCR-based methods such as Random Amplification Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Single Nucleotide Polymorphisms (SNPs), Diversity Arrays (GBS) (Nadeem *et al*. 2018).

DNA fingerprinting in crops using different markers

1. Morphological markers
2. Biochemical markers or protein markers (Isozyme)
3. DNA markers

a) Non PCR based marker or probe based: RFLP
b) Amplification based: RAPD, SSR, ISSR, SCAR, CAPs, STS, VNTRs, SPLAT
c) Probe and PCR based: AFLP, rDNA- ITS d) New generation: SNP, EST, SSCP.

Morphological markers

The earliest approach for identification and differentiation of cultivars was morphological markers based. A specific characteristic found in a genotype was labeled as "fingerprint" for its unique identification. Morphological markers such as fruit shape, color, and size, pubescence of leaves, and the number of flowers per spike were most frequently used for variety identification . In order to identify many crops (Selvi *et al*. 2003, Bhandari *et al.* 2006), morphological descriptors were used. However, with the development of modern technology, the morphological method was seen as an unreliable source for genotypic evaluation and cultivor identification for the reasons listed below. Despite having genetically distinct varieties within a species, they all share a similar morphological appearance. Genetic and environmental interactions may result in morphological traits. Morphological traits that can only express in homozygous form and are governed by recessive genes. These characters are quantitative, so calculating them and genetically mapping them is a difficult task (Bhandari *et al.* 2006).

Proteins markers (Isozymes)

Isozymes were used for fingerprinting after 1960 because of their quick speed, dependability, and relative independence from environmental factors (Nybom *et al.* 2014). Sample collection, enzyme extraction, gel electrophoresis, gel staining, photography, and fingerprint analysis made up isozyme analysis (Sumarani *et al.* 2004). Isozymes were used for fingerprinting and characterization of napier grass (Bhandari *et al.* 2006), paper flower , cassava (Sumarani *et al.* 2004) and garlic. However, Isozymes assay faced some issues i.e., degradation of proteins during samples collection. Protein extraction itself is a tedious and strenuous job (Nybom *et al.* 2014). Due to faulty gel preparation, the wrong kind and quantity of electrophoretic and grinding buffer, grinding and staining techniques, and the physiological and ontogenetic circumstances of tissues, electrophoresis occasionally fails to reveal polymorphism. The outcomes of an isozyme analysis are significantly impacted by variations in sample time and tissue type (Johnson *et al.* 2010).

DNA markers

Research on plant diversification frequently use a variety of DNA marker techniques. Because each person's DNA sequence is distinct, it is possible to study the genetic diversity and relatedness of species using sequence information. First and second generation markers include RAPD, RFLP, AFLP, and SSR markers. First and second generation molecular markers include RFLPs, RAPDs, AFLPs, and SSR, whereas third and fourth generation markers include SNPs, DArT tests, and GBS (Paux *et al*. 2012). Molecular markers are frequently thought of as potentially valuable technologies for raising the yields of pulse crops (Kelly *et al.* 2003) . It is suggested that DNA

markers, in particular RAPD, AFLPs, and SSRs, are a suitable technique for identifying clones, somaclonal variations, breeding lines and hybrids, and cultivars as well as monitoring introgression, mapping QTLs (Paterson *et al.* 2003) and to study genetic diversity in maize crop. RFLP is probe based DNA marker or non PCR based marker. PCR based markers includes RAPD, AFLP, SSR, ISSR, GBS, DArT.

Non PCR based DNA marker

Restriction fragment length polymorphisms (RFLPs)

The earliest DNA markers employed are RFLP. In order to detect DNA fragments of the same size that differ in one base pair, RFLPs use restriction enzymes to cut genomic DNA before hybridising to DNA-labeled probes. RFLP markers are utilized to identify recessive features since they are co-dominant (Uddin and Cheng 2015, Ben-Ari and Lavi 2012). For taxonomic investigations and to comprehend the relationships between the species in several crops, including, tomatoes, peanuts, and RFLPs were employed. A complex, time-consuming, and expensive method of genotyping is RFLP genotyping. DNA probes are not readily available for a lot of plant species. The single locus nature of RFLP makes it challenging to detect base pair changes other than one. Oligonucleotide probe hybridization is a challenging technique that is sensitive to small temperature changes (Ben-Ari and Lavi 2012).

Amplified fragment length polymorphisms (AFLP)

For a more dependable banding pattern, the AFLP marker method combines RFLP and PCR. In that it finds restriction segments in the genome, AFLP is analogous to RFLP. Instead of using southern hybridization to identify genomic restriction fragments, PCR amplification is used instead. This method only detects the presence or absence of restriction fragments, not their length. Mango, sorghum, wheat, and sweet potato genetic diversity have all been studied using AFLPs. Major agronomic traits, fiber quality traits, and fingerprinting investigations have all been done in, sweet potato (Zargar *et al.* 2017). Since AFLPs are dominant markers, they need both high quality and amount of DNA and are unable to distinguish between homozygous and heterozygous individuals.

Randomly amplified polymorphic DNA (RAPD)

William and colleagues developed RAPD markers for fingerprinting studies RAPD markers were mostly used (Nybom *et. al.* 2014, RAPD markers are useful for analyzing the diversity of many plant species (Sinha *et al.* 2013). The small sample size, quick turnaround, lower cost, and lack of need for prior knowledge of the genome sequence are all advantages of RAPD over other methods. Genomic DNA is used to create PCR fragments, which are then electrophoretically examined to create multi-locus banding patterns that may be viewed under a UV transilluminator. Examination of variations in the size range of PCR results is done for genotype characterization and fingerprinting.

Single nucleotide polymorphism (SNPs)

SNPs are an efficient and popular DNA fingerprinting approach which was firstly proposed by Lander in 1996 (AlS amarai and Al-Kazaz 2015).These comprise single base transversions, transitions, deletions, and insertions and describe sequence polymorphisms in the genome that come from changes or mutations in a single nucleotide (A, T, C, or G) at the particular location. The majority of SNP mutations are transitions, compared to other categories. SNPs are genetically abundant, genetically stable and genotyping chips can be automated to enable high throughout analysis. SNPs, like SSRs, can be mined from the genome database, though. SNPs' bi-allelic nature, which results in two alleles per locus, making them an effective data management tool by generating a sizable database of marker data. The necessity for high-density genetic markers for multi-factorial illness investigations and QTL-based mapping has recently given rise. The basis of SNPs is the hybridization of DNA fragments with SNP chips (high density DNA probe arrays), and the subsequent naming of the SNP allele based on the hybridization outcomes (Yang *et al.* 2013).

Inter simple sequence repeats (ISSRs)

SSRs are frequently used in DNA fingerprinting as PCR-based multi-locus molecular markers since

1994. Using chosen 16–20 base pair long microsatellite sequences as polymerase primers, this approach amplifies inter-specific SSR sequences of varying lengths to create multi locus markers. There are more reproducible bands produced by ISSR primers than RAPD primers because their sequences are frequently longer than RAPD primer sequences. ISSR markers are dominant in nature but have several disadvantages, such as poor repeatability when compared to other markers. These markers are extensively used in phylogeny, genetic diversity, linkage studies, gene tagging, genome mapping, and evolutionary biology research due to their high polymorphism, though.

DNA fingerprinting applications in crop improvement

Markers are used in the identification of crop varieties, crop protection, heterosis prediction, seed purity analysis, conservation and assessment of plant germplasm resources, building of genetic maps, genotyping, and molecular marker assisted breeding (MAB).

Crop protection: The characterization of germplasm and varietal protection are two uses for contemporary fingerprinting technology. To demonstrate the potential of fingerprinting approaches to address variety protection challenges, the International Association of Breeders has done model studies in maize and tomato. The International Union for the Protection of New Varieties of Plants (UPOV) has made a conscious effort to develop and employ fingerprinting methods in DUS testing (Archak 2000, He *et al.* 2020) Crop Forensic botany can make use of fingerprinting. To settle disagreements, DNA fragments obtained from suspects' possessions and crime scenes have been analyzed using SSR and RAPD procedures.

Prediction of heterosis: The ability to forecast heterosis is crucial for breeding process and efficiency improvement. Additionally, DNA markers do away with the problem of isozyme-based heterosis prediction, which is too constrained for widespread application. The heterosis of boll number and weight in single cotton was correlated with the genetic distance of the molecular marker.

Identification of cultivar and seed purity analysis: For identifying molecular markers for DNA fingerprinting, previous researchers considered three criteria i.e., codominance, polymorphism and allele uniqueness (Lukman *et al.* 2008). Determining the genetic purity is one of the most important quality control elements in the development of hybrid seeds. The classic field purity test is time-consuming, challenging, and findings are acquired after the growing season. It looks at a number of plant morphological trait. Due to their superior specificity, selectivity, simplicity, precision, and genetic stability, DNA molecular markers may detect changes in DNA levels without having an adverse impact on the environment, which has major advantages in the detection of seed purity.

Germplasm resource evaluation and conservation: The use of DNA molecular markers for germplasm identification, evaluation, and preservation is crucial. DNA markers are utilized to filter the crucial germplasm and protect and maintain the breeding population. The knowledge of their genetic variety, origins, and evolutionary connections would greatly help us use the germplasm resources at our disposal more effectively and serve.

Genetic diversity assessment: Another fruitful use of DNA fingerprinting is to study genetic relatedness among genotypes/species. Parentage analysis is mostly done for this purpose which is an efficient way to find gene flow. SSR markers are mostly used but it is also found that other multi-locus markers can also be used with great confidence such as AFLPs when dominant alleles are present in between frequency of 0.1 to 0.4 .Genetic relatedness also provides valuable information about the domestication process as data is taken from different ecological zones. Since nuclear DNA-generated markers can only produce phenetic analyses rather than phylogenetic analyses, precise determination of genetic relatedness is not achievable, but SSR and AFLPs produce satisfactory results. When studying specific chromosomal areas using a huge collection of SSR markers that span the entire genome of cultivars, the HiDRAS research demonstrated the best method for conducting phylogenetic analysis among genetically comparable apple cultivars. In this manner, genetic relatedness among apple varieties was calculated.

Genotyping: To identify specific cultivars, DNA

fingerprints created using PCR or non-PCR based markers are utilized. Compared to conventional morphological and molecular characterization, these are more trustworthy (Iqbal *et al.* 2021). The Plant Breeders Rights Rules require DNA fingerprinting to safeguard varieties. It is an effective method for identifying closely related species and types as well as for estimating genetic relatedness and assessing genetic diversity.

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