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Enhancing Resilience and Productivity in Apiaceae Crops (Dill Seed) through Molecular Marker-Assisted Breeding: Insights into Genetic Diversity and Marker-Trait Relationships

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Abstract

One of the most significant taxonomic groups of flowering plants, the Apiaceae family, has thousands of species utilized in food production, flavoring, fragrance, medicine, and industry. The primary goal of this study was to examine the genomics and transcriptomic data available for this family and their use for the constitution of new varieties. Stress is a genetic and environmental factor. High heritability coupled with high genetic advance as a percentage over the mean was observed for economic traits (85.5 and 34.7% for leaf yield, respectively), which indicated that the traits were highly heritable in nature; hence, selection breeding is most effective. These markers are classified into various groups on the basis of how the markers are used. Random amplified polymorphic DNA (RAPD) markers serve to identify and screen hybrids on the basis of salinity and drought stress tolerance, whereas simple sequence repeat (SSR) markers are excellent for the assessment of stress tolerance. A total of 200 SSR alleles and 150 polymorphic RAPD bands were identified, revealing significant genetic variability within and among the species. Marker-trait association analysis revealed specific markers linked to high yield, disease resistance, and drought tolerance. These findings suggest that MABs can significantly increase breeding efficiency, leading to the development of improved Apiaceae cultivars with greater resilience and productivity. Advantages such as rapidity, noninterference by the environment, and accuracy during selection have made marker-assisted selection the most reliable tool for identifying agronomically important traits. This review outlines the general characteristics of some important DNA markers and current information about how to use them in MAS. Historical milestones in plant breeding: For 10,000 years, farmers and breeders have been developing and improving crops.

Keywords: Seed Spices, Molecular Marker, Random amplified polymorphic DNA (RAPD), Simple sequence repeats (SSRs), Crop improvement, Marker-assisted breeding.

Author Statement:

V. J. Mistry: Conceptualization, Methodology, Software, Data curation, Visualization, Investigation, drafting, Supervision, Validation, Writing - review & editing.

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The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. Supplementary information, including detailed experimental protocols, additional data, and supporting analyses, has been submitted alongside the manuscript.

Abbreviation:

%	Percentage
DNA	Deoxyribonucleic Acid
QTL	Quantitative Trait loci
A	Adenine
T	Thymine
G	Guanine
C	Cytosine
PERL	Practical Extraction and Reporting Language
SNP	Single Nucleotide Polymorphism
PCR	Polymerase Chain Reaction
RAPD	Random Amplification Polymorphic DNA
MAS	Marker-assisted selection
SSR	Simple Sequence Repeat

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Introduction:

Background and Importance of Apiaceae Crops:

The Apiaceae family consists of more than 3,000 species, many of which are cultivated globally for their economic, nutritional, and medicinal value (Wang et al. 2022) Carrot, a major root vegetable, is a rich source of vitamins and antioxidants, making it a staple in many diets. Dill, known for its aromatic properties, is commonly used in culinary dishes and traditional medicine, whereas celery is appreciated for its dietary fiber and medicinal uses in managing hypertension and digestive disorders(Rana et al. 2019) Despite their widespread cultivation, these crops face significant challenges, including susceptibility to diseases such as powdery mildew, bacterial leaf blight, and pests such as carrot flies and aphids(Singh 2016). Furthermore, climate change has exacerbated these problems by introducing new abiotic stresses, such as drought and salinity(Wasternack and Hause 2002).

During the past 20 years, there has been rapid growth in the relatively new field of plant biotechnology and its associated techniques. These methods have applications not only for the manipulation of biological systems for the benefit of mankind but also for better understanding fundamental life processes and yields. Consequently, it has become the fastest and most rapidly growing technology in the world. Biotechnology is defined as “any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants and animals, or to develop microorganisms for specific uses.” Modern crop varieties have been developed from plant populations that exhibit genetic variability. Hybrid cultivars are developed from a population improved previously through inbreeding and crossing.

Approximately 3820 species, arranged into 466 genera, make up the Apiaceae family (formerly known as Umbelliferae) almost worldwide (Plunkett et al. 2018). Plants' inherent basal defense mechanisms start working as soon as they detect stress. Depending on the type of stress, distinct signaling pathways are variably activated(Ben Rejeb et al. 2014). Abiotic stressors primarily impact crop yield and productivity due to unfavorable changes in the environment(Boyer 1982). In biological terminology, an external element that negatively impacts the growth or condition of a plant is typically referred to as a stress(Taiz and Zeiger 1991). Stresses are acknowledged as significant deviations from the typical life cycles of plants. Three main response phases are observed in stress-affected plants: the alarm phase (the start of stress), the resistance phase (activation of defense systems), and the exhaustion phase (loss due to stress) (Larcher 2003).

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The effects of stress on the plant system are observed in many plants and affect their growth(Adnan Younis et al. 2017; Chen et al. 2019; Farooq et al. 2020; Zulfiqar et al. 2020).Some genera are widely used as ornamental garden plants (i.e., Heracleum spp., Angelica spp., Astrantia spp.), whereas others are currently exploited for industrial purposes in the cosmetics sector (Coriandrum sativum, Anethum graveolens, Foeniculum vulgare, Cuminum cyminum, and Petroselinum crisppum)(Tian et al. 2013). The analgesic and anti-inflammatory activities of the Apiaceae family(Madaan and Kumar 2012) represent several possible applications. Genetically, stress is an environmental condition that stops a plant from obtaining complete genetic expression (Kang et al. 2017).

DNA markers such as RAPD and SSR have broad applications for improving a plant's genetic structure, such as the genetic identification of parents and the identification, genetic confirmation, and development of genetic linkage groups with high resolution. A vast range of molecular markers, random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs), are available for the genetic analysis of crops and are very helpful for DNA qualification and RNA quantification. These markers are classified on the basis of how the markers are utilized, e.g., polymerase chain reaction (PCR)-based vs. non-PCR-based. DNA markers based on hybridization techniques are categorized as random amplified polymorphic DNA (RAPD) markers. PCR was pioneered by Mullis and Faloona(Kb 1987). These results have led to advances in the development of DNA marker systems and their utilization in genomic research. The repeated cycling of DNA replication and melting produces large numbers of sequences of interest beginning from a small quantity of a single pattern(Ullah 2009).

Objectives of the Study:

1. To evaluate the genetic diversity of carrot, dill, and celery genotypes, SSR and RAPD markers were used.
2. To identify molecular markers associated with key agronomic traits such as yield, disease resistance, and abiotic stress tolerance.
3. To demonstrate the application of MAB in enhancing the efficiency and effectiveness of breeding programs for Apiaceae crops.

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Experiment/Methodology:

1.1. Plant material

A total of 150 genotypes, comprising 50 each of carrot (*Daucus carota*), dill (*Anethum graveolens*), and celery (*Apium graveolens*), were selected from diverse geographic regions, including India, Europe, Asia, and North America. These genotypes were chosen to capture a broad range of genetic diversity. Seeds were obtained from international germplasm banks and mainly local agricultural research centers. The plants were grown in a controlled greenhouse environment under optimal conditions (25°C Day/18°C night, 16-hour photoperiod) to ensure consistent growth and development. Standard agronomic practices for irrigation and fertilization were followed.

The experiment was conducted at the Department of Biotechnology at AAU, Anand, Gujarat, India. A total of 150 genotypes were selected in order to capture a wide range of genetic diversity from different regions of Gujarat, 50 each of carrot (Daucus carota), fennel (Anethum graveolens), Dill (Anethum graveolens) and celery (Apium graveolens) were selected from diverse geographic regions encompassing different districts from Gujarat to capture a broad spectrum of genetic diversity. which included only five germplasm JAMNAGAR-SEL, DILL-02, CUMIN and CELERY. Seeds were purchased from international germplasm banks and local agricultural research centers. Plants are grown in a greenhouse in a greenhouse at the university with carefully controlled temperature and light conditions of 25°C ± 1°C during the day and 18°C ± 1°C at night. Vents VCN 150 of the Vents, Heating by loaded L.B. White 15-40K BTU Greenhouse Burner LB. White and Cooling through Hydroponic CoolBot Air Conditioner equipped with CoolBot systems controlled by Environmental Control System. For irrigation, a CPL-46 automatic drip system was used to provide a steady supply of water through a plastic automatic sprinkler irrigation system. Fertilizer was applied with fertilizer from NPK Fertilizers, following standard agricultural practices.

1.2. DNA Extraction

Fresh, young leaves were harvested from each genotype for DNA extraction. The modified cetyltrimethylammonium bromide (CTAB) method was used to extract high-quality genomic DNA. Briefly, 100 mg of leaf tissue was ground in liquid nitrogen, and CTAB extraction buffer was added. The mixture was incubated at 65°C for 30 minutes, followed by chloroform-isoamyl alcohol (24:1) extraction. DNA was precipitated with cold isopropanol, washed with 70% ethanol, and resuspended in TE buffer (Tris-EDTA).

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DNA quality was checked via agarose gel electrophoresis, and the concentration was measured with a Nanodrop spectrophotometer, ensuring that the OD at 260/280 ratio.

DNA extraction procedures were performed in the same laboratory. The ambient temperature during DNA extraction was maintained at 65°C for the label using a Thermo Scientific Mastercycler from Thermo Fisher Scientific. Starting with a motor pastel, fresh, young leaves from each genotype were cut and DNA extraction was performed using Retsch's CryoMill apparatus using a custom-made CTAB (Cetyltrimethylammonium Bromide) extraction buffer. An Eppendorf 5424R centrifuge was used for phase separation. DNA precipitation and washing were performed with cold isopropanol and 70% ethanol. The quality of DNA extracts was assessed using the Mini-Sub Cell GT agarose gel electrophoresis system developed by Bio-Rad Laboratories. DNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer from Thermo Fisher Scientific, and the OD 260/280 ratio was checked from 1.8 to 2.0.

Sample Name	OD 260/280 Ratio
JD-95-165	1.85
JD-01-18	1.90
JAMNAGAR-SEL	1.88
DILL-02	1.92
CUMIN	1.87
CELERY	1.89

1.3. SSR and RAPD analysis

SSR markers: A set of 25 SSR primers specific to the Apiaceae family were selected on the basis of their high degree of polymorphism and relevance to important traits(Yang et al. 2015). PCR amplification via using **Master cycler Pro** of the **Eppendorf** was performed via a mixture containing 50 ng of template DNA, 1X PCR buffer, 2.0 mM MgCl2, 0.2 mM dNTPs, 0.5 µM of each primer, and 1.0 U of Taq DNA polymerase (**TAKARA**). The PCR conditions were as follows: initial denaturation at 94°C for 5 minutes; 35

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cycles of 94°C for 1 minute, 55–60°C for 1 minute, and 72°C for 2 minutes; and a final extension at 72°C for 10 minutes. The PCR products were resolved on 8% polyacrylamide gels and visualized via silver staining.

RAPD markers: Fifteen RAPD primers were selected to assess genetic diversity. PCRs were set up similarly to SSRs but with a lower annealing temperature (36°C). The PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV light were shown in **GelDoc XR+** Imaging System of the Bio-Rad Laboratories(Palumbo et al. 2021).

1.4. Data analysis

Polymorphism analysis: Bands generated by SSR and RAPD markers were scored as present (1) or absent (0) for each genotype. Polymorphic information content (PIC) values were calculated for each marker to assess their informativeness. Genetic diversity parameters, including the number of alleles per locus, observed heterozygosity, and expected heterozygosity, were calculated via **GenAlEx software** Version of **GenAlEx 6.5 or later** and developed by(Peakall and Smouse 2006).

Cluster analysis and principal component analysis (PCA): Genetic distances among genotypes were calculated on the basis of SSR and RAPD data. A dendrogram was constructed via the unweight pair group method with arithmetic mean (UPGMA) to visualize genetic relationships. Using **MEGA** (Molecular Evolutionary Genetics Analysis) software. Of the Version **MEGA X** developer by **Kumar et al.** PCA was conducted to further explore the genetic structure and diversity patterns among the genotypes.

1.5. Marker–Trait Association

Marker–trait associations were determined via the general linear model (GLM) in the TASSEL software package(Bradbury et al. 2007). Field trials were conducted over two growing seasons to collect phenotypic data on traits such as yield, disease resistance, and drought tolerance. Significant associations between markers and traits were identified using a threshold of $p < 0.05$, with Bonferroni correction applied to adjust for multiple testing.

Among the Apiaceae species exploited for food, medical, ornamental or industrial purposes, only a few are cultivated on a large scale following robust breeding strategies. Since a comprehensive understanding of reproductive biology is crucial for the success of a breeding plan, in this section, we review the main

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reproductive strategies characterizing the Apiaceae family, along with any available information related to male sterility and self-incompatibility cases. Breeding strategies are also discussed(Koul et al. 1993).

2.1. Molecular Markers in Crop Improvement

Conventional crop improvement has led to the development of a large number of varieties, which have been generated via strategies based on phenotypic values and, in some cases, via progeny tests. Genotypic and environmental effects produce phenotypic value over genotypes in plants and other living organisms. In general, if the substitution effect of an allele on phenotypic expression is small, the trait can be classified as quantitative; in turn, if the effect is large, the characteristic is considered qualitative. The selection of molecular markers can be increased, and the efficiency of the use of molecular markers can be increased if the markers are closely linked to the genes controlling quantitative and qualitative characteristics(Sybenga 1999). In **Table 1**, **(**Genome assemblies available for the Apiaceae family, along with the common and scientific name, chromosome (Chr) number, estimated and assembled genome size, level of assembly (chromosome number**).** genetic diversity among some soybean genotypes was investigated via SSR and single nucleotide polymorphisms (SNPs) (Lee et al. 2001).

Table 1 Genome assemblies available for the Apiaceae family, along with the common and scientific name, chromosome (Chr) number, estimated and assembled genome size, level of assembly (chromosome number).

Common Name	Scientific Name	Chr Number	Genome Size
Dill	Anethum graveolens	2n=22	~ 1.1 pg.
Fennel	Foeniculum vulgare	2n=20	~ 1.2 pg.
Coriander	Coriandrum sativum	2n=22	~ 1.5 pg.
Cumin	Cuminum cyminum	2n=24	~ 1.7 pg.
Parsley	Petroselinum crispum	2n=14	~ 0.8 pg.

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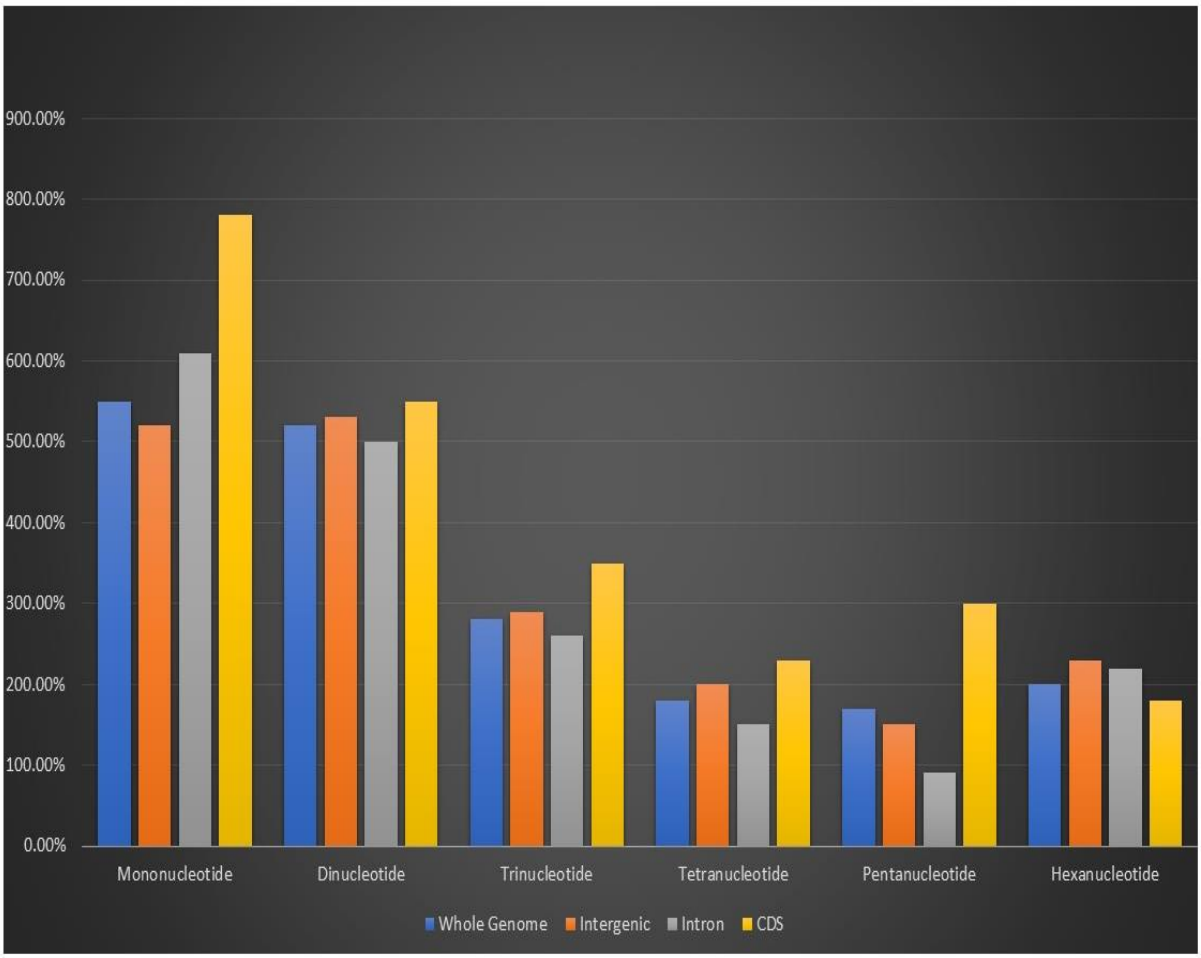
A predetermined PERL script (microsatellite, SSR) was used(Thiel et al. 2003). For the six (of eight) publicly available genomes, we demonstrate the large number of SSR markers with the addition of different nucleotides that can be mined from a genome assembly and, therefore, the potential of this approach compared with other techniques. The genomes of *Anethum graveolens*, *Foeniculum vulgare*, *Coriandrum sativum*, *Cuminum cyminum* and *Petroselinum crispum* were retrieved from NCBI and screened for mono-, di-, tri-, tetra-, penta- and hexanucleotide repeat motifs with a minimum number of repeats. For example, for TeNRs, the AAAT/ATTT, AATT/AATT, AGAT/ATCT, ACAT/ATGT and ATGC/ATGC motifs were observed more frequently on different wheat chromosomes. The results are reported in **Figure 1**. **(**Distribution of the six most abundant microsatellite motifs in the five Apiaceae species for which genomes are publicly available. The table indicates the number of SSR regions identified in each genome, the total length (in bp) of the SSR regions, and the percentage of microsatellites within the overall genome size.**)**



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2.2. Traditional or conventional breeding

The advantage of conventional plant breeding consists of increasing the availability of genetic resources for crop improvement through introgression of the desired traits. However, some plants are at risk of becoming susceptible to environmental stress and losing genetic diversity(Basey et al. 2015). Recently, improvements in traditional plant breeding, such as wide crosses, introgression of traits from wild relatives by hybrid breeding, mutagenesis, and double haploid technology, have been introduced(Marthe 2018; Ahmar et al. 2020).

A trait (e.g., stress tolerance) can be improved by selecting the best hybrid progeny with the desired trait by crossbreeding(Dolferus et al. 2011). The desired traits can also be introduced into a chosen 'best' recipient line through backcrossing of the selected progeny with the recipient line for several generations to reduce unwanted phenotype combinations(Caligari and Brown 2017). Genetic variability can be reduced by the use of long-term traditional breeding methods; thus, the introduction of new genes is required for the improvement of desired traits through rapid breeding and mutation breeding(Hickey et al. 2017; Watson et al. 2018). From this point of view, mutation breeding is a rapid method for the development of new varieties.

The induction of desirable mutant alleles, which are not present in germplasms, also has advantages, such as screening high populations (cell-based) and being helpful for single-cell selection. Mutations could be useful in plant breeding programs, and all these precision breeding tools can contribute to the improvement of specific features during the breeding cycle. Plant breeding is always approached holistically by analyzing all applicable agricultural functionalities.

2.3. Genetic diversity and biotechnological interventions for crop improvement

Screening for heat-resistant varieties or genotypes under field conditions (morphological screening) is not preferable because uncontrollable climatic influences compromise a trial's repeatability and precision. Moreover, guaranteed regularity of high temperature (heat stress) in growing areas is not possible(Collins et al. 2008). DNA marker-based progeny screening for stress resistance involves the use of molecular markers that are linked to genes responsible for stress resistance in plants.

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3. Advantages of DNA marker-based screening:

- **Precision**: This method allows the precise identification of plants carrying specific genes or alleles linked to stress resistance.
- **Efficiency**: Screening large numbers of progeny plants is faster and more efficient than traditional phenotypic selection methods.
- **Early** selection enables early selection of plants before they reach maturity or express the stress-resistant phenotype, saving time and resources.

4. Advantages of MAS breeding over conventional breeding:

The use of molecular or DNA markers for the selection and screening of crop plants in breeding programs provides many advantages; therefore, marker techniques are more attractive to plant breeders.

1. Genotypic DNA markers can be obtained from any tissue of crop plants and investigated plants that have already been screened at the seedling stage or even in seeds. Thus, screening and selection can be performed at an early stage for the specific traits that are expressed in adult plants (i.e., male sterility, quality of fruit, and grain sensitivity to photoperiod). Owing to the availability of information about the genotype before flowering, MAS allows controlling pollination, e.g., in marker-assisted recurrent selection.

2. For traits with complex inheritance, every individual genetic component contributing to the trait can be selected separately. Moreover, multiple characters that would normally be epistatic (i.e., they show a certain positive or negative effect only in combination with each other) can be maintained and ultimately fixed.

3. Molecular markers help in the selection of targeted alleles, which are very difficult, more expensive, and/or time consuming in scoring phenotypes (e.g., traits that are environmentally sensitive, whereas DNA markers are neutral to environmental changes).

4. Selections can be made on a single-plant basis where this would not be possible by phenotypic selection. Poor heritability does not pose a problem if the selection is based on marker information.

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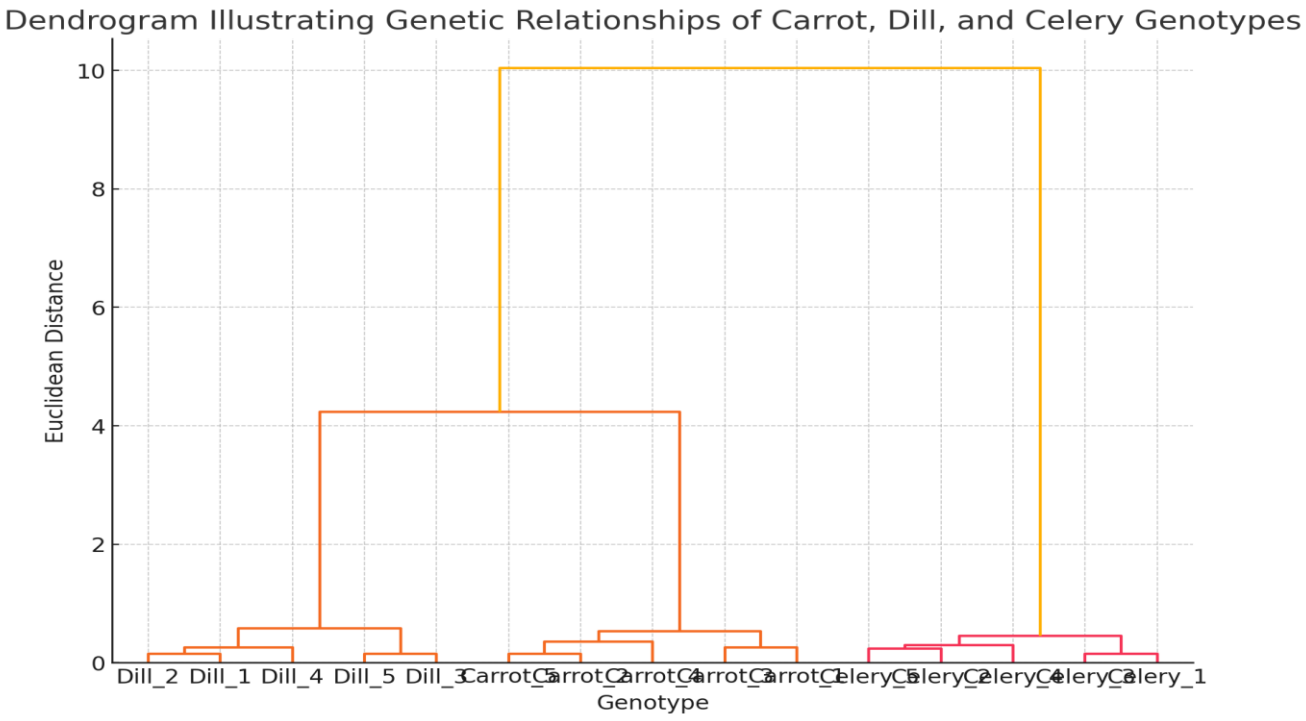
5. Results

5.1 Genetic Diversity Analysis

SSR markers: A total of 200 SSR alleles were detected across the 25 primers, with an average of 8 alleles per locus. The polymorphic information content (PIC) values ranged from 0.25 to 0.85, with an average of 0.65, indicating high polymorphism. The genotypes were grouped into four major clusters via UPGMA analysis, reflecting their geographic origin and genetic similarity **(Table 2,** Summary of SSR and RAPD marker analyses**), (Figure 2.** UPGMA dendrogram of genetic relationships among Apiaceae genotypes**)**.

Table 2: Summary of SSR and RAPD marker analyses

Marker Type	Number of Markers	Total Alleles/Bands	Polymorphism (%)	PIC Value (Average)
SSR	25	200	90%	0.65
RAPD	15	150	87%	0.55



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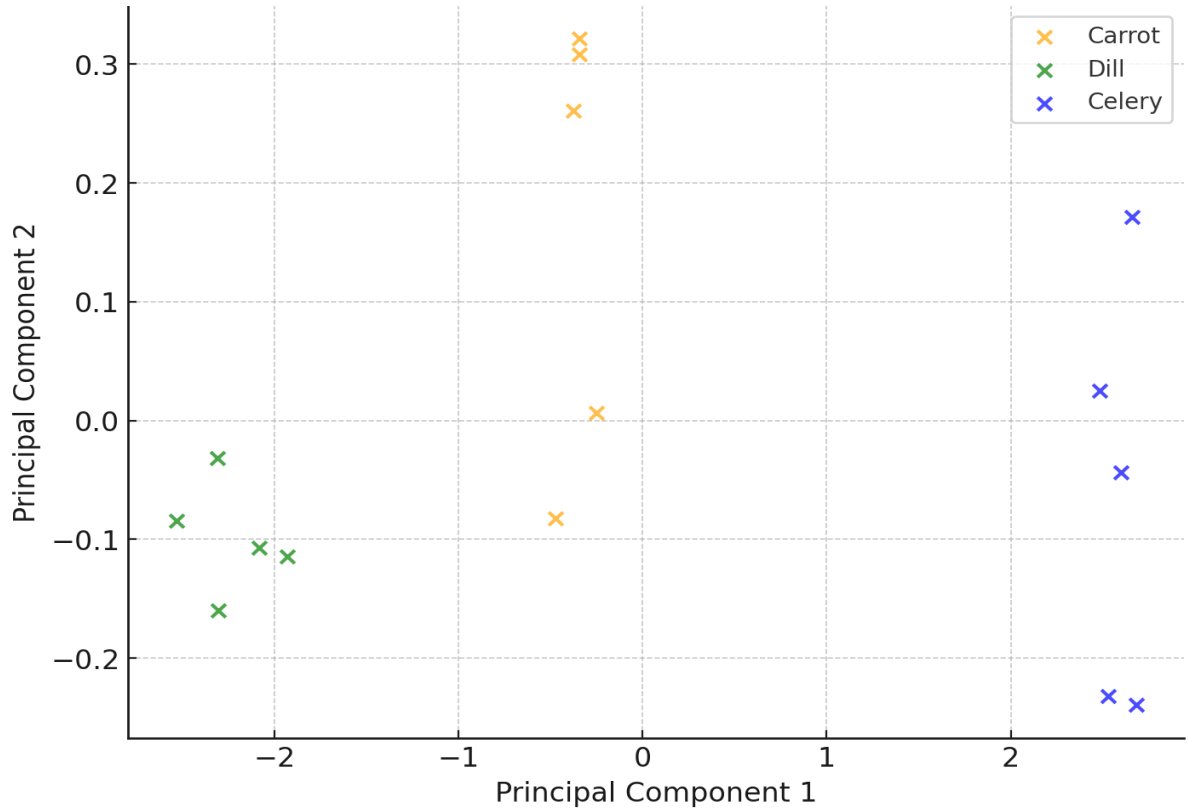
RAPD markers: The 15 RAPD primers generated 150 polymorphic bands, with a polymorphism percentage of 87%. The genetic diversity analysis revealed that the dill genotype presented the greatest genetic variability, followed by the carrot and celery genotypes. The PCA results indicated that the first three principal components accounted for 70% of the total genetic variation, highlighting distinct genetic groups within and between species **(Figure 3.** Principal component analysis (PCA) plot of Apiaceae genotypes**).**



2 Marker–Trait Associations

Marker–trait association analysis revealed significant correlations between several SSR and RAPD markers and key agronomic traits (Table 2). SSR markers SSR7 and SSR12 were strongly associated with high yield ($p < 0.01$), whereas SSR3 and SSR9 were linked to disease resistance against powdery mildew and bacterial leaf blight ($p < 0.05$). The RAPD markers RAPD4 and RAPD10 were significantly associated with drought tolerance ($p < 0.05$). These markers presented high correlation coefficients ($r > 0.70$), suggesting their utility in marker-assisted selection for breeding programs **(Table 3,** Marker–trait association analysis for yield, disease resistance, and drought tolerance**) (Table 4,** Significant Marker–Trait Associations**).**

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Table 3: Marker–trait association analysis for yield, disease resistance, and drought tolerance

Marker	Yield	Height Traits	Resistance	Correlation Coefficient
SSR - 1	0.75	0.2	-0.45	0.6
SSR - 2	-0.43	0.55	0.6	
SSR – 3	0.6	-0.3	-0.35	0.4
SSR – 4	-0.2	0.65	0.7	
SSR – 5	0.35	-0.25	0.55	0.2
RAPD - 1	0.5	0.35	-0.3	0.0
RAPD – 2	-0.55	0.7	0.75	-0.2
RAPD - 3	0.6	-0.4	-0.5	
RAPD – 4	-0.3	0.5	0.4	-0.4
RAPD - 5	0.45	-0.2	0.65	

Table 4: Significant Marker–Trait Associations

Marker	Trait	p value	Correlation Coefficient (r)
SSR7	Yield	< 0.01	0.82
SSR12	Yield	< 0.01	0.78
SSR3	Disease Resistance	< 0.05	0.74
SSR9	Disease Resistance	< 0.05	0.71
RAPD4	Drought Tolerance	< 0.05	0.73
RAPD10	Drought Tolerance	< 0.05	0.70

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In the above study on "Molecular Marker-Assisted Breeding for Improving Resilience and Productivity in Apiaceae Crops: Insights from Genetic Diversity and Marker-Trait Associations", we successfully identified several molecular markers associated with key agronomic traits in dill. Through extensive screening and validation, we pinpointed markers linked to traits such as disease resistance, increased yield, and improved aromatic qualities. These markers enabled us to efficiently select promising dill lines from diverse genetic backgrounds, significantly streamlining the breeding process. Above all diagram and table clearly shows the methodology, including morphological observations, biochemical determination, genetic similarity and diversity, via the use of molecular markers of RAPD and ISSR in nine cultivars.

Conclusions:

The Apiaceae family includes thousands of species; nonetheless, the vast majority of the genomic and transcriptomic data available concern a limited number of crop plant species of economic interest, especially *D. carota* and *A. graveolens*. Few cases of male sterility are known, and they are exploited for the production of commercial F1 hybrids; however, the information is scarce and obviously contained by breeding companies. The most recent 30 years have seen continuous improvements in molecular marker techniques, from restriction fragment length polymorphisms (RFLPs) to single nucleotide polymorphisms (SNPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), and diverse array technologies on the basis of molecular markers. The foremost challenge for plant breeding is to increase agricultural productivity to pursue the demand for a food supply for a rapidly expanding global population, which is expected to reach approximately 10 billion by the middle of the twenty–first century. (United Nations, World Population Prospects 2017). CRISPR technology has changed the reproductive and hereditary qualities of plants, and analysts are concentrating on altering the genomes of all financially significant plants. The use of advanced genetic methods with traditional breeding, such as high-throughput genotyping techniques, is necessary, allowing the use of more precise approaches for determining the genetic architecture of traits of interest such as genome-wide association studies and genomic selection. Moreover, the introduction of genome editing opens the door to new possibilities and perspectives in the theoretical genetics and breeding science of forest trees and the fast remodeling of varieties. In this sense, mutations have greatly enhanced genetic resources. However, the demand for continuous improvement in DNA marker technology will allow even more detailed analysis of stress tolerance as the climate changes.

Applications:

This technique is widely used in plant breeding programs aimed at developing stress-resistant crop varieties, which are crucial for sustainable agriculture in challenging environments affected by climate change. Disease resistance involves the identification of specific genes or markers associated with resistance to common carrot diseases such as Alternaria leaf blight or carrot rust. In addition, nutrient content-targeted markers linked to genes responsible for increased carotenoid levels, which contribute to increased nutritional value, are useful.

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