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Molecular Breeding Advancements and Role of Molecular Markers in Ornamental Plants

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Abstract. **Ornamental plants exhibit the capacity to augment visual appeal through their diverse morphological features, encompassing floral forms, hues, foliage, and numerous other attributes. These botanical specimens find application in landscaping, floral arrangements, container gardening, and various commercial endeavors. As a result, there exists a persistent need for the creation of novel cultivars to satisfy market demands. Traditional plant breeding is slow and takes a lot of work. This has led to the use of biotechnology, especially molecular breeding. Molecular breeding lets breeders target specific, difficult-to-change traits like flower color, scent, and plant shape. It uses advanced methods like marker-assisted selection and genome-wide selection to speed up improvements and reduce the time it takes to develop new varieties. Molecular breeding may revolutionize agriculture in the 21st century by making genetic improvements faster and more efficient. Thanks to advances in genomics, gene editing, and molecular plant breeding, we now understand molecular markers much better. This has improved breeding strategies and given us a deeper understanding of crop diversity. This review elucidates the importance of various commercially valuable ornamental traits and delineates the research endeavors and accomplishments in enhancing floral characteristics through molecular breeding techniques.**

Molecular breeding encompasses a suite of advanced techniques employed in plant breeding to enhance crop characteristics by targeting specific genes or genetic markers associated with desirable traits. This methodology involves the utilization of molecular biology tools, such as DNA markers and genomics, to identify and select plants with desired genetic characteristics more efficiently than conventional breeding methods. The advent of molecular marker development and its applications began in the 1980s, marking a significant milestone in plant genomic research. This was followed by the development of polymerase chain reaction (PCR)–based DNA markers a decade later. Subsequently, numerous molecular markers have been used in various areas of plant molecular breeding, showcasing their widespread applications (Nadeem et al. 2017). The advancement in developing genetic and molecular tools for breeding ornamental crops has been impeded by several factors. These include the extensive diversity of ornamental species, many of which exhibit genetic complexity due to outbreeding, polyploidy, and possession of large genomes (Kuligowska et al. 2016). In recent years, the application of molecular approaches has led to a significant expansion in the diversity of cultivars available across various ornamental species, including roses, carnations, gerbera, and other floral industry staples. Consequently, the floral industry has emerged as a highly promising and economically viable sector on a global scale (Jin et al. 2023). Understanding the genetic makeup and variability within and among plant populations is crucial for their effective utilization and conservation. Several factors influence the level and structure of genetic variation within plant species, including their evolutionary history, population density, mating system, and the mechanisms of gene flow (Hamrick 1989).

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Markers

A "marker" refers to any variation in DNA that can be tracked through crosses, with some markers potentially showing statistical associations with phenotypic traits, or we can say that it is an allelic difference at a given locus in the DNA that can be observed at the morphological, biochemical, or molecular level. These markers serve as signposts, landmarks, and flags, helping scientists and breeders to identify and track desirable traits during the breeding process (Nadeem et al. 2017). These markers can be classified into different types.

Morphological markers, such as seed structure, flower color, and growth patterns, are readily observable visual traits used to distinguish important agronomic characteristics. Their advantage lies in their simplicity, requiring no specialized equipment or complex biochemical/molecular techniques. Plant breeders have long relied on these markers in crop improvement programs (Eagles et al. 2001). Indeed, the use of morphological markers to study variation and guide plant breeding practices has a long history (Lewis 1957). Cytological markers, on the other hand, are associated with variations in chromosome numbers, banding patterns, size, shape, order, and position. These specific patterns serve as chromosome landmarks that can distinguish normal from mutated chromosomes. Additionally, cytological markers are useful in identifying linkage groups and in physical mapping, aiding in the understanding of genetic relationships and chromosome structures (Nadeem et al. 2017).

Before the DNA revolution, biochemical markers or isozymes were the first "molecular" tool to be used for genetic characterization (Tanksley and Orton 1983). Biochemical markers, or isozymes, are multimolecular forms of enzymes that are coded by various genes but have the same functions. Isozymes, as proteins, are the main outcomes of structural genes. They represent distinct variations of an enzyme that perform the same catalytic function but vary in their charge and, consequently, their electrophoretic mobility. Isozymes come with specific drawbacks: (1) there's a restricted number of enzyme loci with accessible staining protocols, (2) enzyme expression can vary based on development and seasonal factors, (3) staining agents used are highly toxic, and (4) interpreting the banding pattern of dimeric loci (enzymes with two subunits) without segregating populations can be challenging (Boyle et al. 1994). The limited number of polymorphic isozyme markers and insufficient biochemical assays to detect these markers restrict the utility of these marker systems in crop improvement programs.

Molecular or DNA markers are nucleotide sequences used to study genetic variation (polymorphisms) between individuals (Nadeem et al. 2017). DNA's versatility, ubiquitous presence, and stability make it an ideal molecule for this purpose. A "genetic marker" refers to a specific, variable nucleotide sequence at a defined genomic location. This sequence exhibits sufficient variation (polymorphism) between individuals to enable tracking of its inheritance across generations. Crucially, these DNA sequence variations are phenotypically neutral (Meerow 2005), meaning they do not directly influence observable traits. DNA markers have

Table 1. Characteristics and relative cost of various marker systems.

Origin	Isozymes	RFLP	RAPD	AFLP	SSR	EST	SNP
Dominance	Codominant	Codominant	Dominant	Dominant	Codominant	Codominant	Codominant
Reproducibility	Very high	High to very high	Low to medium	Medium to high	Medium to high	High	High
Amount of sample required per sample	Several mg of tissues	High to very high 2 to 10 ng of DNA	Low to medium 2 to 10 ng DNA	Medium to high 0.2 to 1 µg DNA	Medium to high 10 to 20 ng DNA	10 to 20 ng DNA	10 to 20 ng DNA
Ease of development	Moderate	Difficult	Easy	Medium	Difficult	Medium	Difficult
Genome and QTL mapping potential	Limited	Good	Very good	Very Good	Good	Good	Good
Candidate gene mapping potential	Limited	Limited	Useless	Useless	Useless	Easy	Easy
Potential for studying genetic variation	Good	Limited	Limited	Limited	Limited	Easy	Easy
Equipment	Inexpensive	Moderate	Moderate	Moderate to expensive	Moderate to expensive	Moderate to expensive	Moderate to expensive

AFLP = amplified fragment length polymorphism; EST = expressed sequence tag; QTL = quantitative trait locus; RAPD = random amplified polymorphic DNA; RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphism; SSR = simple sequence repeat.

become increasingly prevalent in research due to their abundance across genomes and their stability, being largely unaffected by environmental influences during an organism's development (Amiteye 2021). A marker's position on a chromosome is called a "locus," and the different DNA sequences at that locus are known as "alleles." Markers are categorized as either "dominant" or "codominant." Dominant markers only reveal one allele (represented by a band on a gel), masking the presence of the corresponding allele on the homologous chromosome. If an individual has two copies of a recessive allele at a dominant marker locus, no band will be visible on the gel, making direct measurement of heterozygosity impossible. Conversely, codominant markers distinguish both alleles at a locus, allowing direct observation of all genotypes (both heterozygotes and homozygotes). Ideally, a DNA marker should be codominant, evenly distributed throughout the genome, highly reproducible, and capable of detecting high levels of polymorphism (Mondini et al. 2009). Molecular markers are typically classified based on their mode of gene action (codominant or dominant) or their detection method (hybridization-based or PCR-based) (Semagn et al. 2006). Various DNA molecular markers have been created and effectively used in genetics and breeding across agricultural crops. Table 1 offers concise details about molecular markers categorized by their detection method, along with comparisons of key traits of commonly used markers.

Hybridization-Based Markers (RFLP)

For hybridization-based markers, restriction fragment length polymorphism (RFLP) is the sole marker method available. Due to point mutations, translocations, inversions, duplications, and insertions/deletions (InDels), individuals within the same species display polymorphism. In the RFLP process, pure DNA isolation is the initial step. Restrictions enzymes and labeled probe hybridization of the target fragment are used in this process. Its foundation is the creation of various-sized DNA fragments as a result of restriction enzyme digestion (Amom and Nongdam 2017). In the RFLP process, pure DNA isolation is the initial step. Restrictions enzymes that are isolated from bacteria are combined with this DNA and used to cut the DNA at certain loci, also referred to as recognition sites. This leads to an enormous amount of fragments of various lengths. These fragments are separated using agarose or polyacrylamide gel electrophoresis, which generates a sequence of bands (Nadeem et al. 2017). The Southern blot method is used to transfer the isolated DNA fragments to nitrocellulose membrane. Usually, RFLP band score responds to DNA fragments of size range 2 to 10 kb. By hybridizing with a complementary radioactively tagged probe, fragments of interest are found, and following autoradiography, a particular banding pattern is shown (Southern 1975). The amount of probes and restriction enzymes used in the RFLP technique both affect the outcome. The RFLP markers have been employed for genetic diversity and population genetic study in various floricultural genera, alone or in combination with other types of markers (Scovel et al. 1998 in Dianthus; Strommer et al. 2002 in Petunia). Codominant, reliable RFLPs provide an almost infinite number of loci and do not require knowledge of the nucleotide sequence. They may be compared across similar genomes and tested on a variety of detection techniques. The method's drawbacks include its labor intensiveness, relative expense, and frequent slowness. Successful RFLP development requires a high level of laboratory expertise, and the quantity of polymorphism disclosed may be rather little (Meerow 2005). RFLP is now obsolete due to the development of technically less demanding and cheaper PCR-based DNA marker profiling technologies. Extensively, RFLPs have been used to reveal genetic variation and phylogenetic associations in individuals, as well as gene mapping studies.

PCR Based

PCR, invented by Cary Mullis in 1983 (Mullis et al. 1986), enables the amplification of small DNA quantities without requiring living organisms. PCR involves three key steps: denaturation (DNA strand separation), annealing (primer binding), and extension (DNA synthesis). Random amplified polymorphic DNA (RAPD) uses PCR with short, arbitrary primers (8 to 15 nucleotides) to amplify random DNA segments from large genomes (Amiteye 2021). This process uses Taq DNA polymerase, derived from *Thermus aquaticus*, to amplify minute quantities of target DNA. Unlike other PCR methods, RAPD does not require prior sequence knowledge of the target DNA flanking regions. A thermocycler controls the cyclical process: DNA is heated for denaturation, cooled for primer annealing, and then heated again for DNA polymerase–mediated extension using deoxynucleotides (dNTPs) and primers. Each cycle exponentially increases the target DNA copy number. The advent of PCR has spurred the development of numerous molecular markers for applications like genome characterization and mapping (Meerow 2005).

Random amplification of polymorphic DNA (RAPD). RAPD was first presented by Welsh and McClelland (1990) and Williams et al. (1990). It is the method that horticulturists use most frequently, mostly because the results can be produced quite inexpensively and quickly. In this, the polymorphism is dependent on the existence or absence of an amplification result. Population research, genetic linkage mapping, and the identification of horticultural crop cultivars and varieties have all made extensive use of RAPD techniques (Azizi et al. 2021). Despite the many

benefits that RAPDs offer, there are a few things to keep in mind. For example, dominant markers like as RAPDs result in some information loss because the amplification process either happens at a locus or it does not. Scores can therefore only be ascertained by band presence or absence (Kumari and Thakur 2014). Kumar et al. (2016) conducted a study in which 10 RAPD primers were used to analyze the genetic variation and population structure present in 38 Indian chrysanthemum cultivars. Sufficient diversity was obtained among all the chrysanthemum cultivars. All 38 cultivars were separated into two subpopulations. A mixed population ancestry was observed. In RAPD analysis, the pre-existing DNA sequence information is not a necessary prerequisite (Premkrishnan and Arunachalam 2012). A primer sequence chosen for RAPD analysis should have as much GC (guanine and cysteine) as possible, but 50% to 80% of GC is often favored. With this GC content, the primer will be able to function efficiently at the annealing temperature that facilitates the operation of DNA polymerase to effect DNA elongation (Cao et al. 2015). Generally speaking, RAPD primers can simultaneously produce PCR products from 1 to 10 genomic DNA locations. The typical length of a RAPD PCR fragment is between 0.5 and 5 kb. It is important to remember that primers might either fail to amplify PCR fragments or successfully amplify a specific region of DNA. The existence, distribution, and placement of primer complementary sequences on the template genomic DNA determine whether the RAPD PCR is successful or not. There is no PCR fragment produced when the two primer copies anneal too far apart or when their 30 ends do not appear on the template in the correct orientation to one another (Zakiyah et al. 2019). The PCR result is subsequently separated on an agarose gel and stained with ethidium bromide for visibility. By verifying the existence or lack of particular bands in the electrophoresis, polymorphism at or between primer binding sites can be found. The frequency of RAPD markers can be affected by a number of significant parameters, including the amount and quality of DNA, PCR buffer, magnesium chloride concentration, annealing temperature, and Taq DNA (a type of DNA polymerase) (Nadeem et al. 2017).

Amplified fragment length polymorphism. The amplified fragment length polymorphism (AFLP) is a firmly established DNA marker technique adopted to detect relationships among closely related cultivars. AFLPs are DNA fragments that range in size from 80 to 500 bp. They are produced by the restriction enzyme digestion reaction, which is then followed by the oligonucleotide adapter attachment to the restriction fragments and the selective PCR amplification of a subset of the fragments. Therefore, RFLP and PCR technologies are combined in the AFLP marker analysis protocol in part to perform DNA digestion and PCR amplification (Sorkheh et al. 2007). The limitations present in the RAPD and RFLP technique were overcome through the development of AFLP markers (Vos et al. 1995). The four main steps of the AFLP technique including fragmentation of DNA by restriction digestion, attachment of adapters and ligation, restriction fragment selective PCR, and gel electrophoresis analysis. DNA is cut during AFLP using two restriction enzymes: a common cutter and a rare cutter. The oligonucleotides are ligated at both ends of the resultant fragments. Oligonucleotides are brief segments of nucleic acid that are used in PCR ligation. The uncommon cutter (6-bp recognition site) has a designated end, whereas the frequent cutter (3-bp recognition site) has a different end. Only the fragments that these cutters have sliced will be amplified as a result of this. Known adapter sequences are used in the primer production process (Nadeem et al. 2017). After performing PCR, visualization is done in either agarose gel or polyacrylamide gel stained with $AgNO_3$ or by auto radiography.

Simple sequence repeats (SSRs). Simple sequences known as SSRs are found randomly throughout the nuclear, chloroplast, and mitochondrial genomes of many species. They arrange in tandem repeats of mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide motifs that are highly abundant and polymorphic (Amiteye 2021). Microsatellites are also called as SSRs. Studies have also confirmed the presence of SSRs in protein-coding genes and expressed sequence tags (ESTs) (Morgante et al. 2002). It represents less repetition per locus with a higher polymorphism level. This high polymorphism level can be easily identified by PCR and is caused by different numbers of repeats occurring in microsatellite areas. Single-strand DNA slippage, double-strand DNA recombination, mobile element (retrotransposon) transfer, and mismatches can all contribute to the occurrence of SSRs (Nadeem et al. 2017). SSR marker loci are produced using two-primer PCRs as compared with RAPDs or inter–simple sequence repeat (ISSR) analysis, which uses single primers per PCR. While the other primer hybridizes to the DNA template in the opposite orientation, the first primer anneals to the template in a forward manner. When complementary to particular DNA sequences around the SSR sequence, both primers anneal (Mei et al. 2015). SSR PCR fragment separation is accomplished with agarose or polyacrylamide gel electrophoresis. Next, the assessment and evaluation of gel banding patterns are done. Finally, SSR profile analysis and interpretation are performed for investigation of polymorphism. SSRs are incredibly informative, but the marker development or discovery stage, which involves DNA sequencing, can be costly. Since locus-specific SSR markers are more illuminating, their advancement represents a highly valuable genetic resource (Kalia et al. 2011).

Single-nucleotide polymorphisms (SNPs). SNPs are genetic polymorphism that results from specific single-bp positional variations in DNA sequences between members of the same species or distinct species. In contrast to other genetic markers, SNPs are the most common type of DNA sequence polymorphisms found in living things (Manivannan et al. 2021). Depending on which nucleotide is substituted, SNPs can be transversions (C/G, A/T, C/A, or T/G) or transitions (C/T or G/A). Single-base alterations, such as SNPs that are InDel in a single base, are typically seen in mRNA. The lowest unit of heredity is a single-nucleotide base, and SNP can provide the greatest number of markers in an easy-to-understand manner (Xu 2010). In plants in which the genome is relatively unknown or unmapped, such as many floricultural species, it can provide a shortcut to finding closely linked markers to the phenotype of interest. SNPs are widely used in plants for characterizing genetic resources, genetic diversity, genetic diagnostics, phylogenetic analysis, disequilibrium-based association mapping, and variety/cultivar identification, among other uses of genetic markers (Rafalski 2002). Numerous SNP genotyping assays have been created, each based on a distinct molecular process. The most significant ones include allele-specific hybridization, oligonucleotide ligation, intrusive cleavage, and primer extension. Recent advances in high-throughput genotyping techniques, including allele-specific PCR, Genotyping-by-Sequencing (GBS), chip-based next-generation sequencing (NGS), and NGS, have made SNPs the most desirable markers for genotyping (Sobrino et al. 2005).

Expressed sequence tag (EST). ESTs are short DNA sequences derived from the ends of cDNA, which are DNA copies of mRNA. Since mRNA represents actively expressed genes, ESTs provide a snapshot of the genes being used in a specific tissue or at a particular time. In molecular breeding, ESTs are valuable tools for gene discovery, particularly for genes expressed under specific conditions or in certain tissues. They aid in developing molecular markers for tracking desirable traits during breeding, analyzing gene expression levels, and annotating genomes by identifying gene locations and potential functions. Essentially, ESTs help breeders pinpoint and understand the genes controlling important traits like flower color or disease resistance, enabling more efficient and targeted crop improvement (Beruto et al. 2024). Messenger RNA (mRNA) is converted into complementary DNA (cDNA) by reverse transcriptase, which results in EST. To increase the stability of mRNAs, EST generation is required. The reason for this is that the chemistry of nucleic acids makes them more stable in the form of DNA or cDNA molecules than they are in the form of RNA molecules. Sequencing of cDNA produces ESTs. To create 50 ESTs or 30 ESTs, respectively, a few hundreds of nucleotides are sequenced starting at either the 50 or 30 end of the cDNA (Amiteye 2021). Currently, there are several millions of ESTs from a diversity of organisms available in many databases. ESTs have been invaluable in gene discovery, providing significant insights into gene function. In the study of gene function, ESTs have contributed in the construction of DNA microarrays and related probes. On the plus side, numerous extremely valuable EST-based molecular markers have been obtained, including cleaved amplified polymorphic

Table 2. Advantages and disadvantages of some markers.

Markers	Advantages	Disadvantages
Morphological	Easy to use, cheaper, visually characterized	Less polymorphic, influenced by environment, influenced by plant growth stages
Isozymes	No need of specific instrument, easy to use, codominant	Less polymorphic, influenced by environmental factors
RFLPs	Codominant, no need of prior sequence information	Time consuming, high quantity of pure DNA needed, expensive, time consuming
RAPD	Easy to use, lower quantity of DNA is required, polymorphic	Dominant, highly purified DNA is required, low reproducibility.
AFLP	Reliable, high reproducibility, more informative	Dominant marker, highly purified DNA is required, high quantity of pure DNA needed
SSRs	Codominant marker, lower quantity of DNA is required, high reproducibility	High developmental cost, presence of more null alleles, occurrence of homoplasy
SNP	Cost effective, widely distributed in genome, no need of prior sequence information, high reproducibility	High developmental cost
DArT	Cost effective, high throughput, highly polymorphic, prior sequence information not needed, high reproducibility	Dominant marker, high developmental cost

AFLP = amplified fragment length polymorphism; DArT = diversity array technology; RAPD = random amplified polymorphic DNA; RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphism; SSR = simple sequence repeat.

sequences (CAPSs), ISSRs, SNPs, and RFLPs (Xiao et al. 2020). Table 2 showcased the advantages and disadvantages of some markers whereas, in Table 3 comparision between some major markers was done.

Potential Use of Molecular Markers in Ornamental Crops. While ornamental crops have been widely studied using approaches like morphology, physiology, and functional genomics, molecular studies have been comparatively limited (Baliyan et al. 2014). Ornamental breeding has challenges due to polyploidy and its complex genetics. The primary goal is to identify marketable plants with unique aesthetic and ornamental qualities. Breeding aims are generally based on economic significance, as the breeding procedures have lacked sophistication compared with other agricultural crops, but with the advancement in knowledge of molecular basis and high technologies, various techniques are being developed for the thorough study of ornamentals at molecular level like marker-assisted selection (MAS), Genome-Wide Association Study (GWAS), quantitative trait loci (QTLs), and association mapping for various concepts like the phylogenetic relationship, genetic diversity, genetic mapping, disease resistance, and many more aesthetic and economically needed traits. Some of them are discussed in the following paragraphs (Fig. 1).

Genetic links between cultivars and their parentage are valuable for geneticists, breeders, and public policymakers. Morphological characteristics and biochemical and molecular markers can provide genetic links and parentage information. Morphological features are limited in number and easily affected by environmental factors. Biochemical markers can identify cultivars without environmental impact, although their effectiveness depends on the developmental stage of plant tissues. Molecular markers give valuable information and may accurately detect variation among individuals, populations, and species, complementing earlier characterization. Various studies have been conducted to study the genetic diversity and linkage present between different genotypes, species, varieties or cultivars of ornamental plants by using different molecular markers. Martí:n et al. (2002) employed RAPD analysis on 15 commercial chrysanthemum genotypes, using 10 primers that generated 151 markers, 113 of which were polymorphic, indicating substantial variability among the cultivars. Similarly, Mor et al. (2008) used RAPD to assess genetic diversity in marigold genotypes. While they tested 25 primers, only 15 produced amplifications, resulting in 113 bands (an average of 4.5 per primer). Their analysis revealed 95 polymorphic and 18 monomorphic bands, demonstrating 84% polymorphism across the genotypes. This RAPD analysis effectively differentiated between both genotypes and species. Whankaew et al. (2014) also investigated marigold, using ISSR markers and a marigold-specific genomic DNA library to develop SSRs. They analyzed 20 African marigolds (*Tagetes erecta* L.)—comprising 14 commercial varieties and six Thai landraces—and six French marigolds (*Tagetes patula* L.). This analysis identified 38 polymorphic markers and 112 alleles, with two to seven alleles per locus. The observed heterozygosity were 0.48 and 0.32, while polymorphism values ranged from 0.10 to 0.71. Their results demonstrated the effectiveness of SSR markers for assessing genetic diversity, classifying taxa, and identifying individual marigold plants. Tang et al. (2013) investigated genetic diversity and structure in tulip varieties. Using 236 polymorphic SNPs derived from 'Kees Nelis' and 'Cantata', they selected 121 SNPs (minor allele frequency >0.1) for genetic analysis of 72 accessions. Overall heterozygosity (Ho) was 0.35, ranging from 0.22 in *Tulipa fosteriana* to 0.43 in *Tulipa gesneriana* × *T. fosteriana* hybrids. Genetic differences between *T. gesneriana* cultivar groups, based on flowering time and shape, were relatively small.

Furthermore, molecular markers are very valuable for creating genetic maps. Genetic mapping involves arranging markers in order, showing their relative genetic distances, and allocating them to linkage groups (Fig. 2) based on recombination values from pairwise pairings (Meerow 2005). To assess inheritance and recombination patterns, highly polymorphic

Table 3. Comparing some salient characteristics of major markers.

Marker	PCR-based	Mode of inheritance	Level of polymorphism	Reproducibility
RFLP	No	Codominant	Low-medium	High
RAPD	Yes	Dominant	Medium-high	Low
SCAR	Yes	Codominant	High	High
AFLP	Yes	Dominant	High	Medium
SSR	Yes	Codominant	High	High
ISSR	Yes	Dominant	High	Medium
SRAP	Yes	Dominant	Medium-high	Low
SAMPL	Yes	Codominant	High	High
CAPS	Yes	Codominant	High	High
SNP	Yes	Codominant	Extremely high	High
DArT	No	Dominant	High	high
STS	Yes	Codominant	High	High
RAMP	Yes	Codominant	High	High

AFLP = amplified fragment length polymorphism; CAPS = cleaved amplified polymorphic sequence; DArT = diversity array technology; ISSR = inter–simple sequence repeat; PCR = polymerase chain reaction; RAPD = random amplified polymorphic DNA; RAMP = randomly amplified microsatellite polymorphism; RFLP = restriction fragment length polymorphism; SAMPL = selective amplification of microsatellite polymorphic loci; SNP = single-nucleotide polymorphism; SRAP = sequence-related amplification polymorphism; SSR = simple sequence repeat; STS = sequence-tagged site.

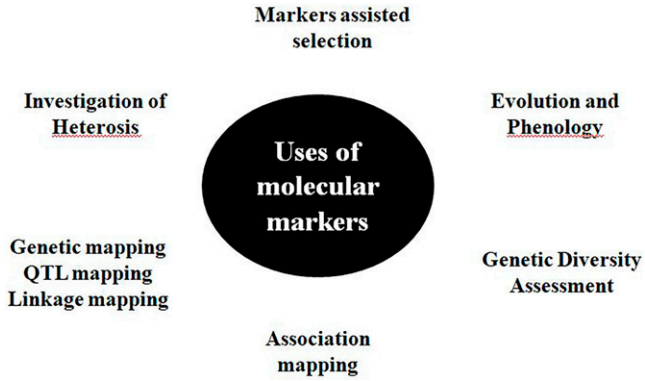

Fig. 1. Uses of molecular markers in different techniques.

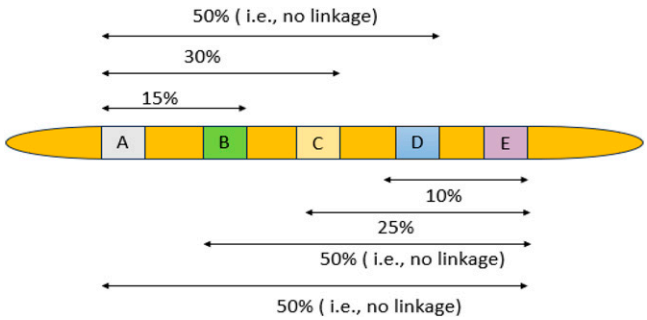

Fig. 2. General representation of linkage on a chromatid.

markers like RFLPs, SNPs, SSRs, or AFLPs are needed, together with a known population (family). Markers that form a linkage group are presumed to be on the same chromosome. Oyant et al. (2007) constructed a consensus linkage map for roses by integrating data from various existing genetic maps. This integrated map facilitated the identification of QTLs associated with flowering date and petal number. Novel rose SSR markers were used to enhance genomic maps of interspecific F1 progeny. Using a pseudo test cross mapping strategy, separate genetic maps were developed for each parent of the progeny. The resulting maps comprised seven linkage groups, encompassing 105 markers spanning 432 cM for the maternal map and 136 markers covering 438 cM for the paternal map. SSR markers were instrumental in establishing homologous relationships between linkage groups on the maternal and paternal maps. These genetic maps successfully localized a major gene and QTLs influencing key flowering traits, specifically petal number and flowering date.

An integrative genetic linkage map of *Catharanthus roseus* was created by Gupta et al. (2007) using an F2 population of 144 plants and various molecular and morphological markers. The map defines 14 linkage groups (LGs) and consists of 131 marker loci, including 125 molecular DNA markers (76 RAPD, 3 RAPD combinations; 7 ISSR; 2 EST-SSR from *Medicago truncatula*; and 37 other PCR-based DNA markers), chosen from a total of 472 primers or primer pairs, and six morphological markers. Similarly, Shahin et al. (2010) in their work tested the feasibility of diversity array technology (DArT) and nucleotide-binding site (NBS) profiling in lily; they developed higher density genetic linkage maps for two lily populations, one of which is a continuation of the mapping study of Van Heusden et al. (2002), and mapped important ornamental traits in the set of lily populations and additionally, resistance to lily mottle virus (LMoV) was mapped. To accurately map traits and initiate marker-assisted breeding (MAB), it is important to have adequate map coverage with markers that target desired qualities and can be easily used for downstream breeding applications. That is the reason of DArT and NBS profiling were used in this study. Jaccoud et al. (2001) developed DArT for lily. Given that resistance to *Fusarium* and LMoV is a primary objective in MAB, and the cultivar 'Connecticut King' serves as the exclusive source of resistance within the studied populations, DNA from this cultivar was used to generate DArT microarrays. This approach facilitated the simultaneous development of both the NBS markers and DArT libraries, a novel achievement. This combined methodology enabled the construction of genetic maps for lily, a species characterized by one of the largest genomes in both the plant and animal kingdoms. NBS markers are well distributed over the linkage groups. Figure 3 presents the work done by Shahin et al. 2010. The construction of genetic linkage maps for lily was achieved using two populations, LA and AA that share one parent Connecticut King. Three different molecular marker systems (AFLPTM, DArT and NBS profiling) were used in generating linkage maps for Connecticut King. The LA and the AA populations consist of 20 and 21 linkage groups (LGs), respectively. Average density between markers was 3.9 cM for the LA and 5 cM for the AA population. Several horticultural traits were mapped for the first time in Lilium and showed to be single gene based. We propose to name these genes as LFCc for flower colour, lfs for flower spots, LSC for stem colour, lal for antherless phenotype and lfd for flower direction whereby upper and lower case names refer to dominant and recessive genes, respectively. Additionally, resistance to Lily mottle virus (LMoV) was mapped as a locus on LG AA10. Peltier created the first genomic map of petunia in 1994. Since then, genetic maps for at least 27 ornamental plants have been created. Traditional markers like AFLPs and SSRs were used in most maps, resulting in poor mapping resolution due to a restricted number of available molecular markers (Yagi 2018). SNP markers from NGS are appropriate for creating high-density genomic maps. A high-density genetic map was constructed for identification of loci controlling flower-type traits in chrysanthemum. Flower type is a significant and complex feature of chrysanthemum. The corolla tube merged degree (CTMD) and relative number of ray florets (RNRF) are major elements influencing chrysanthemum flower types. Few studies have addressed the inheritance of these two complicated features, hindering targeted breeding for flower-type improvement. Song et al. (2020) analyzed 305 F1 hybrids from two parents, with notable variances in CTMD and RNRF performance. They used Specific-Locus Amplified Fragment (SLAF) sequencing technology to create a high-density genetic linkage map with an average distance of 0.76 cM. Over a 2-year period, three key QTLs governing CTMD and four major QTLs underpinning RNRF were consistently identified. They also studied the genetic map's synteny with other Compositae species and found weak collinearity. The genomes of *Helianthus annuus* L. and *Lactuca sativa* L. var. ramosa Hort. were explored in QTL regions with substantial genomic collinearity. Eight genes were identified. These findings provide a basis for molecular marker–assisted breeding and gene exploration in chrysanthemum without a reference assembly.

Another utilization of molecular breeding is in developing disease resistance in crops. Although disease control is of importance in ornamental production, breeding efforts to reach higher levels of resistance in most ornamental breeding programs have been relatively limited. If resistance is considered as a selection criterion, it is often used at a relative late stage in the breeding process or when cultivars are in the trialing stage. This is due to a number of specific problems related to ornamental breeding such as the many different ornamental crops and ploidy level in a number of the most important ornamentals (Debener 2009). MAS and QTLs for disease resistance have been created for several crops

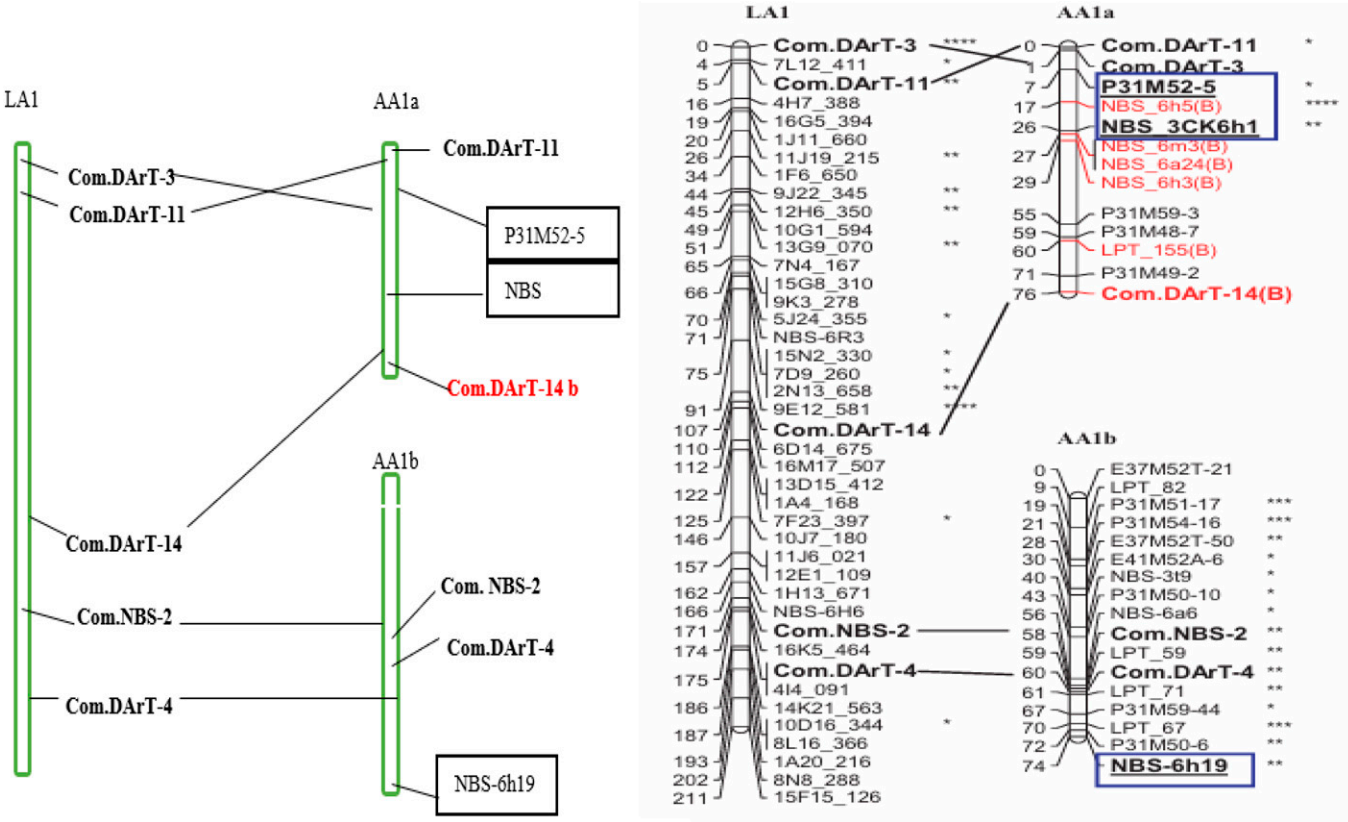

Fig. 3. Linkage groups. Aligned genetic maps of the LA and AA populations. All the bold markers are common markers. The mapped traits (LFCc, lfs, LSC, lal, lfd, and LMoV) are circled. The six putative *Fusarium* quantitative trait loci are placed in rectangles (Shahin et al. 2010).

and diseases. MAS can be useful in plant breeding for features that are difficult to characterize, costly, or time consuming; depend on developmental stage or environment; need backcrossing to select recessive alleles; or involve gene pyramiding (Xu and Crouch 2008). However, the use of MAS in breeding programs is not as widespread as expected. Complex characteristics, cost efficiency relative to phenotyping selection, high-throughput precision phenotyping for QTL mapping, usable computational tools, genotype-by-environment interaction, and epistasis are major challenges. As a result, many crops suffer from a lack of QTL validation into usable MAS (Neale and Kremer 2011).

Since the early 1990s, there has been increasing work at identifying markers. Hundreds of RFLPs, AFLPs, SSRs, RGAs, PKs, CAPs, SCARs, and other markers (Byrne 2009) have been described and used to create both diploid and tetraploid maps (Spiller et al. 2011). On these maps, loci have been placed for a variety of traits including for disease resistance and the related resistant gene analogs (RGAs) and pathogenesis resistance (PR) genes. Nevertheless, in the last few years, a number of new developments have changed the roadmaps for research in the life sciences and also the feasibility of disease resistance mapping and marker assisted breeding in ornamentals. Thus "far markers" have been identified for resistance to diseases like black spot (Rdrs) and powdery mildew in rose and with the ability of genotyping by sequencing to generate thousands of markers, and our ability to identify markers useful in plant improvement have increased exponentially. Transgenic rose lines with various fungal resistance genes inserted have shown limited success, and RNA interference technology has potential to provide virus resistance. Roses, as do other plants, have sequences homologous to characterized R-genes in their genomes, some of which have been related to specific disease resistance. With improving next generation sequencing technology, our ability to do genomic and transcriptomic studies of the resistance related genes in both the rose and the pathogens to reveal novel gene targets to develop resistant roses will accelerate (Debener and Byrne 2014). Arens et al. (2012) examined ornamental plant resistance to diseases such as *Fusarium oxysporum*, Tulip shattering virus, and *Botrytis tulipae*, using SNP markers. Markers for disease resistance have been developed in polyploids, including roses (Leus 2018). Compared with diseases, breeding for pest or mite resistance in ornamentals is still in its early stages. Only a few examples exist, such as mite resistance screening in azalea (Luypaert et al. 2014). Recently, the construction of a genome-wide genetic linkage map and identification of quantitative trait loci for powdery mildew resistance in Gerbera daisy was done by Bhattarai et al. 2023. Powdery mildew (PM) is a widespread fungal disease that affects many key crops. The PM caused by *Podosphaera xanthii* has been the most difficult problem in commercial cultivation of Gerbera worldwide, frequently resulting in significant crop yield and quality losses. A few PM-resistant breeding lines and cultivars have been identified in Gerbera, but the underlying genetics of PM resistance in Gerbera remain unexplained. Therefore, a genome-wide genetic linkage map was created with 791 SNP site markers covering 1912.30 cM over 27 linkage groups and has a marker density of 1 per 2.42 cM. One large consistent QTL was found in LG16, accounting for more than 16.6% of the phenotypic variance in PM resistance. This genetic linkage map will be highly valuable for discovering and tagging QTLs for other critical features in Gerbera, and the newly identified QTL and SNP markers will enable the construction of molecular markers for enhancing Gerbera's resistance to PM.

Ornamental plants are known for their beautiful foliage. They can have different colors, sizes, and shapes, which all together add a sense of beauty to a place. Why do they differ so much? The differences can be primarily because of genetic diversity and selective breeding. Ornamental plants belong to a vast array of species and varieties, each with a unique genetic makeup. Natural selection in diverse environments over millennia has driven variation. Like leaves, they have adapted different shapes (e.g., broad, needle, fern-like) and sizes to optimize light absorption, water conservation (by reducing surface area), and protection against herbivores in their native habitats. Similary with color (Pigments): Different colors are due to the presence and

concentration of various pigments. Selective Breeding: Horticulturists deliberately crossbreed and stabilize naturally occurring mutations (like variegation) to enhance aesthetic appeal, resulting in the extreme and unique foliage seen in commercial markets today. Feng et al. 2020 identified the genes that influence leaf shape in ornamental kale. This would provide insight into the mechanism underlying leaf development. They created F1, F2, BC1P1, BC1P2, and F2:3 populations of ornamental kale from a cross between F0819, a feathered-leaved inbred line, and S0835, a smooth-leaved inbred line. Genetic analysis revealed that the feathered-leaved trait was controlled by a semidominant gene, BoFL. The gene was mapped on chromosome C9 using bulked segregate analysis sequencing. Fine-scale mapping between the two flanking markers IN was used to narrow down the candidate region. CAPS4610, the first nonsynonymous SNP, was found to be associated with leaf form. BoALG10, a homolog of ALG10, encodes an α-1,2-glucosyltransferase in *Arabidopsis thaliana* and may be responsible for the feathery-leaved characteristic. This research is crucial for understanding leaf growth mechanisms and breeding ornamental kale with unique leaf forms. Gu et al. (2002) and Zhang and Shen (2007) found that the feathered-leaved trait has partial dominance over the smooth leaf trait after conducting genetic crosses. Ren et al. (2015) investigated the genetic basis of red leaf color in ornamental kale, a commercially important trait driven by anthocyanin accumulation, researchers created a large F2 population by crossing red- and white-leaved double haploids. The inheritance pattern revealed that the red leaf trait is controlled by a single dominant gene (Re). Using the white-leaved plants for genetic mapping, the 'Re' gene was successfully mapped to Linkage Group C09 of Brassica oleracea. The gene's locus was precisely positioned between six SSR markers, with the closest one being only 0.3 cM away. These results are critical for implementing marker-assisted selection (MAS) to accelerate red-leaf breeding and for the future map-based cloning of the 'Re' gene.

Diversity in flower color is another aspect that has to be studied at molecular level. There are hundreds of thousands of flowers having various degrees of color pigments. Previously, flower color was determined through visual observation or measurement using the Royal Horticultural Society Color Chart (RHSCC) (Voss 1992). Analyzing the relationship between flower color and pigmentation, as well as understanding the genetic mechanism of floral coloring, can serve as a guide for directional breeding of ornamental plant flower colors. The study of the links between floral color and pigments requires a quantitative and accurate determination of flower color (Lu et al. 2021). Thus, molecular markers help in discovering the genetic basis of different flower color and inheritance pattern and identifying QTLs related to these traits.

Orchids are among the most beautiful flowers, with diverse genera classification like *Phalaenopsis*, dendrobiums, oncidium, cymbidiums, cattleya, and many more. The orchid's diverse and beautiful flower colors depend on the presence of pigments like carotenoid and anthocyanin (Hsiao et al. 2011). Key enzymes like PSY, CHS, and DFR play important roles in the formation of carotene and anthocyanin pigments (Sudarsono et al. 2017). Flower colors vary greatly across *Phalaenopsis* species and have been used to identify commercial orchids. *Phalaenopsis* with unique, fresh, and novel traits are in high demand, with buyers willing to pay premium prices. The diversity of *Phalaenopsis* spp. can be understood through their morphological, biochemical, and molecular characteristics (Haristianita et al. 2017). Molecular markers can be effectively used to distinguish genotypes, to analyze genetic relationships between genotypes or to infer phylogenetic relationships between species and closely related genera (Weising et al. 1995). As *Phalaenopsis* species exhibit varying flower hues. DNA sequence diversity was analyzed, and SNPs were discovered by Sudarsono et al. (2017). The objective was to isolate and describe PSY (for carotenoid), CHS, DFR, F3'-H, F3-H, and F3'-5′-H (all five for anthocyanin) genes from *Phalaenopsis* species, analyze nucleotide sequence variability, and construct gene-specific molecular markers to promote breeding for unique *Phalaenopsis* flower colors. The primary goal of *Phalaenopsis* breeding is to improve blossom color diversity, which includes white, yellow, purple, red, and other unique colors. Short, single-stranded DNA fragment primers (SNAPs) were designed based on nonsynonymous SNP sites identified in six pigment biosynthesis genes. These primers were used to generate SNAP markers. Three sets of primers were designed for CHS, four for PSY, and one for other genes. The genetic diversity of 30 *Phalaenopsis* species and commercial hybrids was evaluated using 10 SNAP markers based on six genes linked with color production. The investigation revealed considerable genetic variety among investigated species that are linked to changes in flower color. Future research could help forecast flower color in *Phalaenopsis* progeny by analyzing the primary flower color characteristics and genotype grouping of the examined species.

Yan et al. (2005) clarified the genetic basis of inheritance of flower color in *Salvia miltiorrhiza* and also constructed a high-density genetic linkage map. For this, the two color extremes found in this species, *S. miltiorrhiza* with dark violet flowers and *S. miltiorrhiza f. alba* with white flowers, were selected. The genomic map includes 605 SNPs spanning 738.3 cM across eight linkage groups, with an average distance of 1.22 cM between two markers on the created genetic map and the RGB values of the flowers, two QTLs for blossom color were identified.

Table 4. Work done in some ornamental crops using molecular markers.

Ornamental crops	Marker types	Achievements	References
Rose	RFLPs, AFLPs, RAPD, SSR, ISSRs	Phylogenetic relationship	Yan et al. 2005
	SSR	Genetic linkage maps, QTL	Oyant et al. 2007
	EST-SSR, SSRs	Diversity analysis in 48 rose genotypes	Qi et al. 2018
	SSRs	Characterization of Indian-bred rose cultivars	Veluru et al. 2020
Chrysanthemum	RAPDs, ISSRs and AFLPs	Identification of different cultivars, genetic diversity	Zalewska et al. 2007
Lilium	RAPD, ISSR	Parental linkage maps	Abe et al. 2002
	AFLP, DArT and NBS profiling	Mapping of major genes and QTL for several ornamental traits and disease resistances	Shahin et al. 2010
Gladiolus	ISSR	Genetic diversity evaluation	Kumar et al. 2016
Marigold	RAPDs	DNA fingerprinting	Mor et al. 2008
Carnation	RAPDs, SRAPs and ISSRs	Mapping of plant chromosomes	Fu et al. 2008
Jatropha	RAPDs	Genetic diversity	Subramanyam et al. 2009
Ornamental kale	SNPs, CAPS, InDelS	Mapping and identification of the leaf shape gene BoFL	Feng et al. 2020
Tulip	SNPs	Genetic diversity and structure in a collection of tulip cultivars	Tang et al. 2013
Phalaenopsis	RAPD	Genetic diversity	Goh et al. 2005
	AFLP	Genetic map for leaf traits	Xu et al. 2012
	SNAP	Genetic diversity	Sudarsono et al. 2017

AFLP = amplified fragment length polymorphism; CAPS = cleaved amplified polymorphic sequence; DArT = diversity array technology; EST = expressed sequence tag; InDel = insertions/deletion; ISSR = inter–simple sequence repeat; NBS = nucleotide-binding site; RAPD = random amplified polymorphic DNA; RAMP = randomly amplified microsatellite polymorphism; RFLP = restriction fragment length polymorphism; SAMPL = selective amplification of microsatellite polymorphic loci; SNAP = short, single-stranded DNA fragment primer; SNP = single-nucleotide polymorphism; SRAP = sequence-related amplification polymorphism; SSR = simple sequence repeat.

These two QTLs are placed on LGs 4 and 5. The study's findings establish the groundwork for cloning genes that govern flower color and investigating the molecular mechanisms of floral color regulation in *S. miltiorrhiza*. Genetic studies (GWAS, QTL mapping) are revealing genes that control ornamental plant traits like petal number. Advanced molecular breeding tools, including CRISPR-Cas9, TALENs, and ZFNs, are revolutionizing the field by boosting cold and heat tolerance in these crops (Husain et al. 2024).

Flowers like rose, jasmine, tuberose, marigold, and lavender are also used in perfume and in the cosmetic, pharmaceutical, and food industries. Although cultivated for centuries, the gene pool of these flowers' cultivars used for industrial cultivation has been poorly characterized. Annual market demand for different flowers drives scientists and breeders to develop new varieties (cultivars) that boast better aesthetics, higher quality, and enhanced resilience to stress. Developing new cultivars typically involves conventional breeding methods, such as crossbreeding and mutation. Traditionally, breeders pick superior progeny or mutants based on phenotypes, which can be time consuming and unproductive for traits with significant genotype–environment interactions. New breeding lines were developed through clonal selection of vegetatively propagated industrial populations. In the rose, the industry avoided cross-breeding with other rose species due to concerns about potential changes in rose oil composition. Research on rose molecular genetics and scent has advanced significantly during the last decade. Molecular markers have been successfully used to characterize rose genetic diversity and identify cultivars (Rusanov et al. 2009). Microsatellite markers are particularly informative for detecting polymorphism and studying the diversity of rose cultivars. SSR utilization can help identify cultivars, safeguard them from unauthorized commercialization, and document genetic variability for the conservation of Indian rose germplasm. Research on the genetic diversity of *Rosa damascena* is crucial for understanding its origins and initiating cross-breeding programs to improve oil-bearing damask roses Kiani et al. (2008). Work done in some ornamental crops using molecular markers is displayed in Table 4.

Conclusions

Molecular marker technology has dramatically advanced in the past 30 years, progressing from RFLPs to SNPs and array-based markers. Coupled with the rise of cost-effective, high-throughput NGS, these advancements offer immense potential for ornamental plant improvement. Adopting these cutting-edge techniques is essential for overcoming existing limitations and introducing desirable traits. While valuable cultivars exist, challenges like complex genetic backgrounds, long life cycles, polyploidy, and self-incompatibility impede breeding efficiency. Traditional breeding methods, reliant on recombination and hybridization, are often slow, inefficient, and unpredictable. Genetic manipulation offers a powerful alternative to address these shortcomings. The increasing accessibility of genetic marker technology in research settings promises an expanded role in evaluating and improving floricultural germplasm. This has significant economic implications. The ability to precisely select for desirable traits minimizes resource expenditure on undesirable plants. This leads to faster release of improved cultivars to market, capturing potential economic gains more quickly. Furthermore, developing disease-resistant and pest-resistant varieties reduces the need for costly chemical interventions, benefiting both growers and the environment. Enhanced product quality, including novel flower colors, shapes, and fragrances, can command premium prices, boosting profitability. Future molecular breeding efforts will require a deeper understanding of the molecular networks controlling target traits to broaden the gene pool and unlock further innovation. Sequencing-based technologies will be central to future ornamental crop research, driving cultivar development and facilitating the study of complex biological phenomena. Ultimately, elucidating the genetic basis of complex traits will not only enhance breeding efficiency but also drive economic growth within the ornamental plant industry.

References Cited

Abe H, Nakano M, Nakatsuka A, Nakayama M, Koshioka M, Yamagishi M. 2002. Genetic analysis of floral anthocyanin pigmentation traits in Asiatic hybrid lily using molecular linkage maps. Theor Appl Genet. 105(8):1175–1182. https://doi.org/10.1007/s00122-002-1053-7.

Amiteye S. 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon. 7(10):e08093. https://doi.org/10.1016/j.heliyon.2021.e08093.

Amom T, Nongdam P. 2017. The use of molecular marker methods in plants: A review. Int J Curr Res Rev. 9:17.

Arens P, Bijman P, Tang N, Shahin A, van Tuyl JM. 2012. Mapping of disease resistance in ornamentals: A long haul. Acta Hortic. 953:231–237. https://doi.org/10.17660/ActaHortic.2012.953.32.

Azizi OMA, Lau HY, Abu-Bakar N. 2021. Integration of advanced technologies for plant variety and cultivar identification. J Biosci. 46:91. https://doi.org/10.1007/s12038-021-00214-x.

Baliyan D, Sirohi A, Kumar M, Kumar V, Malik S, Sharma S, Sharma S. 2014. Comparative genetic diversity analysis in *Chrysanthemum*: A pilot study based on morpho-agronomic traits and ISSR markers. Sci Hortic. 167:164–168. https://doi.org/10.1016/j.scienta.2013.12.029.

Beruto M, Dhooghe E, Dunn B. 2024. Advancement in propagation, breeding, cultivation, and marketing of ornamentals. Horticulturae. 10(7):747. https://doi.org/10.3390/horticulturae10070747.

Bhattarai K, Sharma S, Verma S, Peres NA, Xiao S, Clark D, G, Deng Z. 2023. Construction of a genome-wide genetic linkage map and identification of quantitative trait loci for powdery mildew resistance in Gerbera daisy. Front Plant Sci. 13:1072717. https://doi.org/10.3389/fpls.2022.1072717.

Boyle TH, Menalled FD, O'Leary MC. 1994. Occurrence and physiological breakdown of self-incompatibility in easter cactus. J Am Soc Hortic Sci. 119(5):1060–1067. https://doi.org/10.21273/JASHS.119.5.1060.

Byrne DH. 2009. Rose structural genomics, p 353–379. In: Folta K, Gardiner S (eds). Genetics and genomics of Rosaceae, Springer, New York, NY, USA. http://doi.org/10.1007/978-0-387-77491-6_17.

Cao X, Wu Z, Zhou R, Jiang FL, Yang ZE. 2015. A novel random amplified polymorphic DNA-based strategy for genetic diversity analysis and identification of tomatoes. Genet Mol Res. 14(1):1650–1661. https://doi.org/10.4238/2015.March.6.11.

Debener T. 2009. Current strategies and future prospects of resistance breeding in ornamentals. Acta Hortic. 836:125–130. https://doi.org/10.17660/ActaHortic.2009.836.17.

Debener T, Byrne DH. 2014. Disease resistance breeding in rose: Current status and potential of biotechnological tools. Plant Sci. 228:107–117. https://doi.org/10.1016/j.plantsci.2014.04.005.

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH, Carter M. 2001. Implementation of markers in Australian wheat breeding. Aust J Agric Res. 52(12):1349–1356. https://doi.org/10.1071/AR01067.

Feng X, Li X, Yang X, Zhu P. 2020. Fine mapping and identification of the leaf shape gene BoFL in ornamental kale. Theor Appl Genet. 133(4):1303–1312. https://doi.org/10.1007/s00122-020-03551-x.

Fu X, Ning G, Gao L, Bao M. 2008. Genetic diversity of *Dianthus* accessions as assessed using two molecular marker systems (SRAPs and ISSRs) and morphological traits. Sci Hortic. 117(3):263–270. https://doi.org/10.1016/j.scienta.2008.04.001.

Goh MWK, Kumar PP, Lim SH, Tan HTW. 2005. Random amplified polymorphic DNA analysis of the moth orchid, *Phalaenopsis* (Epidendroideae: Orchidaceae). Euphytica. 141(1–2):11–22. https://doi.org/10.1007/s10681-005-4814-y.

Gu W, Zheng J, Zhang Y, Liu Z. 2002. A preliminary study on selection and breeding of new lines and main genetic characteristics of ornamental kale. J Shanghai JiaoTong Univ (Agric Sci). 20:129–132.

Gupta S, Pandey-Rai S, Srivastava S, Naithani SC, Prasad M, Kumar S. 2007. Construction of genetic linkage map of the medicinal and ornamental plant *Catharanthus roseus*. J Genet. 86(3):259–268. https://doi.org/10.1007/s12041-007-0033-8.

Hamrick JL. 1989. Isozyme and the analysis of genetic structure in plant populations, p 87–105. In: Soltis DE, Soltis PS (eds). Isozymes in plant biology. Dioscorides Press, Oregon, Portland, OR, USA.

Haristianita MD, Handini AS, Sukma D. 2017. Molecular marker development based on diversity of genes associated with pigment biosynthetic pathways to support breeding for novel colors in Phalaenopsis. Acta Hortic. 1167:305–312. https://doi.org/10.17660/ActaHortic.2017.1167.44.

Hsiao YY, Pan ZJ, Hsu CC, Yang YP, Hsu YC, Chuang YC, Shih HH, Chen WH, Tsai WC, Chen HH. 2011. Research on orchid biology and biotechnology. Plant Cell Physiol. 52(9):1467–1486. https://doi.org/10.1093/pcp/pcr100.

Husain R, Pandey S, Singh D, Gard P, Bose SK, Vikram N, Khan M, Khan NA, Kumar D, Shamim M, Gahlaut V. 2024. Molecular breeding in ornamental crops: Current trends and future prospects in the genomic era. In: Bhargava B, Kumar P, Verma V (eds). Ornamental horticulture: Latest cultivation practices and breeding technologies. Springer, Singapore.

Jaccoud D, Peng K, Feinstein D, Kilian A. 2001. Diversity arrays: A solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29(4):E25. https://doi.org/10.1093/nar/29.4.e25.

Jin C, Dong L, Chang W, Wani MA, Yang C, Li S, Li F. 2023. Creating novel ornamentals via new strategies in the era of genome editing. Front Plant Sci. 14. https://doi.org/10.3389/fpls.2023.1142866.

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK. 2011. Microsatellite markers: An overview of the recent progress in plants. Euphytica. 177(3):309–334. https://doi.org/10.1007/s10681-010-0286-9.

Kiani M, Zamani Z, Khalighi A, Fatahi R, Byrne DH. 2008. Wide genetic diversity of *Rosa damascena* Mill. germplasm in Iran as revealed by RAPD analysis. Sci Hortic. 115(4):386–392. https://doi.org/10.1016/j.scienta.2007.10.013.

Kuligowska K, Lutken H, Muller R. 2016. Towards development of new ornamental plants: Status and progress in wide hybridization. Planta. 244(1):1–17. https://doi.org/10.1007/s00425-016-2493-7.

Kumar P, Kumar M, Naresh RK, Navneet K, Chaudhary P, Sharma S. 2016. Evaluation of genetic diversity among gladiolus (*Gladiolus hybridus* Hort.) Germplasm using ISSR markers. Int J Agric Stat Sci. 12(1):277–283. https://doi.org/10.1007/s12298-018-0519-2.

Kumari N, Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. Am J Anim Vet Sci. 9(1):6–13. https://doi.org/10.3844/ajavsp.2014.6.13.

Leus L. 2018. Breeding for disease resistance in ornamentals, p 97–125. In: Van Huylenbroeck J (ed). Ornamental crops. Springer Nature, Berlin, Germany. https://doi.org/10.1007/978-3-319-90698-0_5.

Lewis WH. 1957. Revision of the genus *Rosa* in Eastern North America: A review. Am Rose Annu. 42:116–126.

Lu C, Li Y, Wang J, Qu J, Chen Y, Chen X, Huang H, Dai S. 2021. Flower color classification and correlation between color space values with pigments in potted *Chrysanthemum multiflora*. Sci Hortic. 283(4):110082. https://doi.org/10.1016/j.scienta.2021.110082.

Luypaert G, Van Huylenbroeck J, De Riek J, De Clercq P. 2014. Screening for broad mite susceptibility in *Rhododendron simsii* hybrids. J Plant Dis Prot. 121(6):260–269. https://doi.org/10.1007/BF03356521.

Manivannan A, Choi S, Jun TH, Yang EY, Kim JH, Lee ES, Lee HE, Kim DS, Ahn YK. 2021. Genotyping by sequencing-based discovery of SNP markers and construction of linkage map from F5 population of pepper with contrasting powdery mildew resistance trait. BioMed Res Int. 2021(1):6673010. https://doi.org/10.1155/2021/6673010.

Martí:n C, Uberhuaga E, Pérez C. 2002. Application of RAPD markers in the characterization of *Chrysanthemum* varieties and the assessment of somaclonal variation. Euphytica. 127(2):247–253. https://doi.org/10.1023/A:1020215016347.

Meerow AW. 2005. Molecular genetic characterization of new floricultural germplasm. Acta Hortic. 683. http://doi.org/10.17660/ActaHortic.2005.683.4.

Mei Z, Zhang C, Khan AM, Zhu Y, Tania M, Luo P, Fu J. 2015. Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in Angelica sinensis (Oliv.) Diels varieties of China. Electron J Biotechnol. 18(2):6–102. http://doi.org/10.1016/j.ejbt.2014.12.006.

Mondini L, Noorani A, Pagnotta MA. 2009. Assessing plant genetic diversity by molecular tools. Diversity (Basel). 1:19–35. https://doi.org/10.3390/d1010019.

Mor VS, Deswal DP, Mann A, Dahiya BS, Beniwal BS. 2008. Characterization of marigold (*Tagetes* spp.) genotypes using SDS PAGE and RAPD markers. Seed Sci Technol. 36(3):757–766. https://doi.org/10.15258/sst.2008.36.3.25.

Morgante M, Hanafey M, Powell W. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat Genet. 30(2):194–200. https://doi.org/10.1038/ng822.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. 1986. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 51:263–273. https://doi.org/10.1101/sqb.1986.051.01.032.

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H, Chung G, Baloch FS. 2017. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnol Equip. 32(2):261–285. https://doi.org/10.1080/13102818.2017.1400401.

Neale DB, Kremer A. 2011. Forest tree genomics: Growing resources and applications. Nat Rev Genet. 12(2):111–122. https://doi.org/10.1038/nrg2931.

Oyant LSH, Crespel L, Rajapakse S, Zhang L, Foucher F. 2007. Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. Tree Genet Genomes. 4(1):11–23. https://doi.org/10.1007/s11295-007-0084-2.

Premkrishnan BV, Arunachalam V. 2012. Silico RAPD priming sites in expressed sequences and iSCAR markers for oil palm. Int J Genomics. 2012:1–5. https://doi.org/10.1155/2012/913709.

Qi W, Chen X, Fang P, Shi S, Li J, Liu X, Cao X, Zhao N, Hao H, Li Y, Han Y, Zhang Z. 2018. Genomic and transcriptomic sequencing of *Rosa hybrida* provides microsatellite markers for breeding, flower trait improvement and taxonomy studies. BMC Plant Biol. 18(1):119. https://doi.org/10.1186/s12870-018-1322-5.

Rafalski JA. 2002. Novel genetic mapping tools in plants: SNPs and LD-based approaches. Plant Sci. 162(3):329–333. https://doi.org/10.1016/S0168-9452(01)00587-8.

Ren J, Liu Z, Niu R, Feng H. 2015. Mapping of Re, a gene conferring the red leaf trait in ornamental kale (*Brassica oleracea* L. var. acephala). Plant Breed. 134(4):494–500. https://doi.org/10.1111/pbr.12286.

Rusanov K, Kovacheva N, Atanassov A, Atanassov I. 2009. *Rosa damascena* Mill., the oil-bearing Damask rose: Genetic resources, diversity and perspectives for molecular breeding. Floric Ornam Biotechnol. 3:14–20.

Scovel G, Ben Meir H, Ovadis M, Itzhaki H, Vainstein A. 1998. RAPD and RFLP markers tightly linked to the locus controlling carnation (*Dianthus caryophyllus*) flower type. Theor Appl Genet. 96(1):117–122. https://doi.org/10.1007/s001220050717.

Semagn K, Bjørnstad A, Ndjiondjop MN. 2006. An overview of molecular marker methods for plants. Afr J Biotechnol. 2540:25–68.

Shahin A, Arens P, Adriaan W, Van H, Linden G, Kaauwen MV, Khan N, Schouten H, Weg WEV, Richard G, Visser F, Jaap M. 2010. Genetic mapping in *Lilium*: Mapping of major genes and quantitative trait loci for several ornamental traits and disease resistances. Plant Breed. 130(3):372–382. https://doi.org/10.1111/j.1439-0523.2010.01812.x.

Sobrino B, Brion M, Carracedo A. 2005. SNPs in forensic genetics: A review on SNP typing methodologies. Forensic Sci Int. 154(2–3):181–194. https://doi.org/10.1016/j.forsciint.2004.10.020.

Sorkheh K, Shiran B, Aranzana MJ, Mohammadi SA, Martínez-Gomez P. 2007. Application of amplified fragment length polymorphism (AFLPs) analysis to plant breeding and genetics: Procedures, applications and prospects. J Food Agric Environ. 5(1):197–204.

Southern EM. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 98(3):503–517. https://doi.org/10.1016/s0022-2836(75)80083-0.

Spiller M, Linde M, Hibrand-Saint Oyant L, Tsai C-J, Byrne DH, Smulders MJM, Foucher F, Debener T. 2011. Towards a unified genetic map for diploid roses. Theor Appl Genet. 122(3):489–500. https://doi.org/10.1007/s00122-010-1463-x.

Strommer J, Peters J, Zethof J, de Keukeleire P, Gerats T. 2002. AFLP maps of *Petunia hybrida*: Building maps when markers cluster. Theor Appl Genet. 105(6–7):1000–1009. https://doi.org/10.1007/s00122-002-1009-y.

Subramanyam K, Muralidhararao D, Devanna N. 2009. Genetic diversity assessment of wild and cultivated varieties of *Jatropha curcas* (L.) in India by RAPD analysis. Afr J Biotechnol. 8(9):1900–1910.

Sudarsono, Haristianita MD, Handini AS, Sukma D. 2017. Molecular marker development based on diversity of genes associated with pigment biosynthetic pathways to support breeding for novel colors in Phalaenopsis. Proc. I International Symposium on Tropical and Subtropical Ornamentals. Acta Hortic. 305–312.

Tang N, Shahin A, Bijman P, Liu J, Tuyl J, Arens P. 2013. Genetic diversity and structure in a collection of tulip cultivars assessed by SNP markers. Sci Hortic. 161:286–292. https://doi.org/10.1016/j.scienta.2013.07.016.

Tanksley SD, Orton TJ. 1983. Isozymes in plant genetics and breeding. Elsevier Science, Amsterdam, The Netherlands.

Van Heusden AW, Jongerius MC, van Tuyl JM, Straathof TP, Mes JJ. 2002. Molecular assisted breeding for disease resistance in lily. Acta Hortic. 572:131–138. https://doi.org/10.17660/ActaHortic.2002.572.15.

Veluru A, Bhat KV, Raju DVS, Prasad KV, Tolety J, Bharadwaj C, Rama Mitra SV, Banyal N, Singh KP, Panwar S. 2020. Characterization of Indian bred rose cultivars using morphological and molecular markers for conservation and sustainable management. Physiol Mol Biol Plants. 26(1):95–106. https://doi.org/10.1007/s12298-019-00735-8.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23(21):4407–4414. https://doi.org/10.1093/nar/23.21.4407.

Voss DH. 1992. Relating colorimeter measurement of plant color to the Royal Horticultural Society Colour Chart. HortScience. 27(12):1256–1260. https://doi.org/10.21273/HORTSCI.27.12.1256.

Weising K, Nybom H, Wolff K, Meyer W. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, FL, USA.

Welsh J, McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18(24):7213–7218. https://doi.org/10.1093/nar/18.24.7213.

Whankaew S, Hasthanasombut S, Thaikert R, Tappiban P, Smith DR, Triwitayakorn K. 2014. Molecular characterization and genetic relationship of marigolds (*Tagetes* spp.) based on simple sequence repeat markers. Plant Genet

Res. 12(3):317–322. https://doi.org/10.1017/S1479262114000100.
Williams GK, Kubelik AR, Livak KL, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18(22):6531–6535. https://doi.org/10.1093/nar/18.22.6531.
Xiao N, Wang H, Yao W, Zhang M, Ming R, Zhang J. 2020. Development and evaluation of SSR markers based on large scale full-length transcriptome sequencing in sugarcane. Trop Plant Biol. 13(4):343–352. https://doi.org/10.1007/s12042-020-09260-5.
Xu Y. 2010. Molecular plant breeding. CABI, Wallingford, UK.
Xu Y, Crouch JH. 2008. Marker-assisted selection in plant breeding: From publication to practice. Crop Sci. 48(2):391–407. https://doi.org/10.2135/cropsci2007.04.0191.
Xu S, Liu X, Liu J, Wang G, Lian F, Liao F. 2012. Analysis of QTL for three leaf traits in *Phalaenopsis* based on AFLP molecular genetic map. Plant Gene Trait. 3(7):34–42. http://doi.org/10.5376/pgt.2012.03.0007.
Yagi M. 2018. Recent progress in whole genome sequencing, high-density linkage maps, and genomic databases of ornamental plants. Breed Sci. 68(1):62–70. https://doi.org/10.1270/jsbbs.17080.
Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB. 2005. Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Theor Appl Genet. 110(4):766–777. https://doi.org/10.1007/s00122-004-1903-6.
Zakiyah N, Handoyo T, Kim KM. 2019. Genetic diversity analysis of Indonesian aromatic rice varieties (*Oryza sativa* L.) using RAPD. J Crop Sci Biotechnol. 22(1):55–63. https://doi.org/10.1007/s12892-018-0271-0.
Zalewska M, Lema-Rumińska J, Miler N. 2007. In vitro propagation using adventitious buds' technique as a source of new variability in *Chrysanthemum*. Sci Hortic. 113(1):70–73. https://doi.org/10.1016/j.scienta.2007.01.019.
Zhang L, Shen X. 2007. Preliminary study on genetic regularity of kale leaf shaped characters. North Hortic. 2:108–109.
Zhang Y, Wang C, Ma H, Dai S. 2013. Assessing the genetic diversity of chrysanthemum cultivars with microsatellites. J Am Soc Hort Sci. 138(6):479–486. https://doi.org/10.21273/JASHS.138.6.479.