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DNA Fingerprinting of Crops and Its Significance in Crop Improvement

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

One of the improvements made possible by the era of genomics is DNA fingerprinting. DNA fingerprinting can be used to detect a variety of genetic changes. In breeding programmes fingerprinting is used to improve productivity and performance. Variety differentiation was relied on morphological characteristics prior to the introduction of genomics and proteomics approaches. Although morphological markers have been used for decades, protein-based markers like isozymes were discovered in the middle of the twentieth century and used for fingerprinting and crop genetic variety assessments. But nowadays, the emergence of genome sequencing has led to the widespread use of genetic markers for crop fingerprinting. The discovery of DNA-based genetic markers has improved genetic research. For genetic diversity estimation, crop evolution, gene mapping and phylogeny, diploid/haploid crop appraisal, heterosis analysis, varietal identification by using marker assisted selection (MAS), highly polymorphic DNA markers are generated. The use of DNA markers for crop fingerprinting started with RFLPs that did not involve PCR and subsequently advanced to PCR amplifiable markers such as AFLPs, RAPDs,

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SSRs, ISSRs, SNPs, and GBS. This review will provide an outlook on several types of markers and their significance in DNA fingerprinting of crops and possible applications, as well as suggestions for further research.

Keywords: Crop fingerprint; markers; genetic variability; applications.

1. INTRODUCTION

Somatically stable DNA fingerprints that are truly different to a person were first shown by Alec Jeffreys and colleagues in a series of studies published in 1985. These portions of DNA, known as tandem repeats or minisatellites, provide the basis for DNA fingerprinting [7,9]. Throughout breeding programmes, it is essential to have proper seed cultivation, marketing, and product supervision in addition to proper cultivar identification, cataloguing, and sustainability. By using morphological characteristics and the distinctness, uniformity, and stability (DUS) method, formerly species and variations were defined [67]. Morphological features are less efficient for variety identification due to unpredictable genetic regulation and environmental variation. The advent of modern biotechnology has provided us with new resources that may assist us in enhancing our breeding techniques for plants and gaining a deeper understanding of their genetics [31,33]. Smith and Wilcox discovered restriction enzymes, while Karry Mullis and his colleagues discovered the polymerase chain reaction (PCR), which made it easier to examine the makeup of organisms at the DNA level and generate a genetic fingerprint. DNA fingerprinting, among various molecular methods, has emerged as the most advanced and innovative approach for identifying plant genotypes [49]. A DNA fingerprint is a definite pattern of cultivars determined by DNA markers. A short section of DNA that displays variability between organisms in the form of base deletions, insertions, and substitutions is known as a DNA marker. DNA markers provide different benefits over morphological and biochemical [53] markers for obtaining a genome-specific profile [34,39]. DNA markers are a reliable and cost-efficient method for distinguishing plant genotypes. They also provide an effective explanation for the genetic diversity and variety that exists among different varieties and species.

The use of DNA fingerprinting has allowed for the classification of inbred lines into elite populations and to differentiate cultivars [32]. However, it is unaffected by environmental factors and changes in gene expression throughout time [38,43]. Although there are non-PCR methods for identifying genetic diversity, such as Restriction Fragment Length Polymorphisms (RFLPs), the majority of novel DNA marker methods rely on amplification by PCR (polymerase chain reaction). These include Random amplification polymorphic DNA (RAPD), Genotyping by sequencing (GBS), Inter Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR) and Diversity Arrays Technology (DArT) [48]. This review aims to provide an extensive analysis of the basic concepts of DNA fingerprinting, as well as a comprehensive evaluation of the different methodologies based on their potential for repeatability, accuracy, cost-effectiveness, and discriminatory power. Furthermore, in addition to the use of DNA fingerprinting, its applications in crop development are also thoroughly analysed, along with its potential in the future.

Types of markers used for fingerprinting in crops

Genetic markers are classified into three groups:

I) Morphological markers (classical or visible markers)
II) Protein markers (also known as biochemical markers or Isozymes)
III) Molecular markers (or DNA markers)

2. MORPHOLOGICAL MARKERS

In the beginning, scientists used morphological markers to identify and separate different varieties. A "fingerprint" is a specific characteristic of a genotype that allows for easy and rapid identification. Traits such as color, Fruit form, size, and pubescence of the leaves, as well as the number of flowers on each spike were the most widely utilized morphological markers for variety identification [68]. As technology has progressed, however, the morphological technique has been discredited as a valid means of cultivar recognition and genotypic evaluation. The expression of morphological traits is restricted to the homozygous state and is thus controlled by recessive genes. Due to the

quantitative nature of these characteristics, evaluating and genetic mapping them may be a difficult job. Many crops i.e., sugarcane [58], sugarcane, and peas [64], Napier grass [11], bougainvillea [41] were fingerprinted using morphological markers.

3. PROTEIN MARKERS (ISOZYMES)

Isoenzymes were first discovered in plant research in the early 1960s, and their relevance grew rapidly during the 1970s and 1980s. Isozymes are distinct molecular weights and electrophoretic mobility variations of an enzyme that have the same function or catalytic activity. On the other hand, protein extraction was usually difficult, particularly for plants with a high phenolic composition in their leaves. This was one of the most typical issues that occurred. Another significant issue was the seldom presence of allozyme polymorphism across similar variants. Isozymes were used for fingerprinting around 1960 due to their rapidity, precision, and lack of dependency on external factors [49]. Many methods were used in the isozyme examination. They included sample collection, enzyme isolation, gel electrophoresis, gel colouring, photography, and fingerprinting. The Isozymes assay, on the other hand, has certain problems, such as the breakdown of proteins during the sample collection process. The process of protein extraction in and of itself is laborious and time-consuming. Isozymes were employed in the process of fingerprinting as well as differentiating grapevine [56], Napier grass [11] and garlic [28].

4. MOLECULAR MARKERS (DNA MARKERS)

Molecular markers, also known as DNA markers, are specific compounds that show simple differences between different races of the same species or between distinct species. Several polymorphism-detecting procedures or technologies, such as southern blotting—nucleic acid hybridization, the PCR technique, and the sequencing of DNA, have led to the development of a wide variety of systems for using DNA as a marker [16]. There are two main categories of DNA markers: those that don't rely on polymerase chain reaction (PCR), and those that rely on PCR. On the other hand, DNA sequencing-based markers, such as Single Nucleotide Polymorphism (SNP) on the basis of expression, were divided into two groups; i.) Dominant markers: The marker is exclusively associated with the one particular form of the trait that the user has chosen to designate. On the contrary, the character's alternate form is not associated with any kind of marker [17], such as RAPD. ii.) Co-dominant marker: Both forms of the designated character have a relationship to the marker [17] such as RFLP (Restriction fragment length polymorphisms), AFLP (Amplified fragment length polymorphisms), SSR (Simple sequence repeat markers)**,** SNP (Single nucleotide polymorphism), EST (Expressed sequence tag), etc. The number of molecular markers is almost uncountably high, and these markers are not influenced either by environment or the phase of the plant's growth in which they are located [72]. Examining the DNA of crops using PCR, non-PCR, and sequence-based markers are summarised in Table 1.

5. NON-PCR BASED MARKERS

Restriction fragment length polymorphisms (RFLPs): In the early 1980s, the Restriction Fragment length Polymorphism (RFLP) system was created as the first and most frequently used marker system .In RFLPs, genetic material is first digested using restriction enzymes like EcoR1, then separated on a gel, and then hybridized (southern hybridized) to DNA-labelled probes in order to identify those with single base differences. Since RFLPs are co-dominant markers, they are able to distinguish DNA fragments from all homologous chromosomes, which enables them to recognize the phase of DNA molecules during which they are coupled. However, DNA analysis with RFLPs is a complicated process that also takes a significant amount of time and money. During hybridization, probes (oligomers) are susceptible to minor temperature fluctuations, making it a difficult procedure [10]. RFLPs require vast amounts of high-quality DNA, which may limit their application under circumstances where the source material is limited.

PCR-based markers: Single-locus and multi-locus approaches are the two categories into which PCR-based methodologies are separated [49].Here, we provide a quick summary of the two PCR-based marker techniques.

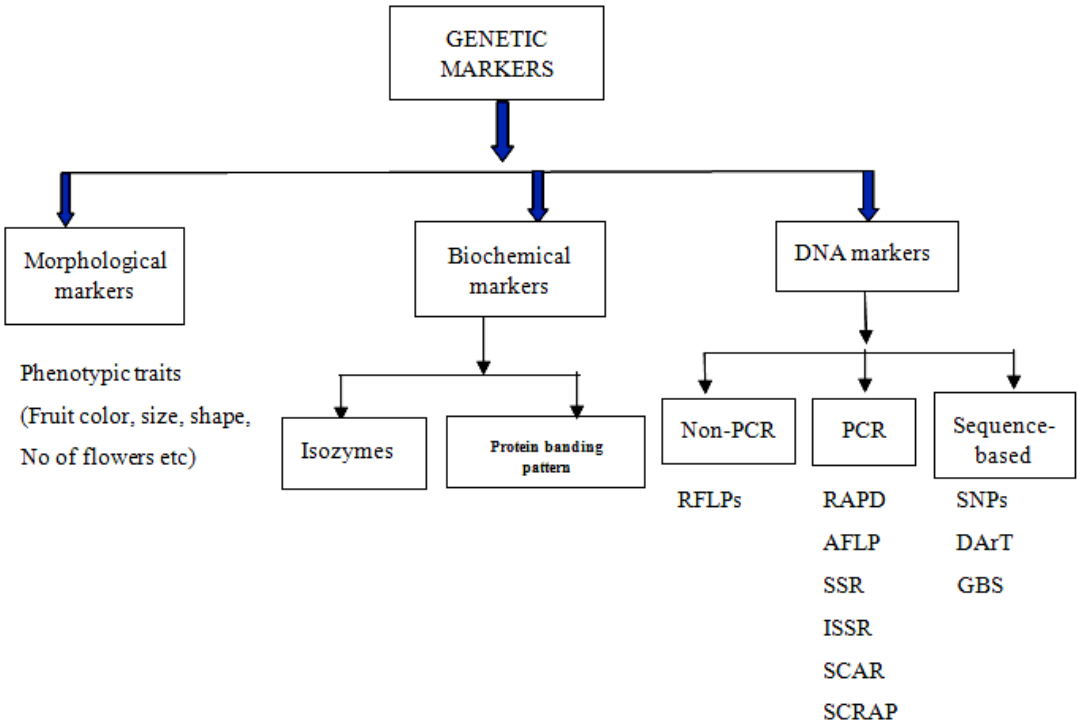

Fig. 1. Different kinds of genetic markers used in crop improvement [3 & 29]

Table 1. A brief history of the distinct marker systems utilized for fingerprinting the DNA of several crops that are economically significant

Type of marker	Crops	References
Morphological marker	Wheat, Sugarcane	[65,51]
Isozyme marker	Napier grass	[11]
NON-PCR based markers		
RFLP	tomato, Rice, peanuts	[47,24,50]
PCR based markers		
RAPD	Potato, Wheat, Maize, Ginger, Rice	[45,57,62]
AFLP	Cotton, Rice, Wheat	[1,12,63]
SSR	Soybean, Wheat, Maize, Sugarcane	[4,26,32,70]
ISSR	Tomato, Rice, Maize, Lemon grass, Chickpea, Wheat	[42,62,40,2,23,20]
Sequence based markers		
SNPs	Potato, Maize, Ginger, Wheat, Potato, Chickpea	[61,66,30,44,18]
DArT	Groundnut, Wheat	[50,19]
GBS	Maize	[69]

Amplified fragment length polymorphisms (AFLP): To provide a more precise banding pattern, the Amplified fragment length polymorphism marker approach involves PCR & RFLP. In this method, the DNA in a sample is broken up into pieces using restriction enzymes, just like in RFLP analysis. However, only a small number of these pieces are then tested using a more specific type of PCR [42].Primers are used in this strategy because they have the ability to bind themselves efficiently to respective target regions (restriction sites and adapter), in addition to a few nucleotides that are located nearby the restriction sites. This needs a large amount and good quality of DNA. AFLPs are less expensive than RAPDs, have greater repeatability, and can identify larger levels of polymorphisms than RFLPs. AFLPs were applied as a helpful approach to monitor genetic diversity in sweet potato, cotton, soybean, and Bt rice. Additionally, AFLPs were utilized for fiber-quality attributes, tagging of essential agronomic factors, and

fingerprinting studies in sorghum, wheat, mango, and sweet potato [73].

Simple sequence repeat markers (SSRs): Short tandem repeats and Microsatellites are other names for SSRs. Short tandem repeats (SSRs) are repeating sequences of nucleotides that are between 6 and 10 base pairs long and can be found all over the genome [35]. DNA fingerprinting by SSRs is a straightforward method that requires only two components: a set of primers or adjacent markers and gel electrophoresis to differentiate the products of PCR. Silver nitrate staining, radiography, ethidium bromide staining, and fluorography are used to verify the banding patterns [49]. In comparison to RFLPs and RAPDs, this marker system is locus-specific, repeatable, and has a high level of polymorphism. SSRs are co-dominant in nature. These markers have emerged as the standard marker for many plant-related advancements because they are highly variable and cover a large part of the genome.

Randomly amplified polymorphic DNA (RAPD): Under Polymerase chain reaction assisted markers, RAPD markers were commonly applied in fingerprinting research [25,49] William and his co-workers improved RAPD [71].This method amplifies random sequences from a DNA template by utilizing one random, short primer (2–10 base pairs) that binds to many different genomic regions[71]. This results in PCR fragments from the whole genetic material. Later, the generated multilocus banding patterns were observed by using an UV transilluminator, which had previously been examined by electrophoresis. Since sequence variations in either of the binding sites of primers create polymorphisms between individuals, RAPD markers are dominant. The use of RAPDs as systematic characteristics is constrained since it can be difficult to establish trait similarities and because of their low repeatability [8,57,59].

Genotyping by sequencing (GBS): GBS is a novel method that has emerged with the advent of NGS (next-generation sequencing) to take advantage of the large range of genetic variation present in plants [15].This bioinformatically intensive approach has the potential to provide a comprehensive analysis of genetic variation throughout the whole genome (i.e., including exons & introns). During the preparation of the sample, steps like isolating and measuring the DNA, digesting it with restriction enzymes, joining it with the appropriate adapters, and amplifying it with PCR primers that are specific to the adapters are done. Grouping samples, selecting a size range, adjusting the concentration, and making a sample sheet are the steps that make up the library's construction. For the subsequent two phases, sequencing and SNP recognition, several bioinformatic methods have been applied e.g., Minia, Bowtie 2, Fastx-collapse, BCF, SAM tools [52].Genotyping by sequencing may be the most appealing technology for crop fingerprinting due to parallel identification of SNPs because it is an easy, economical, accurate, highly reproducible, and rapid method.

Inter simple sequence repeats (ISSRs): Since 1994, for DNA profiling, ISSR markers have been commonly used. ISSR are multi-locus genetic markers that can be amplified with PCR. By using particular microsatellite segments between 16 and 20 bases in length as primers, this approach amplifies inter-specific SSR sequences of varying lengths to generate multi-locus markers. ISSRs are highly polymorphic, highly repeatable, and easy than RAPD. These are usually non-transferable and dominating. However, as polymorphic nature is high in this type of marker, these are often used in phylogenetics, genetic variability, whole-gene analysis, gene marking, and research on genetic linkage.

Single nucleotide polymorphism (SNPs): Single nucleotide polymorphisms (SNPs) are a common kind of genetic variation in individuals. Lander first presented the concept of SNPs in 1996 [5].This type of marker is widely used and has proven to be accurate. In SNPs, among genotypes, there is variation in the sequences of DNA or one nucleotide base. Based on nucleotide alterations, SNPs are classified as transversions and transitions. In plants, the levels of SNPs are typically in the range of one SNP per one hundred to three hundred base pairs. SNPs can be found at varying rates in different chromosomal locations inside gene coding regions, regions of DNA sequence that occur between genes on a chromosome and non-coding portions of genes. The genetic stability and abundance of SNPs, along with the technology of sequencing chips, make it possible to do large-scale screening. It is now very easy and affordable to find many SNPs in a short period in wide range of cultivated plants. Since there are two alleles per locus, SNPs are a remarkable tool for data management as they make a massive collection of marker information. SNPs are less significant per locus than microsatellites because they lack information per locus [69].

Table 2. A review of several marker systems for fingerprinting of DNA

Marker description	RAPD	SSR	RFLP	AFLP	SNP	ISSR
Genomic coverage	Whole genome	Whole genome	Low-moderate	Whole genome	Whole genome	Whole genome
Genomic abundance	High	Moderate to high	High	High	Very high	Moderate
Reproducibility	Low	High	High	High	High	Moderate
Multiplex ratio	Moderate	Medium/high	Low	High	Moderate to high	Moderate
Quality of DNA required	Low	Moderate	High	Moderate	High	Low
Level of polymorphism	High	High	Moderate	High	High	Moderate
Marker index	Moderate	Moderate to high	Low	Moderate to high	Moderate	Moderate
Automation	Moderate	Moderate/ High	Low	Moderate/ High	High	Moderate
Cost per assay	Low	Moderate to high	Moderate to high	Moderate	High	Low

Source: [29]

6. DNA FINGERPRINTING APPLICATIONS

Genotyping, assessing genetic diversity, and protecting food crops are just a few of the many uses for DNA fingerprinting in plant sciences that are discussed here.

7. GENOTYPING OF CULTIVARS

DNA fingerprinting helps to find out how pure a variety is, which in turn stops the sale of contaminated seeds. Genetic markers are also an accurate way to trace the origin of newly developed cultivars. New crop varieties can be registered under the Breeders' Rights Guidelines to safeguard their intellectual property [32].Plant Breeders Rights Rules require DNA fingerprinting in order to secure varieties. SSR markers are ideal for genetic analysis of asexually propagated cultivars since they are extremely repeatable. SNP markers have recently received a lot of interest as a method to recognize vegetatively propagated species. Because of the high amount of cross-pollination, particularly during the seed cycle, when alien pollen pollinates the egg and introduces new genetic variants, genotyping seed propagated plants through marker-assisted selection is complicated. For this, innovative and reliable technology is necessary; The DArT technology is being utilized to differentiate between seed propagated varieties [36].SSR assessment is applied to detect chimera clones and genotype somatic mutations [46].AFLPs, on the other hand, are utilized to identify *In-vitro* generated crops since these changes are hereditary, beneficial, and have prolonged regeneration cycles [37].

8. ANALYSIS OF GENETIC VARIABILITY

In molecular marker technology, recent advancements have become a significant approach to understanding genetic variation and expanding breeding techniques [54].Pedigree assessment is commonly used for this purpose, as it is an effective method of determining genetic drift. Markers are employed in collection of genetic resources, improved breeding resources, and other cultivars to estimate or quantify genetic diversity, which aids in the identification of germplasm, the development of PGR data systems, and the evolution of varietal information structures. Plant breeders may utilize genetically profiled and DNA fingerprinted breeding material, which helps in the classification of breeding lines and pure lines into different heterotic classes and allows them to choose the most effective crossover method for improving hybrid vigour. These findings are useful in cross-pollinated crop breeding for identifying different parents and exploiting maximal heterosis [60].For drought resistance, sixteen different genotypes of rice were examined using SSR markers in order to determine their level of genetic diversity. Drought tolerance was discovered in the cultivars Giza 178, Giza179, and GZ1368- S-5- 4 [21].

9. FOOD/CROP PROTECTION

Cultivars protection and germplasm assessment can both benefit from advanced genotyping techniques. The UPOV (International Union for the Protection of New Varieties of Plants) is constantly developing and applying new fingerprint methods to make sure that new plant varieties are unique, uniform, and stable [6,27].It is well accepted that fingerprinting is an effective technique for identifying malpractices in food products as well as medications. A good illustration of this would be the practise of combining more costly basmati varieties with less expensive non-basmati varieties. To address this problem, numerous markers for adulteration studies have been recommended [6,22].Microsatellite markers are often used to map the genomes of several plant types, and microsatellite linkage maps are now accessible in some significant crops (maize, wheat, soybean, rice, chickpea) for genetic analysis. Further, MAS uses fingerprinting methods to enable breeders to speed up the assessment of traits in fewer generations [14].

10. CONCLUSION AND FUTURE PROSPECTS

As early as the nineteenth century, variety differentiation was identified on the basis of the external morphology of plants. The development of NGS technology in the 21st century has made genetic analysis by sequencing the benchmark for identifying individuals by using their DNA. Gene sequencing, genetic diversity estimation, crop diversification and phylogeny, heterosis analysis, and evaluation of diploid/haploid crops, as well as cultivar differentiation, have all benefited from advances in molecular markers combined with increased performance technologies. In genetic diversity assessments and gene sequencing, RAPDs and AFLPs have been frequently employed. When it's necessary to test sites over the entire genome, both approaches come in handy. Therefore, there are certain limits. The rapid changes in gene expression, high polymorphism level, and great repeatability of SSR markers have led to their increased adoption. Genetic studies and sequence analysis, as well as the advancement of powerful bioinformatic tools, have made it possible to quickly identify unique genomic areas for crop DNA fingerprinting. Advances and discoveries in sequencing technologies should be promoted, as well as the development of cost-effective genotyping-by-sequencing technologies.

In the future, a worldwide DNA fingerprinting library for all crops should be created, with DNA fingerprints of all major crop types registered under PBR rules. As both Pakistan and India appeal to the trademark "Basmati Rice," this system will also be useful in settling trademark conflicts between countries [13].Such problems will be avoided in the future when it comes to the creation of a world library of agricultural types. The fingerprinting of newly developed plant varieties is proposed as a method for securing plant breeders' rights and ensuring the continued availability of novel varieties across national borders. Therefore, for reliable protection of cultivars and fingerprinting of DNA in developing and less developed nations, cost-effective large DNA fingerprinting methods are needed [55].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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