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Molecular Marker Techniques and Genotypic Characterization Approaches in Plant Breeding

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ABSTRACT

Plant breeding, often known as the science of plant development, is the study and practice of modifying a plant's genetic makeup through a variety of breeding techniques to produce higher-quality, more prolific, and more resistant to harsh environmental circumstances. Classical breeding programs are indispensable techniques for increasing yields and improving plant characteristics, but they are progressing too slowly to meet the increasing food demand of the rapidly growing world population alone. Considering that the development periods of plants are generally long in traditional plant breeding, the opportunity to develop higher quality and more productive species that are more resistant to abiotic and biotic stress factors is very limited. Because there are multiple steps required in producing new plant varieties, including hybridization, selection, and testing, the process of creating a new variation takes several years. However, it is important to rapidly develop plant varieties with desirable characteristics to meet the increasing food demand of the rapidly growing world population, so the application of biotechnological methods integrated into plant breeding and combined with traditional methods can help reduce food shortages. Today, with the quick acceleration of biotechnology, molecular DNA marker technology has been developed in plant breeding and very important developments have been experienced. Thanks to the development of molecular tools for genetic research aimed at improving agricultural traits in plants related to crop yield, crop quality, or tolerance to adverse environmental conditions, we now have a much better understanding of plant genetics and the architecture and function of plant genomes. Therefore, it is of critical importance to revise current breeding procedures by incorporating molecular markers into breeding programs in the future.

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Introduction

Plant breeding is an exciting field of applied science that employs choice and crossbreeding to progressively improve plants for traits and attributes that breeders and consumers are looking for (Zuurbier, 1994). Finding and introducing novel genetic material (such as genes conferring disease resistance) from external sources, such as gene bank accessions, related plant varieties and native varieties, is a key objective of plant breeding (van Berloo, 2000). Although conventional breeding procedures have proved to be extremely efficient in continuously creating superior varieties, current breakthroughs in biotechnology and molecular biology have demonstrated that they may be leveraged to improve plant breeding efforts and hasten variety creation (Moose et al., 2008). New techniques and processes for introducing genetic material from intra- or interspecific plants have become possible thanks to molecular approaches in biotechnology and plant breeding, without the problems commonly associated with the introduction of "wild genes" using old methods (van Berloo, 2000).

While Mendel demonstrated the essential link between genotype and phenotype, Darwin articulated the scientific principles of selection, and those are the discoveries that paved the way for a more scientific approach to plant breeding. The scientific foundation of plant breeding has been strengthened by subsequent developments in the fields of molecular biology, biotechnology, cytogenetics, quantitative genetics, plant biology, and most recently genomics (Varshney et al., 2009). In many countries across the world, new improved agricultural products emerged in the second half of the 20th century, leading to an increase in crop productivity systems. This was made possible by developments in genetic engineering, molecular biology, and molecular breeding research (García-Gonzáles et al., 2010; James, 2008; Navarro Mastache, 2007; Christou et al., 2006; Vasil, 1994). The potential benefits of marker-assisted selection in plant breeding, along with the theoretical advantages of using genetic markers in conjunction with the selection of plants carrying genes of interest that offer a way to introduce genetic information

encoding desired traits into plant cells, were first documented about eighty years ago. Without the development of these techniques, it would have been impossible for us to eventually integrate advanced and new agricultural products into production systems (Pareek, 2006; Crouch and Ortiz 2004).

Following the 1980s, cultivated plants were successfully developed using some biotechnological techniques. Later, conventional plant breeding techniques and the latest advancements in genomic research and molecular marker applications were combined to create a plant breeding process that was suitable for many agricultural systems worldwide (Karakas, 2021). As a new era in plant molecular research begins, molecular markers have become widely used in traditional breeding programs. Molecular markers have made the use of DNA fingerprinting to identify polymorphism in various individuals an essential tool for agricultural progress (Ahmad et al., 2010; Çakır, 2023). Base sequence polymorphisms within a species are incredibly common at the DNA level. By using restriction enzymes that only cut sections with specific sequences, it is possible to identify these polymorphisms. This can be achieved by first determining the various lengths of the DNA fragments that electrophoresis produces. Electrophoresis detects alterations in the DNA fragments produced when polymorphism involves the insertion or deletion of DNA between two conserved restriction sites (Flavell, 1989). Genomics, plant breeding, taxonomy, and genetic engineering have all been shown to benefit from the versatility of DNA-based molecular markers (Joshi et al, 1999). Certain breeding companies have been employing markers for the past 20 years to increase breeding efficiency and cut down on the time it requires to create a novel variety. Molecular marker-assisted selection is viewed by plant geneticists as a useful support for plant breeding initiatives (Lammerts van Bueren et al., 2010; Joshi et al., 2011).

Plant Breeding: Applications of Morphological, Biochemical and Cytological Markers

Morphological, cytological, and biochemical markers were employed to distinguish one genotype from others before molecular (DNA) markers were created (Dar et al. 2017; Dar et al., 2019). Morphological markers are phenotype-based indicators that are easily recognized by the eye and are genetically regulated by one or more genes (monogenic and oligogenic) (Eagles et al., 2001). Examples of morphological markers are morphological markers related to semi-dwarfing, high seed productivity in wheat and rice, low shell ratio, high oil ratio in sunflower seeds, and light seed coat colour, high oil, low protein ratio in sesame. These markers may be employed as indirect selection criteria in breeding programs rapidly, inexpensively, and reliably (Baydar, 2020; Jiang, 2013). Cytological markers are markers related with differences in chromosomal size, shape, location, and banding levels (Nadeem et al., 2018). Cytological markers are particularly useful in diagnosing normal and mutant chromosomes, identifying linkage groups, and physical mapping (Jiang, 2013). When studying biochemical markers, we refer to the synthesis of the same enzyme by various genes as isoenzymes. Isoenzymes such as phosphoglucomutase, esterase, and peroxidase were the first genetic markers discovered, and it has been claimed that the basic need for an isoenzyme to be utilized as a genetic marker is to generate polymorphism. Examples of these markers are cannabinoids in hemp (De Meijer et al., 2003) and terpenoids in mint (Baydar, 2020).

These markers, which have characters that can be monitored in terms of inheritance patterns at the morphological (such as flower colour), biochemical (such as isoenzymes) and DNA level (molecular markers), are called genetic markers (Walton, 1993). These traits carry – albeit indirectly – genetic information about other traits of interest in the organism under study, so these traits are considered markers. The notion of a genetic marker is not new. Morgan (1910) and his students investigated the fruit fly Drosophila to see how numerous mutant characteristics they developed interacted with one another. As a result, they developed the concept of linear gene map. The real concept of marker emerged as a result of Sax's (1923) studies on the connection of characters showing qualitative expansion with characters showing quantitative expansion. The understanding of markers was shaped in the early 20th century by observing the connection between bean seed pigmentation and seed size. In this study, seed pigmentation was used as a marker to determine bean grain weight (Yıldırım and Kandemir, 2001). However, in later years, the practical applicability of these markers remained limited due to negativities and deficiencies such as the small number of these markers and the absence of mutant characters obtained through mutation studies in natural populations. Thanks to subsequent research, the idea of using DNA itself as a direct marker emerged (Paterson, 1996; Yıldırım and Kandemir, 2001; Mondini et al., 2009).

An Overview of DNA Marker Techniques

Today's green revolution is biotechnology, which provides strong instruments for the effective genetic growth of agricultural plants and other living things via continuous and fast expanding technologies targeted at the efficient utilization of biological systems for the benefit of humanity. The potential uses of genetic marker linkage maps and marker-assisted selection in plant breeding, as well as the theoretical benefits of employing genetic markers, were first put forth approximately 80 years ago (Crouch and Ortiz 2004). There has been a revolution in the field of plant genomics with the increasing interest in the subject in recent decades. Molecular markers have played a crucial role in this revolution as they are well-suited for studying plant genomes. Research on genome structure and in particular the genetic mechanisms supporting commercially important traits has increased the greatest advances in agricultural biotechnology. In many higher plant species, single gene markers have been found, mapped, and catalogued by researchers. The rapidly advancing studies of genome or molecular biology, is revealing information about the identity, location, impact, and function of the genes controlling the traits (Jonah et al., 2011).

The first records of transgenic plants created using the Agrobacterium process date back to 1980, specifically marking the beginning of the era of plant biotechnology. In later years, molecular marker systems were created and incorporated into plant breeding to create high-resolution genetic maps and to benefit from genetic relationships between markers and important plant traits (Koziel et al., 1993; Delannay et al., 1995). Many molecular markers have been developed since the 1980s after molecular studies gained momentum. These developed molecular markers and their properties are examined in Table 1. Compared to morphological, cytological, or biochemical markers, new generation DNA-based molecular markers have the advantages of high polymorphism detection and rapid detection, as well as not being affected by environmental conditions or plant development processes (Fang et al., 2016).

Molecular markers are also DNA fragments linked with any gene area in the genome, as well as nucleotide sequences revealed through polymorphisms in nucleotide sequences from distinct genotypes (Baydar, 2020). Polymorphisms in the population are induced by nucleotide sequence deletions, insertions, gene mutations, duplications, and translocations; nonetheless, these markers have no influence on gene function (Mondini et al., 2009). Molecular markers are more trustworthy than morphological, cytological, or biochemical markers because they are not affected by environmental conditions or plant developmental stages (Devran, 2003). Molecular markers are used in genetic mapping, conservation of plant genetic resources, genetic diversity analysis, evolutionary genetic studies, cloning of agriculturally important genes, and marker-assisted breeding research because they indicate differences in nucleotide sequences in the genome (Liu et al., 2014; Gonçalves et al., 2009; Fang et al., 2016; Çifci and Yağdı, 2010).

Classification of Hybridization and PCR-Based Molecular (DNA) Markers

Today, according to traditional breeding program approaches, it is recommended to use molecular breeding procedures together with classical breeding methods. It is now more common to incorporate molecular approaches into traditional programs, that is, to employ them in conjunction, in regions where traditional breeding methods are insufficient. Molecular markers are segments of DNA that indicate a specific field of genetic information. Sections of an organism's genetic code associated to desirable characteristics can be detected using molecular markers. Each sort of molecular marker has its own set of application procedures and is classified into distinct groups (Yılmaz, 2021). For example, polymerase chain reaction (PCR) or hybridization-based molecular markers are classified according to the detection mode, while they are classified as dominant or codominant markers according to the principle of inheritance (Table 1) (Semagn, 2006; Williams et al., 1990).

Uses of DNA markers in plant molecular breeding; characterization of genetic resources (Dar et al., 2019), determination of genetic similarities and distances between varieties (Vianna et al., 2019), determination of parents to be used in the breeding program (Mert et al., 2003), protection of newly developed varieties (Wang et al., 2010), determination of qualitative and quantitative trait loci (Xu et al., 2017), genetic mapping (Begna et al., 2021), bulk segregant analysis (Zhao et al., 2023), F_1 identification and in many other molecular studies (Bianco et al., 2011).

RFLP (Restriction Fragment Length Polymorphism)

The RFLP marker technique was the first molecular marker technology and the only hybridization-based marker system (Miller and Tanksley, 1990; Bark and Havey, 1995). The codominant marker class includes RFLP, the first non-PCR-based marker system developed (Bark and Havey, 1995). It has been proven that characterization of heterozygous individuals can be achieved using this feature (Desplanque et al., 1999). RFLP technique is based on the hybridization of a tagged DNA fragment (probe DNA) to DNA of a similar or different sequence in a DNA sample under study (Yıldırım and Kandemir, 2001). Prior to RFLP analysis, the genomic DNA to be examined is cut to certain sizes using restriction enzymes that identify 4-6 nucleotides, and the cut fragments are passed through an electrophoresis equipment. Then, the DNA fragments are classified according to their size and transferred to nylon filters using the Southern Blot technique, and radioactive 32P or non-radioactive chemiluminescent is applied to the filters. Consequently, if the probe pieces and the DNA pieces on the filter are identical, the probe DNA clings to the piece with a same nucleotide sequence, and polymorphism may be detected with an autoradiogram by exposing the filter to an X-ray film (Baydar, 2020).

Table 1. Classification and Comparison of Molecular Markers (Korzun, 2002; Wolfe and Liston, 1998; Vardar-Kanlıtepe et al., 2010; Genişel, 2013; Amiteye, 2021).

Molecular Marker Technique	Being PCR Based	Polymorphism Level	Inheritance Type	Automation Level	Cost Level	Reproducibility
RFLP	Hybridization based	Low/Medium	Co-dominant	Low	High	High
RAPD	PCR Based	Medium/High	Dominant	Medium	Low	Low
AFLP	PCR Based	High	Dominant	Medium/High	Medium	Medium
SSR	PCR Based	High	Co-dominant	Medium/High	Low	High
ISSR	PCR Based	High	Dominant	Medium/High	Low	Medium
SNP	PCR Based	High	Co-dominant	High	Medium	High
SCAR	PCR Based	High	Co-dominant	Medium	Medium	High
DArT	Hybridization based	Extremely High	Dominant	High	Medium	High

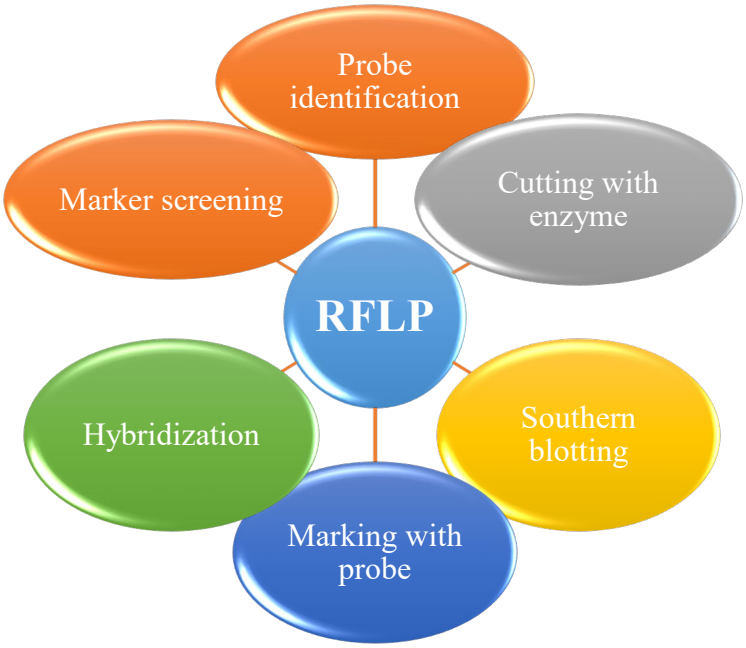

Figure 1. Application stages and sequence of the RFLP technique (Yang et al., 2015)

Differences between and within species are determined with RFLP markers. It is reliable, co-dominant and its polymorphism rate is at a medium level (Grover and Sharma, 2015). The most important disadvantages are that their analysis is expensive, time-consuming, labor-intensive, and requires a large amount of high-quality DNA. Codominant markers such as RFLP are useful for MAS and evolution studies but are time-consuming, can be relatively expensive, and require a high degree of technical expertise (Young et al., 1992).

RAPD (Random Amplified Polymorphic DNA)

When we look at RAPD marker technology, we see that it employs PCR to randomly amplify DNA areas of huge genomic DNA using random oligonucleotide short primers that are generally 6-10 or 8-15 nucleotide long (Babu et al., 2021; Amiteye, 2021). Because the reaction conditions are not particular, this approach allows for random amplification; also, unlike other PCR applications, a single primer is used instead of two, and this single primer is used for DNA amplification by functioning as both forward and reverse primers in both directions. This initiator primer amplifies the sections of the genome where it may bind to two closely spaced segments of DNA. When the amplified DNA fragments are run on an agarose gel, it is observed that certain fragments are generated in some genotypes but not in others (Welsh and McClelland, 1990; Liu, 2007). The variety of primer binding sites and the ensuing DNA fragments of various lengths produce polymorphism. When these applications are performed in a population, genotype analysis of the offspring is performed by looking at the production patterns of the parents (Williams et al., 1990).

Previous DNA sequence information is not necessary for RAPD analysis because random primers are employed (Premkrishnan and Arunachalam, 2012). The RAPD marker system also has the benefit of requiring a small amount of DNA, being simple to make primers for, and having a large number of markers that are dispersed throughout the genome (Kesawat and Das, 2009). This marker system stands out because it gives quick results, is cheap, requires little labor, and has a high polymorphic band ratio. The downside of the RAPD main system, on the other hand, is its limited repeatability and informative power. This system's dependability is limited, and different findings might be achieved in different laboratory conditions (Yorgancılar et al., 2015). A number of critical elements that impact the consistency of RAPD markers include the concentration and quality of the DNA, the type and quantity of DNA polymerase employed, the PCR buffer, the annealing temperature, the concentration of magnesium chloride (Wolff et al., 1993).

AFLP (Amplified Fragment Length Polymorphism)

AFLP marker approach is a molecular marker technique that cuts genomic DNA using two restriction enzymes, each can identify three bases, after that ligates adapters to both ends of cut DNA and then uses AFLP primers of 17-21 nucleotides in length for PCR amplification. Examples of these restriction enzymes are EcoRI, Hind-III, and Msel. After cutting the genomic DNA, adding adapters to the sticky ends of DNA fragments ranging in size from 80 to 500 bps occurs through ligation. It is a marker technique that uses PCR to subsequently amplify a subset of fragments after preliminary and selective amplification processes (Vos et al., 1995; Amiteye, 2021; Baydar, 2020). The PCR procedure consists of an initial selective amplification using primers with one selective nucleotide added to the adapter, and after a final selective amplification using primers with three selective nucleotides added, the resulting AFLP particles appear on a polyacrylamide gel. The technique produces between 50 to 100 particles in a single reaction, which results in a rather high polymorphism rate (Althoff et al., 2007; Mian et al., 2002; Filiz and Koç, 2011). Thus, the AFLP marker analysis technique combines RFLP and PCR techniques to achieve DNA digestion and PCR amplification (Sorkheh et al., 2007).

SSR (Simple Sequence Repeat) and ISSR (Inter Simple Sequence Repeat)

SSRs are sequences that are repeated frequently and a certain number of times in the living genomes, and their repeat unit sizes vary between 1 and 6 nucleotides. Each repeat unit is repeated 5-50 times creating an SSR up to a size of 300 bases, and the total size of all SSRs in a genome ranges from 4 to 20 kb (Jeffreys et al., 1985). These are sequences that differ among species in terms of their location in which part of the genome and the number of times they are repeated. SSR technique was developed based on whether these sequences exist among individuals within the same species. SSRs, or microsatellites, are clusters of sequences in which one to six nucleotide patterns are repeated in tandem across eukaryotic genomes (Somers et al., 2004). For example, these groups are represented as (A)n, (AT)n, (GT)n, (ATT)n, and (GACA)n; where n indicates the number of tandem repeats (Koelling et al., 2012; Weber, 1990). Selection of overlapping SSRs from different genotypes in SSR marker analyses is possible by PCR primer amplification because the DNA sequences surrounding microsatellites are frequently conserved among members of the same species.

SSR technique is widely used in genetic mapping in plants. Because they are highly polymorphic, SSRs provide a high amount of information in plants (Röder et al., 1995). Nevertheless, sequence skipping, improper base pairing, and uneven crossing-over events during DNA replication are the primary causes of variation in numbers, and gel electrophoresis can detect these (Matsuoka et al., 2002). It has been stated that SSR markers make them more attractive and reliable in genetic studies such as genomic selection (Poland and Rife, 2012), with the advantages of abundance in the plant genome, reproducibility, high polymorphic structure, cost effectiveness and high-throughput analysis (Cavanagh et al., 2013; Röder et al., 1995; Allwright and Taylor, 2016). Therefore, SSRs are frequently used in studies (Bhattarai et al., 2020; Dal Canbar, 2023) such as in mapping and DNA fingerprinting studies (Kumar et al., 2016; Parker et al., 2002), biodiversity studies (Muzzalupo et al., 2014), and genetic relatedness studies (Röder et al., 1995), variety testing (Tommasini et al., 2003; Pan, 2006) and relationship mapping (Racedo et al., 2016). Additionally, QTL detection (Jun et al., 2008), genetic variation resulting from selection (Stachel et al., 2000) and its use in many subjects and fields reveal how widely used and preferred it is.

While its codominant and reproducible properties are its most important advantages (Rongwen et al., 1995), the need for sequence knowledge of the studied genome is its shortcoming. However, the other shortcoming of the system is that the high mutation rates and changes in the primer binding regions allow the formation of different and meaningless alleles (Ridout and Donini, 1999; Gökalp, 2022).

When we examine the ISSR technique, it is a type of DNA-based marker frequently used in molecular biology studies in plants (Karaca and Izbriak 2008). The ISSR method is a highly reproducible approach based on the locus-independent random distribution of two, three, four and five repetitive nucleotide units in eukaryotic genomes (Zietkiewicz et al., 1994). This PCR-based molecular marker technique is based on the principle of replicating a spaced DNA segment reproducibly between 2 identical microsatellite repeat segments oriented in opposite directions. This process is known as inter simple sequence repeat (ISSR) methodology. (Amom and Nongdam, 2017). In this approach, a single primer targeting multiple genomic loci is often used to amplify inter-SSR sequences of varying widths (Pradeep Reddy and Siddiq, 2002). These primers used in the PCR reaction are typically 15–30 bp long and are also called microsatellites (Gupta et al., 1994; Nadeem et al., 2018). After PCR amplification, amplified products ranging in size from two hundred to two thousand bp are separated by gel electrophoresis, and the resulting ISSR banding patterns can be visualized by autoradiography or $AgNO_3$ staining. The banding patterns obtained are then scored and transferred to special statistical programs, and polymorphism rates are determined and frequently used in molecular breeding studies. Compared to RAPD, ISSRs are simpler, easier to understand, and more reproducible. Therefore, it becomes easier to perform molecular analyses (Yorgancılar et al., 2015; Chatterjee et al. 2004; Yılmaz, 2021).

SNP (Single Nucleotide Polymorphism)

SNPs, in their simplest definition, are single base differences in a particular piece of DNA between two individuals. It can also be defined as a nucleotide that shows a high rate of substitution among sample individuals in a population (Wang et al., 1998). Brookes (1999) defined SNPs differently: SNPs are single base changes in genomic DNAs that have distinct sequence alternatives, or alleles, which are present in normal individuals in certain populations (with an allele frequency of at least 1% or greater) (Sönmezoğlu et al., 2010). These markers, which are rather prevalent across the genome, occur 500–1000 bp frequently in the exon and intron regions. SNPs are located mostly in non-coding regions of DNA and occur frequently in the genome. The higher the single nucleotide polymorphism, the easier it is to identify variations between individuals. DNA single nucleotide polymorphism can be caused by one or more base deletions or insertions. Genes, non-coding sections between genes, and non-coding parts of the gene (introns) can all contain SNPs. SNPs in the coding region alter the sequences of amino acids, whereas those in the non-coding region have no effect on inheritance or changes in genes (Gözel et al., 2016).

SNP markers are applied to genomes for which AFLP yields insufficient response and which are not amenable to SSR analysis in breeding populations. The methodology involves the sequencing and comparison of DNA fragments that are dispersed over certain sections of the genome. It is very repeatable and amenable to automation (Ching et al., 2002). The primary rationale behind the widespread adoption of SNP markers in molecular plant breeding research in recent times is their exceptional polymorphism, co-dominance, accuracy, high resolution, affordability, and informative value (Lombardi et al., 2014). These days, there are several targeted or whole genome sequencing-based SNP genotyping platforms that use DNA sequencing (Fan et al., 2006). SNP technique in plants with new techniques such as RT-PCR; it is used extensively in genetic diversity, full genome sequencing, population analysis, kinship relationships, QTL, and MAS studies (Beissinger et al., 2013).

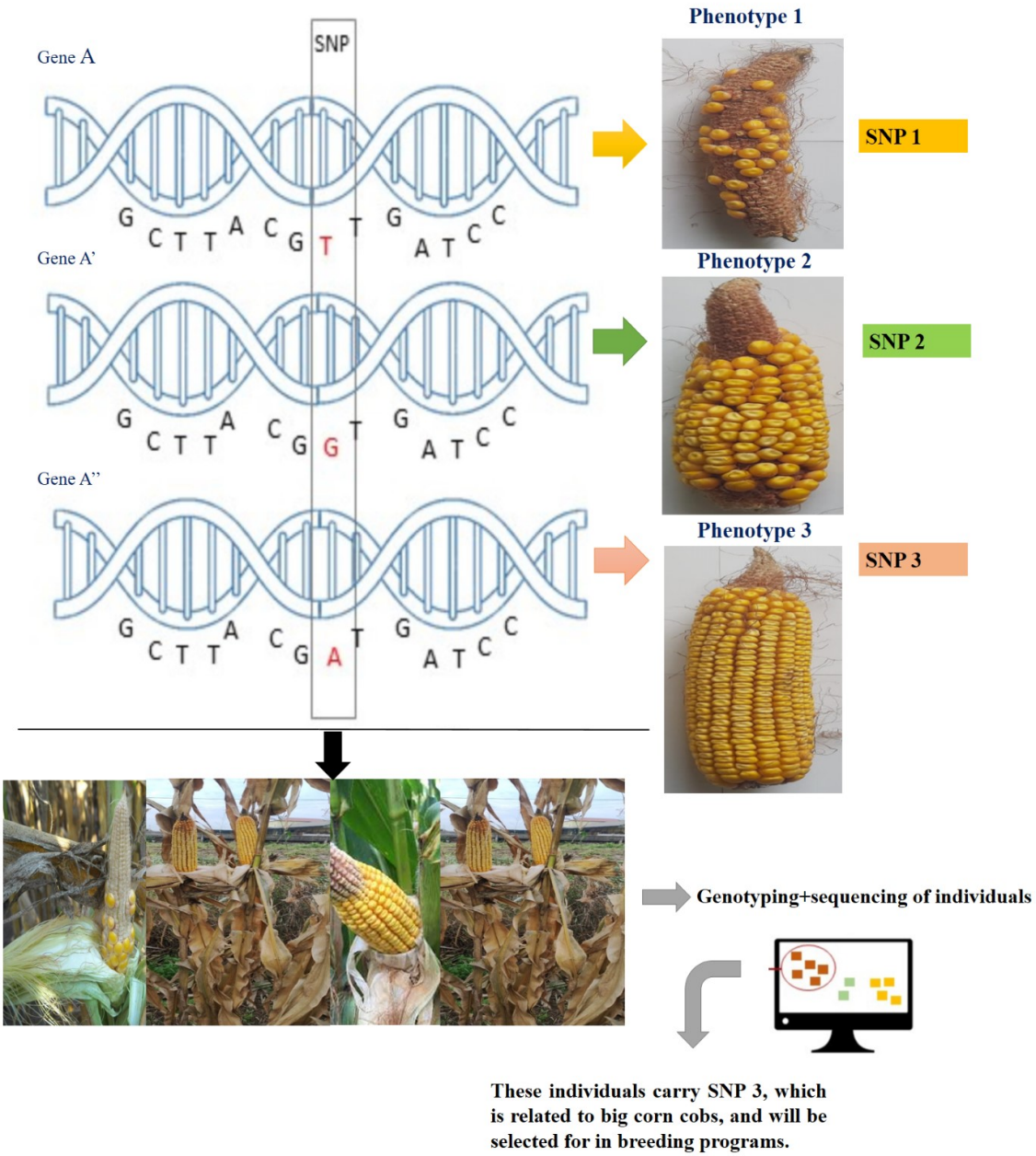

Figure 2. Marker-assisted selection of a quantitative characteristic in maize with an SNP marker (Turcotte et al., 2022, it has been modified).

SCAR (Sequence Characterized Amplified Region)

They are dominant or codominant markers developed from a specific region of the genome associated with a gene or trait. Due to problems with the RAPD marker, the SCAR technique was developed (Paran and Michelmore, 1993). SCAR marker is obtained from RAPD bands (AFLP etc.). This marker mostly originates from a single region in the genome, and SCAR markers are developed based on bands such as RAPD-AFLP detected in connection with a specific gene or trait in the genetic map. The RAPD or AFLP band in question is cloned and sequence analysed. After the RAPD or AFLP primer is determined in the sequence, a new primer is developed by extending it from the 3' end to a length of 24-26 bp. The newly developed primer is used to create a sequence-specific marker (McDermott et al., 1994). SCARs, which are generally dominant markers, can be converted to codominant markers by digestion with four base pair restriction enzymes and identification using Denaturing Gradient Gel Electrophoresis or Single Strand Conformation Polymorphism methods (Rafalski and Tingey 1993; Shidfar, 2014; Chawla, 2002).

DArT (Diversity Arrays Technology)

A hybridization-based molecular marker approach called Diversity Arrays Technology (DArT) can generate thousands of sequence-specific markers in plants without requiring genome-level sequence information (Badea et al., 2011). In plant genome research, this marker technology provides highly effective DArT markers that can be rapidly generated with the same hybridization-based applications for almost any

genome (Jaccoud et al. 2001). Advantages of DArT marker technology are as follows; I) it is amenable to automation, thus eliminating the limitations of molecular approaches, II) it is highly efficient and reproducible, III) many loci are screened simultaneously and therefore it is cost-effective (Milczarski et al., 2011). DArT technology in the different facilities examined; it has been successfully used in plants such as sorghum (Mace et al., 2008), wheat (Akbari et al., 2006), triticale (Alheit et al., 2011), and barley (Wenzl et al., 2004).

Genotypic Identification, Systematics and Characterization with Molecular Markers in Plant Breeding

Plant genetic resources are the most valuable resources that have economic, scientific, or social value both today and in the future, providing farmers and breeders with the genetic diversity necessary to develop new varieties with higher yields, higher quality, greater abiotic stress tolerance and resistance to pests and infections (Ramanatha and Hodgkin 2002). In plant production, it is critical to protect and use plant genetic resources for food security and forestry, including field and garden crops, medicinal and aromatic plants (Laurentin, 2009). Germplasm conservation studies, which specifically involve the preservation of genetic diversity within a species, include methods that program the characterization and evaluation of species. Characterization studies are a crucial step as they determine the genetic identity of each individual in the plant germplasm collection and provide a lot of information to breeding programs. Characterization studies in plants are based on morphological or molecular markers to identify genetic diversity inter- and intra-species and to determine the genetic relationships between genotypes (Rao, 2004). Plant breeding studies on genetic diversity that employ molecular marker systems can assess the family links among complex components of species at every level of the genetic structure, beginning with the origin of specific genotypes (Kilian et al., 2007; Arystanbek Kyzy, 2019).

Because they were simple to analyse, morphological markers were widely utilized in numerous plant species for many years prior to the invention of biochemical and molecular markers in order to identify genotypes and understand the genetic relationships between genotypes (Dar et al. 2017, Sharma and Sharma, 2018; Scarano et al., 2002). Several factors make morphology-based characterization studies less accurate because I) there are a few numbers of characters available, II) most of the material studied shows no variation in highly heritable traits, and III) environmental factors have a significant impact on quantitative traits. Furthermore, despite being helpful in mitigating the impacts of the environment, biochemical markers such isozymes, allozymes, and storage proteins only cover a tiny portion of the genome, making them insufficient to identify subtle alterations (Karp et al., 1997; Rao 2004).

Morphological markers can effectively determine the level of genetic differences between species, genera, and families. Nevertheless, due to shortcomings like their susceptibility to mutation and environmental influences, morphological markers helped pave the way for the use of molecular markers in genotypic identification following the development of the latter. As a result, the use morphological markers has nearly entirely disappeared in recent years, since molecular markers more effectively determine differences between species, genera and families at the DNA level (Federici et al., 1998). When we look at the researchs on genotypic identification, systematics, and characterization, we see that marker technology is the main focus of many studies in molecular approaches (Aksu and Çevik, 2015).

Ramakrishnan et al. (2014), examined plant regeneration by considering the genotypes of 17 maize lines and performed genetic similarity analyses using ISSR markers. As a result of molecular variance analysis between genotypes, it was observed that there was 93% genetic variance within the population and 7% between the populations. do Amaral Júnior et al. (2011) examined genetic diversity with 52 maize lines (49 maize lines, 2 ancestral lines, 1 local maize variety), and it was stated that 122 (89.05%) of the 137 bands obtained using 15 ISSR primers were polymorphic and showed parallelism with previous studies.

Table 2. Examples of Genetic Diversity Analysis with Molecular Markers in Plants

Plant	Marker Type	Analysis Type	References
Barley (*Hordeum vulgare*)	125 RAPD and 228 ISSR	Genotype Identification and Genetic Diversity	Fernandez et al., 2002.
Wheat (*Triticum aestivum*)	48 SSR	Genetic Diversity	Salem et al., 2008.
Wheat (*Triticum aestivum*)	43 SSR	Genetic Diversity within Salt Tolerance Detection	Al-Ashkar et al., 2020
Lavandula Species	RAPD	Genetic Diversity	Ibrahim et al., 2017.
Corn (*Zea mays*)	30 SSR	Genetic Diversity	Lopes et al., 2015.
Corn (*Zea mays*)	22 RAPD	Genetic Diversity	Bruel et al., 2007.
Corn (Zea mays)	11 RAPD and 6 SSR	Genetic Variation	Sebbenn et al., 2005.
Soybean (*Glycine max* L. Merrill)	SNP and DArT	Genetic Diversity and Population Structure	Shaibu et al., 2021.
Turkish pepper (*Capsicum annuum* L)	AFLP	Genetic diversity	Aktas et al., 2009.
Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*	DArTseq-based SNPs	Genetic Diversity and Population Structure	Yang et al., 2016.
Sorghum (*Sorghum bicolor* L. Moench)	SSR	Genetic Diversity Analysis for Drought Tolerance	Rajarajan et al., 2011.
Turkish oregano (*Origanum onites* L.)	RAPD	Genetic Variability	Tonk et al., 2010.
Rapeseed (*Brassica napus* L.)	18 ISSR	Genetic diversity and morpho-physiological assessment of drought tolerance	Motallebinia et al., 2024.

As a result of banding, 9 groups were created according to their genetic similarities. The results showed that the ISSR technique was successful in showing the similarities and differences between current maize lines and ancestral lines. In the study of Karcıcıo (2006), 25 different durum wheat varieties were analysed using the RAPD technique, 26 of 42 randomly selected primers were found to be informative. Of the total 176 PCR products obtained from polymorphic primers, 81 were found to be polymorphic and the polymorphism rate in the studied durum wheat varieties was determined as 46.02%. Uslu (2022) morphologically and molecularly analyzed sixteen local leek (Allium ampeloprasum var. porrum L.) genotypes collected from various locations in Turkey. Although there were no morphological changes among the sixteen local leek genotypes, statistically significant differences were found between the genotypes. Additionally, it was shown that 13 markers out of 18 SSR markers examined in leek genotypes under molecular characterization provided reliable and polymorphic findings among genotypes. A total of 69 alleles were obtained, of which 45 were polymorphic and 24 were monomorphic.

Conclusion and Recommendations

The idea of using molecular markers in plant breeding programs and applied genetics research is not a new paradigm. However, before the development of molecular markers, many of these suggestions were technically impossible in traditional breeding programs. Traditional plant breeding programs benefit greatly from the integration of DNA markers and the rapid development of molecular breeding techniques utilizing contemporary technology. Use of molecular markers in plant breeding; the development of new varieties can be accelerated by increasing the success rate in classical breeding by providing advantageous studies such as genotypic identification, systematic and characterization analyses, early and effective selection, and creation of genetic maps of plant genomes. In particular, the integration molecular markers into breeding programs will ensure more reliable, more effective, and successful results in a shorter time than classical breeding studies. Knowing the genetic diversity and the relatedness of genotypes is crucial for making informed decisions about the appropriate use of gene resources, including those containing uncommon genes. To pick ideal genotypes as parents for the plant breeding programs, the information regarding their genetic diversity is a crucial component.

Although molecular marker systems are not used alone instead of classical breeding, they are considered complementary and supportive systems that increase success in classical breeding. It is of great importance to carry out molecular marker studies and breeding studies together in order to obtain faster and more effective results. In molecular plant breeding, different marker systems are used to create linkage groups and genetic maps, depending on differences at the DNA level. By using one or more of these markers, genetic maps are created, and it is possible to determine the locations (loci) of genes on chromosomes. The development of marker-assisted selection methods has been made possible by genetic maps produced using molecular marker technology and the determination of the chromosomal locations of the genes in these maps. Compared to classical breeding, plant molecular breeding research can be carried out more efficiently, and successful results can be obtained faster with the development and use of molecular marker technologies in breeding. Molecular marker technology is expected to continue advancing in the coming years to better understand the biology underlying different traits of interest. It is anticipated that these developments will make the use of technology in plant breeding programs more accurate, efficient, and economical.

Declarations

The authors declare no conflict of interest in this paper.

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