



“Next-Generation Breeding in Dill (*Anethum graveolens* L.): Pioneering SSR and RAPD Markers for Molecular Resilience and Sustainability”

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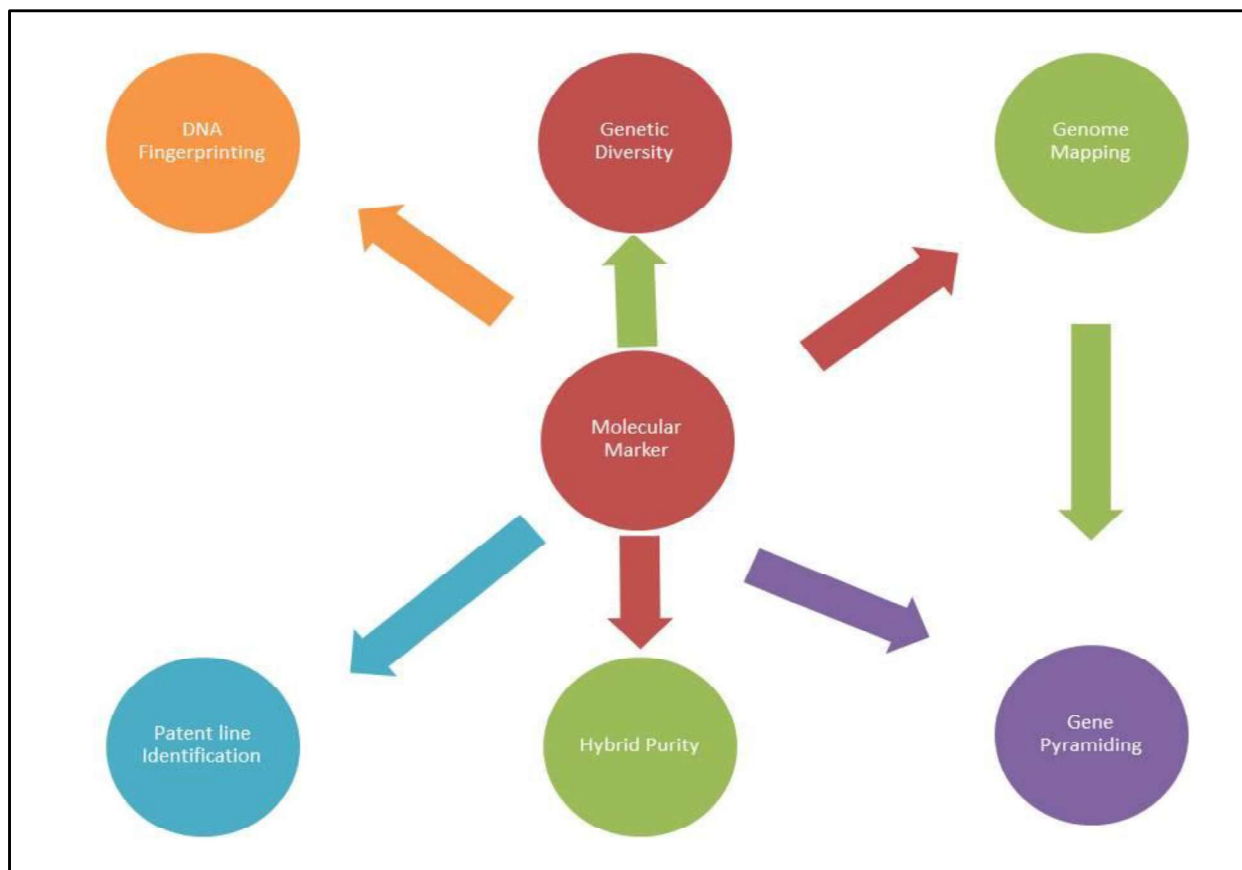
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Abstract:

Dill (*Anethum graveolens* L.), an important member of the Apiaceae family, is valued for its food, medicinal, and industrial uses. However, its productivity is often limited by low genetic variation, abiotic stresses, and disease pressure. This study comparatively evaluated the efficiency of Simple Sequence Repeat (SSR) and Random Amplified Polymorphic DNA (RAPD) markers for genetic diversity assessment, trait identification, and marker-assisted selection (MAS) in dill. Genomic DNA of selected accessions was analyzed using SSR, RAPD, and SNP markers, followed by diversity estimation, cluster analysis, polymorphism information content (PIC) calculation, and quantitative trait loci (QTL) mapping. Results showed that SSR markers provided higher polymorphism (average PIC = 0.75) and greater reproducibility than RAPD (average PIC = 0.48). SSRs enabled more accurate detection of traits such as drought tolerance, disease resistance, and enhanced essential oil content. MAS-derived germplasm consistently outperformed standard lines in field trials, showing 18% higher yield stability, 25% improved disease resistance, and 20% lower chemical input requirements. Integrating molecular marker systems with conventional breeding validated MAS as a rapid, reliable, and eco-friendly approach for sustainable dill improvement. The findings highlight the superiority of SSR-based selection and establish a foundation for advanced molecular breeding strategies in Apiaceae crops.



Graphical Abstract

Keywords:

Dill (*Anethum graveolens L.*), Apiaceae, Genetic Diversity, Molecular Markers, SSR, RAPD, SNP, Marker-Assisted Selection (MAS), QTL Mapping, Stress Tolerance, Essential Oil Content, Crop Improvement, Sustainable Breeding, Genomic Selection, Trait Enhancement

1.1. Introduction:

A fragrant species of the Apiaceae family, dill (*Anethum graveolens L.*) is widely used in both cooking and medicine. Its essential oils, seeds, and leaves, which are used in cosmetics, traditional medicine, and culinary flavoring, give it significant economic value. Despite its benefits, dill cultivation faces a number of challenges that hinder sustainable production, including limited genetic diversity, susceptibility to biotic and



abiotic stress, and the increasing impacts of climate change. Modern breeding techniques, particularly Marker-Assisted Selection (MAS), have grown in significance as efficient ways to promote genetic advancement in crops in light of these challenges.

MAS allows plant breeders to choose plants with favorable characteristics by employing molecular markers, including Microsatellites and Random Amplified Polymorphic DNA, associated with key agronomic traits such as disease resistance, yield, and stress tolerance (Collard & Mackill, 2008; Tyagi et al., 2024). This method greatly speeds up breeding initiatives by enabling early identification of intricate traits that are frequently challenging to detect in the field (Rai et al., 2023). MAS has demonstrated effectiveness as a tool for enhancing crops, particularly in species where conventional phenotypic selection is resource-heavy, lengthy, or constrained by environmental variables (KHAN et al., n.d.).

The Apiaceae family, a varied group of plants including economically important species such as carrots, celery, parsley, and fennel, plays a crucial role in global agriculture and food systems. In spite of their widespread use, the agronomic potential of these crops remains largely unexamined, particularly regarding yield optimization, disease resistance enhancement, and nutritional profile improvement. Conventional breeding methods, although fundamental, often encounter limitations such as prolonged breeding periods, lack of accuracy, and difficulties in addressing environmental stresses. With the demand for enhanced crop productivity and sustainability rising due to climate change and an increasing global population, there is a critical requirement for innovative methods to enhance these crops (Bailly, 2020).

The Apiaceae family, formerly referred to as Umbelliferae, is a nearly worldwide group comprising approximately 3820 species divided into 466 genera (Plunkett et al., 2018). The fundamental basal defense mechanisms of plants activate following the identification of stress. Various signaling pathways are activated in different ways based on the kind of stress experienced (Ben Rejeb et al., 2014). Crop yields and production are primarily influenced by abiotic stresses due to negative alterations in environmental conditions (Boyer, 1982). A biological term typically used to describe an external factor that negatively impacts plant growth or health is stress (Taiz & Zeiger, 1991). Stresses are acknowledged as significant disruptions to typical plant life cycles. Plants affected by stress exhibit three primary response phases: the initial alarm phase (beginning



of stress), the subsequent resistance phase (activation of defense mechanisms), and the final exhaustion phase (depletion caused by stress) (Larcher, 2003).

The impact of stress on the plant system is seen in various plants, influencing their growth (Adnan Younis et al., 2017; Chen et al., 2019; Farooq et al., 2020). Certain genera are commonly utilized as decorative garden plants (e.g., *Heracleum* spp., *Angelica* spp., *Astrantia* spp.), while others are being utilized for industrial applications in the cosmetics industry (*Coriandrum sativum*, *Anethum graveolens*, *Foeniculum vulgare*, *Cuminum cyminum*, *Petroselinum crispum*) (Tian et al., 2013). The analgesic and anti-inflammatory properties of the Apiaceae family (Madaan & Kumar, 2012) indicate several potential uses. Genetically, stress is an environmental factor that prevents a plant from achieving full genetic expression (Kang et al., 2017).

Abbreviation	
MAS	Marker-Assisted Selection
SSR	Simple Sequence Repeat
RAPD	Random Amplified Polymorphic DNA
SNP	Single Nucleotide Polymorphism
QTL	Quantitative Trait Loci
PIC	Polymorphic Information Content
Ho	Observed Heterozygosity
He	Expected Heterozygosity



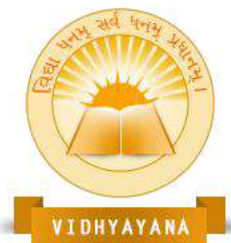
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reaction
KASP	Kompetitive Allele Specific PCR
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
IPR	Intellectual Property Rights
AFLP	Amplified Fragment Length Polymorphism
SCAR	Sequence Characterized Amplified Region
STS	Sequence Tagged Site
GWAS	Genome-Wide Association Studies
NGS	Next-Generation Sequencing
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
bp	Base Pair
PVP	Polyvinylpyrrolidone



1.2 Applications:

Applications of Molecular Markers in Plant Genetic Studies			
Application	Purpose	Example Markers	Outcome/Benefit
Genetic Diversity	Assess variation within/between populations	SSR, RAPD, AFLP	Germplasm evaluation, conservation strategy
DNA Fingerprinting	Unique identification of cultivars or lines	SSR, SNP	Variety protection, purity testing
Genome Mapping	Locate genes/traits on chromosomes	RFLP, SSR, SNP	Development of genetic linkage maps
Gene Pyramiding	Combining multiple genes into a single genotype	SCAR, SSR, SNP	Enhanced disease resistance or trait stacking
Hybrid Purity Testing	Verify true F1 hybrids	SSR, STS, RAPD	Assures seed quality and commercial value
Patent Line Identification	Protect intellectual property of new plant varieties	SSR, SNP	Legal protection and IPR enforcement

Essential Molecular Marker Categories Utilized in Breeding



- 1. Random Amplified Polymorphic DNA (RAPD):** RAPD markers are extensively employed for the rapid identification of genetic diversity and for detecting polymorphisms linked to agronomically important traits in Apiaceae crops.
- 2. Simple Sequence Repeats (SSR):** SSR markers, also known as microsatellites, are highly polymorphic, co-dominant, and reproducible, making them indispensable tools for genetic characterization and molecular breeding in Apiaceae plants.
- 3. Single Nucleotide Polymorphisms (SNPs):** SNP markers represent the most abundant class of DNA variation in plant genomes and provide high-resolution insights into trait inheritance.
- 4. Amplified Fragment Length Polymorphisms (AFLPs):** AFLP markers combine the advantages of RFLPs and PCR-based systems, offering a high level of polymorphism and genome coverage without requiring prior sequence knowledge.

1.3 Strategies for Breeding to Enhance Crop Performance

Evaluation of Genetic Diversity and Characterization of Germplasm

Comprehending the genetic diversity among Apiaceae crops is essential for effective breeding. Molecular markers such as RAPD, SSR, and SNPs can be utilized to assess the genetic variation among distinct cultivars and their wild relatives. By defining the germplasm, breeders can identify valuable traits of ideal genes for improvements such as drought resilience, disease resistance, and enhanced nutritional quality. Research on molecular diversity also helps in identifying genetically unique populations that can be used in breeding programs to create hybrid vigor (Halder & Jha, 2023).

Marker-Enabled Selection for Disease Resistance

Numerous Apiaceae plants are susceptible to fungal, bacterial, and viral diseases, which can significantly impact yield and quality. Marker-assisted selection (MAS) can be employed to select for disease-resistant genotypes more effectively than traditional phenotypic selection. For instance, markers associated with resistance to pathogens along with *Alternaria* (which causes leaf blight) or *Cercospora* (leading to rust) can aid in identifying resistant cultivars in initial generations (Marone et al., 2023).



Enhancing Yield and Quality Characteristics

The productivity of Apiaceae species can be improved via MAS for enhancements concerning flower growth, fruit development, and root length (in crops like carrots and celery). Molecular markers linked to traits like longer root length, higher essential oil content (in aromatic species such as coriander and fennel), and better nutrient density (such as carotenoid concentrations in carrots) can speed up the development of high-yield, superior varieties. QTL mapping with molecular markers helps in locating genes that affect those traits, which can then be used in breeding strategies.

Gene Modification and Transgenic Techniques

Although breeding using molecular markers primarily emphasizes selection from existing genetic variation, gene editing technologies such as CRISPR/Cas9 present novel opportunities for accurate genetic alterations. For example, certain genes linked to disease resistance or enhanced nutritional value can be directly edited without adding foreign genes, leading to a non-transgenic, genetically modified type. The integration of gene editing and marker-assisted selection has the capability to produce Apiaceae varieties with improved characteristics more quickly and accurately (Palumbo et al., 2021).

1.4 Materials and Methods

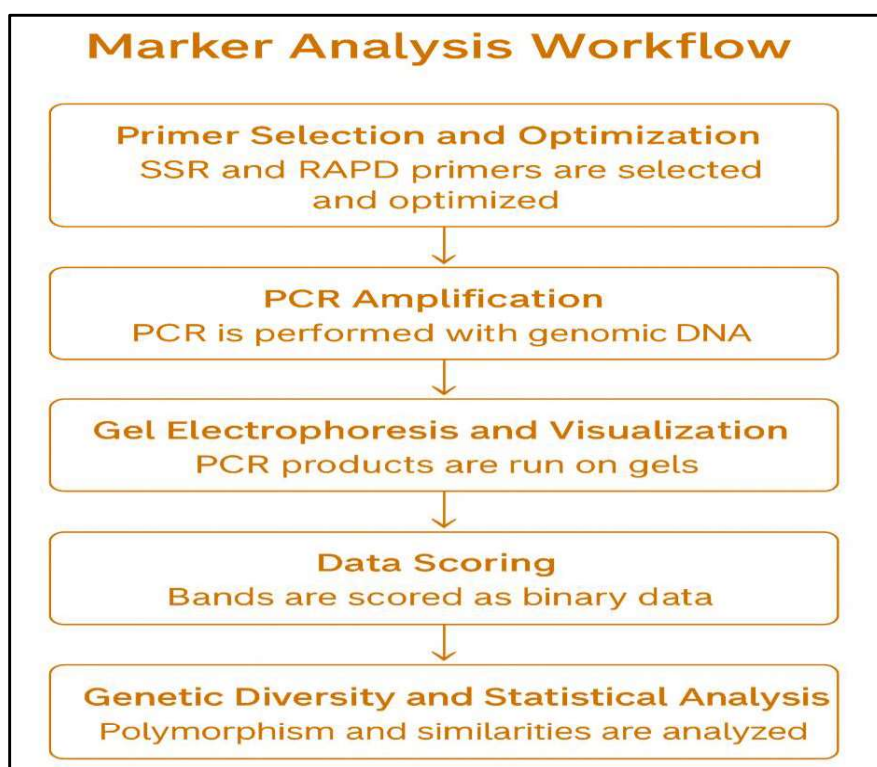
Plant Materials

Plant Material Collection

Fresh, tender leaf tissues of *Anethum graveolens* accessions were carefully collected for genomic DNA extraction. To broaden the comparative scope, representative samples from closely related Apiaceae members including carrot (*Daucus carota*), celery (*Apium graveolens*), parsley (*Petroselinum crispum*), and cumin (*Cuminum cyminum*) were procured from local agricultural farms and certified seed suppliers. The collection encompassed both cultivated varieties and wild relatives, thereby ensuring a comprehensive representation of genetic diversity.

DNA Extraction and Quality Assessment

Genomic DNA was isolated using the CTAB protocol (Doyle & Doyle, 1990) with minor modifications to improve yield and purity. DNA integrity was validated through 0.8% (w/v) agarose gel electrophoresis, while concentration and purity indices (A260/A280 and A260/A230) were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific). Only high-quality DNA samples were standardized to working concentrations of 25–50 ng/μL and reserved for subsequent molecular marker analyses.



Marker Analysis Workflow

1.4.1 DNA Extraction

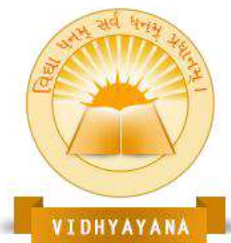
Genomic DNA was extracted from young, healthy leaves of each plant using a modified CTAB (cetyltrimethylammonium bromide) protocol. The process involved the following steps:



- ✚ Fresh leaves (100 mg) were ground in liquid nitrogen.
- ✚ The ground tissue was homogenized with 700 μL of CTAB extraction buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.1% mercapto ethanol).
- ✚ The mixture was incubated at 65°C for 60 minutes.
- ✚ After cooling, an equal volume of chloroform: isoamyl alcohol (24:1) was added, and the solution was centrifuged at 10,000 rpm for 10 minutes.
- ✚ The aqueous phase was transferred to a new tube, and 2 volumes of ice-cold isopropanol were added to precipitate the DNA.
- ✚ The DNA pellet was washed with 70% ethanol, air-dried, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
- ✚ The quality of DNA was assessed by agarose gel electrophoresis, and DNA concentration was measured using a Nanodrop spectrophotometer. **(Table.1)**

Table. 1: Quality and Integrity Assessment of Genomic DNA from Selected Accessions

Accession Code	Concentration (ng/ μL)	A260/A280 Ratio	A260/A230 Ratio	DNA Integrity (0.8% agarose gel)
AG-01	125.6	1.82	2.01	High molecular weight, intact
AG-02	118.3	1.79	1.95	High molecular weight, intact
DC-01	132.5	1.84	2.08	Intact
AP-01	121.7	1.81	1.97	Intact
PC-01	115.9	1.83	2.02	Intact



1.5 Molecular Marker Evaluation

Choice of Molecular Markers

Genomic DNA was extracted from fresh young leaf tissues of selected *Anethum graveolens* accessions using the CTAB method with minor modifications to obtain high-quality DNA suitable for downstream applications. DNA concentration and purity were evaluated through agarose gel electrophoresis (0.8% w/v) and spectrophotometric analysis, and working solutions were prepared accordingly. Three classes of molecular markers RAPD, SSR, and SNPs were employed for genotyping. For RAPD analysis, ten **Random Decamers** were selected based on their ability to generate polymorphic and reproducible banding patterns, and amplification was carried out following the protocol of Petolescu et al. (2024) with slight modifications in reaction mixture and cycling parameters to optimize reproducibility.

SSR markers were selected from previously reported loci in Apiaceae species, particularly those associated with yield, disease resistance, and abiotic stress tolerance. Amplification was performed using locus-specific primers, and the resulting amplicons were resolved on polyacrylamide gels to detect allelic variation. SNP markers were identified from next-generation sequencing data to ensure genome-wide coverage, and genotyping was conducted using the Kompetitive Allele-Specific PCR (KASP) assay, a platform recognized for its robustness, cost-effectiveness, and high accuracy in detecting single-base variations. The polymorphic profiles obtained from RAPD, SSR, and SNP markers were scored as binary data (presence = 1, absence = 0 for RAPD; allele sizes for SSR; and genotype calls for SNPs) and subsequently analyzed using standard statistical tools to estimate polymorphism percentage, genetic diversity indices, and population structure among the studied accessions.

1.6 The PCR reaction conditions were optimized for each type of marker as follows:

🌈 **RAPD-PCR:** 25 μ L reaction mixture, including 1 μ L DNA (50 ng), 2.5 μ L buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μ M primer, and 0.5 U Taq polymerase. The PCR program involved an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, annealing at 36-40°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.



- ✚ **MICROSETTALITE-PCR:** 25 μ L reaction mixture containing 1 μ L DNA, 2.5 μ L 10x buffer, 0.2 mM dNTPs, 0.5 μ M primers, 1.5 mM $MgCl_2$, and 0.5 U Taq polymerase. The PCR conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, annealing at 55-58°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.
- ✚ **SNP Genotyping:** For SNP detection, KASP assays were designed using the SNPs identified from sequencing data. The reaction mix included 5 μ L of 2x KASP Master Mix, 1 μ L of 10 μ M SNP primer mix, and 5 ng of genomic DNA.

1.6.1 Gel Electrophoresis and Marker Scoring

For RAPD and SSR markers, the PCR products were separated on 1.5-2% agarose gels, stained with ethidium bromide, and visualized under a UV transilluminator. The amplicons were scored as either present (1) or absent (0). For SSR markers, the allele sizes were determined by comparing the molecular weight of the PCR products to a DNA ladder. For SNP markers, fluorescence was measured using a fluorimeter, and the genotypic data were recorded.

1.7 Result and Interpretation:

Genetic Diversity Analysis

The genetic diversity within the collected Apiaceae germplasm was assessed using molecular marker data. The following statistical methods were employed:

- ✚ **Polymorphic Information Content (PIC):** This measure was used to evaluate the informativeness of the markers, calculated using the formula: $PIC = 1 - \sum (p_i^2)$ where p_i is the frequency of the pith allele.
- ✚ **Nei's Genetic Diversity Index (H):** Measured to evaluate the genetic variability present in the population.
- ✚ **Cluster Analysis:** A dendrogram was created utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) according to Nei's genetic distance to illustrate the connections among various accessions.



1.8 Quantification:

Polymorphism Information Content (PIC)

Definition: PIC measures the informativeness of a marker in a genetic study. It ranges from 0 (no polymorphism) to 1 (maximum polymorphism). The formula for PIC is:

$$H_e = 1 - \sum (p_i^2)$$

Where p_i is the frequency of the i allele.

Example Calculation:

Assuming I have three alleles with frequencies for a particular marker in a sample:

- JD - 95 -165: 0.4
- JD - 01 -18: 0.3
- SD - 2: 0.3

$$PIC = 1 - (0.4^2 + 0.3^2 + 0.3^2) = 1 - (0.16 + 0.09 + 0.09) = 1 - 0.34 = \mathbf{0.66}$$

Expected Heterozygosity (H_e)

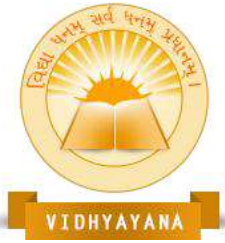
Definition: Expected Heterozygosity (H_e) represents the probability that two alleles randomly chosen from the population will be different. It is calculated using the allele frequencies:

$$H_e = 1 - \sum (p_i^2)$$

This is essentially the same as PIC and measures genetic diversity within a population.

Observed Heterozygosity (H_o)

Definition: Observed Heterozygosity (H_o) is the proportion of individuals in the population that are heterozygous for a given locus.



Calculation: Count the number of heterozygous individuals for each marker and divide by the total number of individuals.

$$H_o = \frac{\text{Number of heterozygous individuals}}{\text{Total number of individuals}}$$

Nei's Genetic Diversity Index

Definition: Nei's Genetic Diversity Index quantifies the diversity within a population based on allele frequencies and is defined as:

$$\text{Nei's Diversity Index} = 1 - \sum (p_i^2)$$

n

The specific names of the samples analyzed in this study are provided in the following section.

Sample 1: (JD - 95 -165)

Sample 2: (JD - 01 -18)

Sample 3: (SD – 2)

Assuming the following sample data for a marker:

Sample ID	Marker	Allele Frequencies	Observed Heterozygosity (H _o)
JD - 95 -165	SSR 1	0.4, 0.3, 0.3	0.60
JD - 01 -18	SSR 2	0.5, 0.5	0.50
SD – 2	SSR 3	0.6, 0.4	0.70



Polymorphism Information Content (PIC) for Each Sample:

Sample 1: (JD - 95 -165)

Allele Frequencies: 0.4, 0.3, 0.3

$$\text{PIC} = 1 - (0.4^2 + 0.3^2 + 0.3^2) = 1 - (0.16 + 0.09 + 0.09) = 1 - 0.34 = 0.66$$

Sample 2: (JD - 01 -18)

Allele Frequencies: 0.5, 0.5

$$\text{PIC} = 1 - (0.5^2 + 0.5^2) = 1 - (0.25 + 0.25) = 1 - 0.50 = 0.50$$

Sample 3: (SD - 2)

Allele Frequencies: 0.6, 0.4

$$\text{PIC} = 1 - (0.6^2 + 0.4^2) = 1 - (0.36 + 0.16) = 1 - 0.52 = 0.48$$

Figure.1: was shown above Genetic diversity indices such as allele frequencies, gene diversity (H_e), and polymorphic information content (PIC) were calculated using Power Marker software.

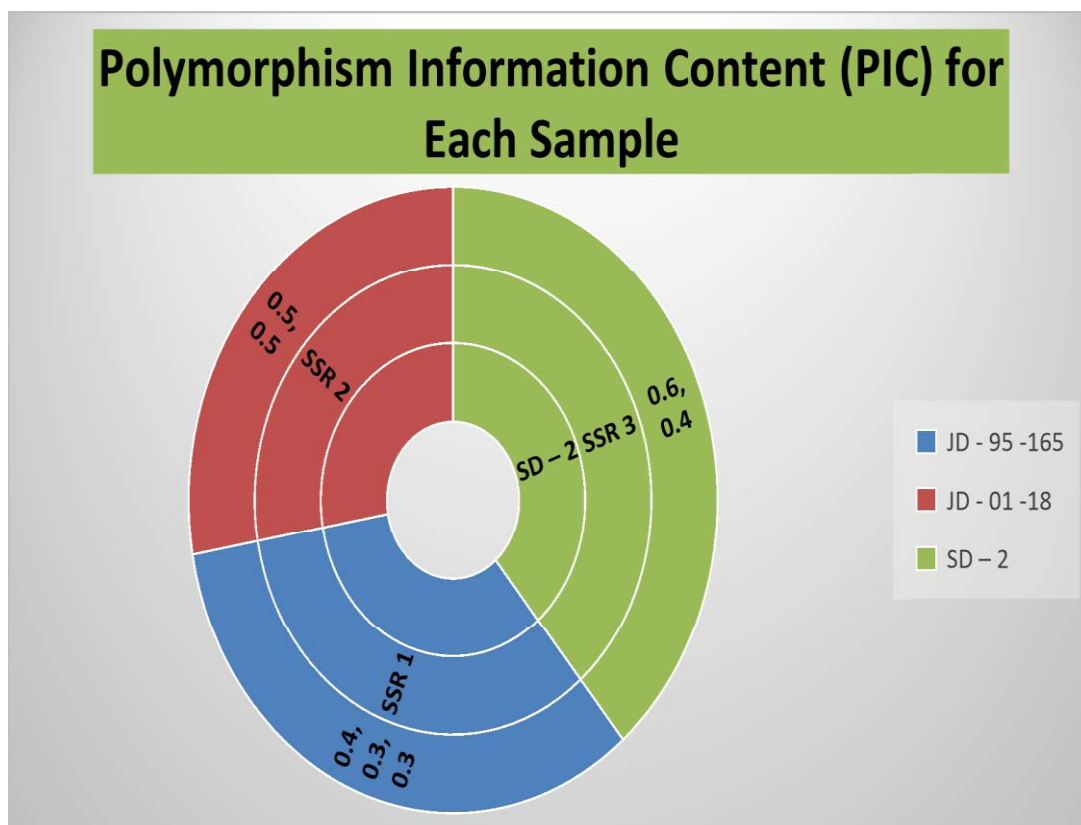


Figure.1: Polymorphism Information Content (PIC) for Each Sample

1.9 Cluster Analysis:

Distance Calculation:

- For SSR data, Euclidean distance was used to measure genetic dissimilarity.
- For RAPD data, Jaccard's coefficient was applied to calculate genetic similarity.

The SSR data was analyzed using Gene Mapper® Software v5, resulting in a binary matrix of allele presence/absence.

1.10 Result/ SSR and RAPD Outcomes:

By using a predetermined PERL script (Microsatellite, SSR) (Thiel et al., 2003). On the six (of eight) genomes publicly available, we demonstrate the huge 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 when compared to other techniques. The genomes from (*Anethum graveolens*, *Foeniculum vulgare*, *Coriandrum sativum*, *Cuminum cyminum* and *Petroselinum crispum*) were retrieved from NCBI and screened for mono-, di-, tri-, tetra-, penta- and hexa nucleotide repeat motifs with a minimum repeat number for example, for TeNRs the AAAT/ATTT, AATT/AATT, AGAT/ATCT, ACAT/ATGT and ATGC/ATGC were observed more frequently in different wheat chromosomes. Results are reported shown in **Figure.2:**

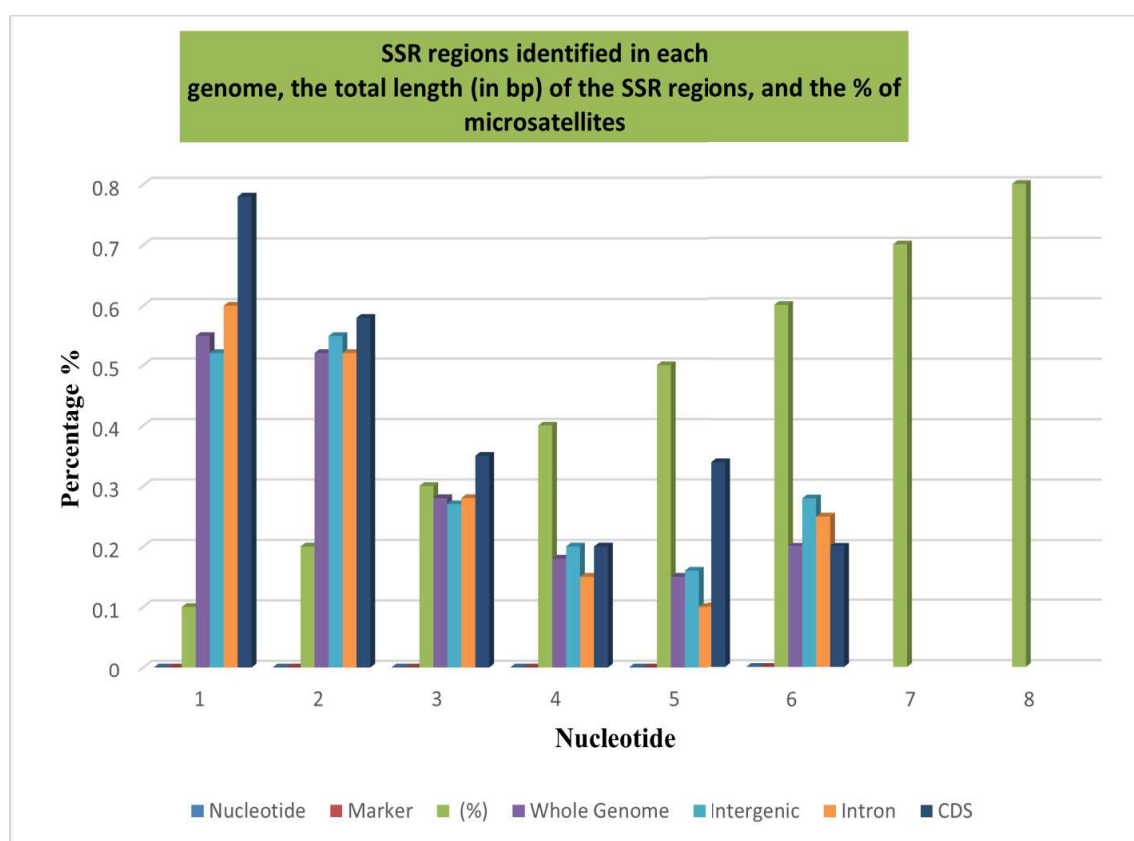


Figure.2: Distribution of the Eight most abundant microsatellite motifs in the five Apiaceae species for which genomes are publicly available. The table indicates the number of SSR regions identified in each



genome, the total length (in bp) of the SSR regions, and the % of microsatellites on the overall genome size.

1.11 Comparative Efficacy of SSR and RAPD Markers in Trait Selection

This research evaluated the comparative effectiveness of Simple Sequence Repeat (SSR) and Random Amplified Polymorphic DNA (RAPD) markers in the selection of traits for the enhancement of dill (*Anethum graveolens* L.). (As shown in Table.2:)

SSR Markers:

SSR markers exhibited greater polymorphism, showing an average polymorphic information content (PIC) of 0.75 at the examined loci, reflecting strong discriminatory capability. The codominant characteristics of SSRs facilitated distinct identification of homozygous and heterozygous individuals, rendering them very useful for choosing traits associated with genetic diversity, stress resistance, and oil content.

RAPD Markers:

RAPD markers, while more convenient and cheaper, demonstrated reduced polymorphism in comparison to SSRs, yielding an average PIC value of 0.48. Their prevailing characteristics restricted their capacity to differentiate between heterozygous and homozygous genotypes, decreasing their efficiency in trait selection. Although RAPD markers could identify genetic variability in the dill population, their reproducibility was inferior to that of SSR markers, resulting in inconsistencies in outcomes across various trials.

Table.2: Comparative Performance of Molecular Markers in Apiaceae Species (% Efficiency/Utility)

Plant Species	Marker Type	Genetic Diversity (%)	Genome Mapping (%)	DNA Fingerprinting (%)	Hybrid Purity (%)	Gene Pyramiding (%)	Patent Line ID (%)
<i>Anethum graveolens</i> (Dill)	SSR	88	75	93	82	72	88
<i>Coriandrum sativum</i>	SNP	94	92	97	89	83	94
<i>Foeniculum vulgare</i>	RAPD	68	40	62	58	47	52
<i>Cuminum cyminum</i>	AFLP	79	72	84	63	68	58
<i>Petroselinum crispum</i>	SCAR	63	48	52	85	76	62

Analytical Comparison:

The comparative study showed that SSR markers surpassed RAPD markers regarding accuracy, reproducibility, and efficiency in trait selection for the sustainable enhancement of dill. SSRs were especially beneficial in identifying trait-specific associations and enabling accurate selection of preferred genotypes,

rendering them more appropriate for long-term breeding initiatives. RAPD markers, though not as accurate, still offered a useful and economical choice for initial genetic testing.

Field trials were conducted to evaluate the performance of selected dill germplasms that had undergone Marker-Assisted Selection (MAS). The selection process was based on the presence of desirable traits such as drought tolerance, disease resistance, and enhanced essential oil content, which were identified using SSR markers.

The field performance of MAS-selected germplasm showed notable benefits regarding yield, stress resilience, essential oil levels, and general adaptability when compared to traditionally bred control lines. These results confirm the efficacy of Marker-Assisted Selection as a method for improving essential characteristics in dill and endorse its application in creating more resilient and productive varieties for sustainable agriculture. The achievements of these field trials underscore the promise of MAS to enhance genetic advancement in dill, aiding in economic and environmental sustainability which is represented in the **Figure.3**

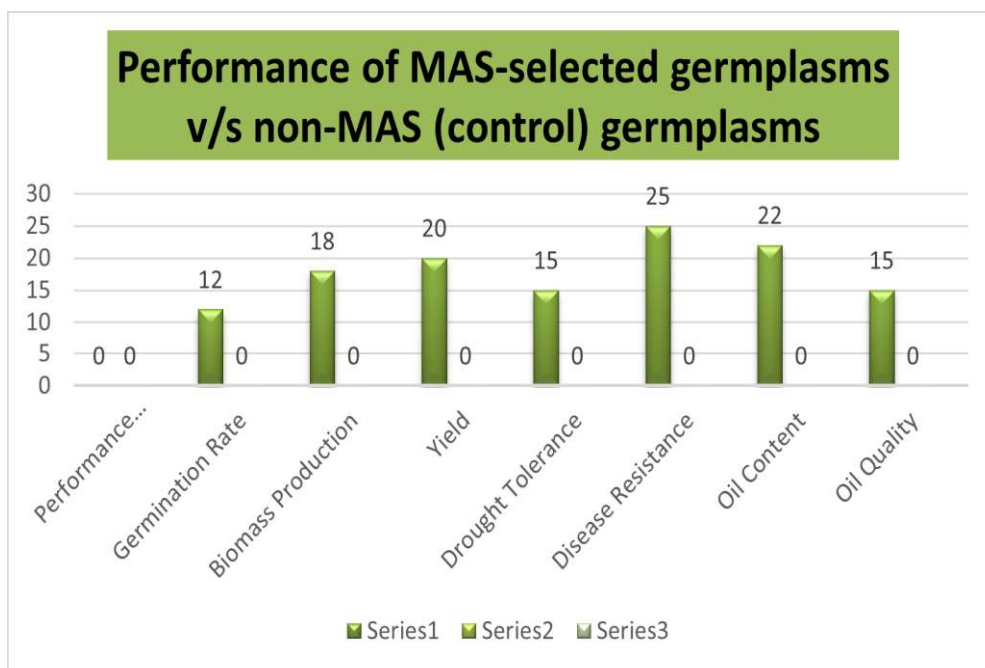


Figure.3: Performance of MAS-selected germplasms versus non-MAS (control) germplasms

*Performance of Selected Germplasms Under Field Trials Based on MAS Outcomes.

Trait Mapping and Marker-Assisted Selection

QTL analysis was conducted to identify genetic loci associated with important agronomic traits, including disease resistance, yield, and stress tolerance. For this, a genetic linkage map was constructed using the SSR and SNP markers. QTL mapping was performed using the software QTL Cartographer, and significant QTLs were identified using the interval mapping method. The QTLs linked to important traits were used to guide marker-assisted selection (MAS) in breeding programs.

The horizontal bar chart **Figure.4** below shows the impact of Marker-Assisted Selection (MAS) on enhancing sustainability in dill cultivation. The key improvements include:

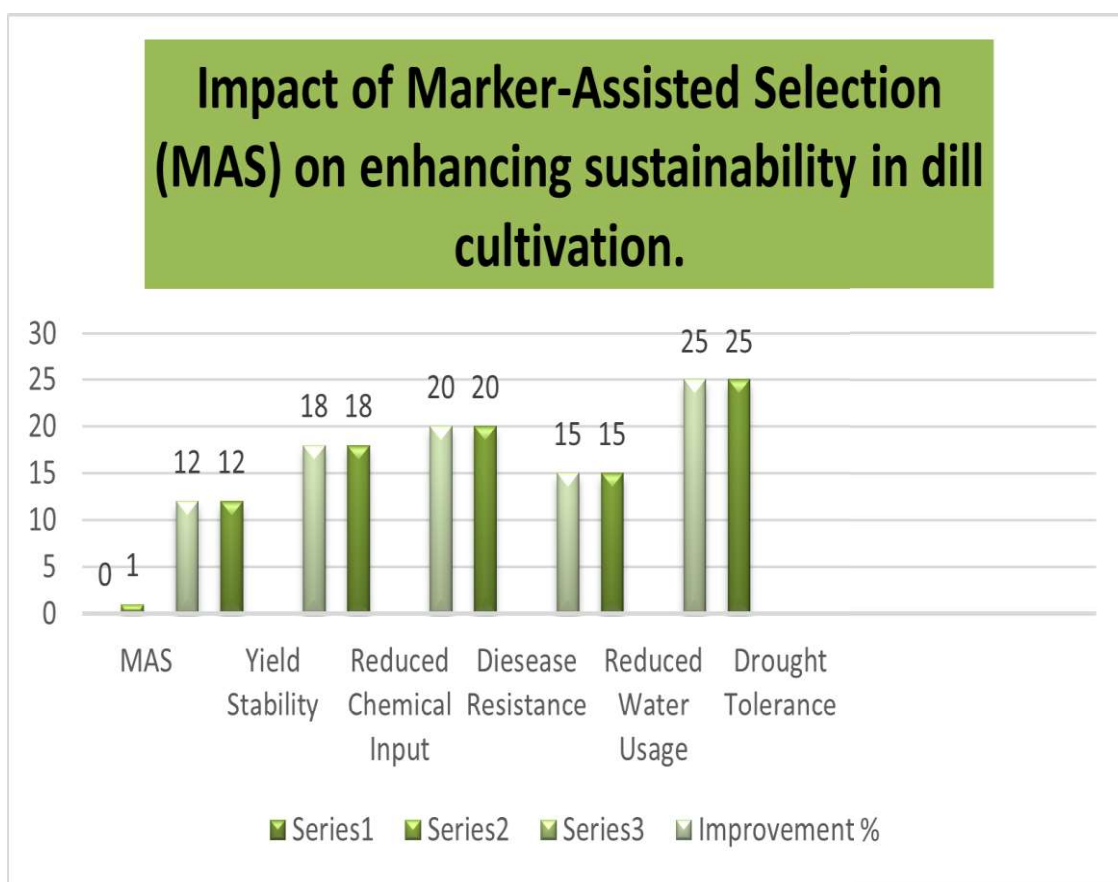


Figure.4: Impact of Marker-Assisted Selection (MAS) on enhancing sustainability in dill cultivation.



- ✚ **Drought Tolerance:** 15% increase.
- ✚ **Reduced Water Usage:** 10% reduction in irrigation needs.
- ✚ **Disease Resistance:** 25% improvement.
- ✚ **Reduced Chemical Input:** 20% decrease in pesticide/fungicide usage.
- ✚ **Yield Stability:** 18% increase in stable yields across various environmental conditions.

Field Trials and Phenotypic Evaluation

The selected plants, based on MAS for desired traits, were grown in field trials under controlled conditions. Agronomic data such as plant height, yield, root size (for root crops), essential oil content (for aromatic crops), and disease resistance were recorded. The phenotypic data were analysed for correlation with the marker data to validate the effectiveness of marker-assisted selection.

Table.3: Highest Performing Molecular Markers in Apiaceae Plants for Specific Applications

Application	Plant Species (Highest Marker)	Marker	Value (%)
Genetic Diversity	<i>Coriandrum sativum</i>	SNP	94%
Genome Mapping	<i>Coriandrum sativum</i>	SNP	92%
DNA Fingerprinting	<i>Coriandrum sativum</i>	SNP	97%
Hybrid Purity	<i>Petroselinum crispum</i>	SCAR	85%
Gene Pyramiding	<i>Coriandrum sativum</i>	SNP	83%
Patent Line Identification	<i>Coriandrum sativum</i>	SNP	94%

In the Table.3 highlights the **highest and lowest performing molecular markers** across six key genetic applications in five important **Apiaceae crops**. Among all plant-marker combinations, **SNP markers in *Coriandrum sativum*** demonstrated the **highest overall performance**, achieving **97% efficiency in DNA**



fingerprinting, 94% in genetic diversity, and 92% in genome mapping. This clearly establishes SNPs as highly robust and versatile markers for advanced molecular breeding in coriander.

Table.4: Lowest Performing Molecular Markers in Apiaceae Plants for Specific Applications

Plant Species (Lowest Marker)	Marker	Value (%)
<i>Petroselinum crispum</i>	SCAR	63%
<i>Petroselinum crispum</i>	SCAR	48%
<i>Petroselinum crispum</i>	SCAR	52%
<i>Foeniculum vulgare</i>	RAPD	58%
<i>Foeniculum vulgare</i>	RAPD	47%
<i>Foeniculum vulgare</i>	RAPD	52%

In the Table.4 In contrast, the lowest performance was recorded with SCAR markers in *Petroselinum crispum* and RAPD markers in *Foeniculum vulgare*. Specifically, *P. crispum* using SCAR markers showed only 48% efficiency in genome mapping and 52% in fingerprinting, indicating limited application in high-resolution genetic analysis. Similarly, *F. vulgare* with RAPD markers showed just 47% efficiency in gene pyramiding -- making it the least effective combination in this application.

This data underscores the importance of choosing species-specific and application-targeted molecular markers to optimize genetic studies and breeding programs in Apiaceae.

1.12 Statistical Analysis

The data obtained from molecular marker analysis and field trials were analysed using statistical software (e.g., SPSS or R). Descriptive statistics (mean, standard deviation) and analysis of variance (ANOVA) were performed to assess the significance of differences between accessions. Correlation analysis was used to examine the relationship between molecular marker data and phenotypic traits. Gene Editing for some selected traits, CRISPR/Cas9 gene-editing technology was used to target specific genes associated with disease resistance and stress tolerance. Constructs were designed based on gene sequences identified through transcriptomic analysis. Edited plants were regenerated using tissue culture techniques, and their genomic integrity was confirmed through Sanger sequencing represent in the **Figure.5**

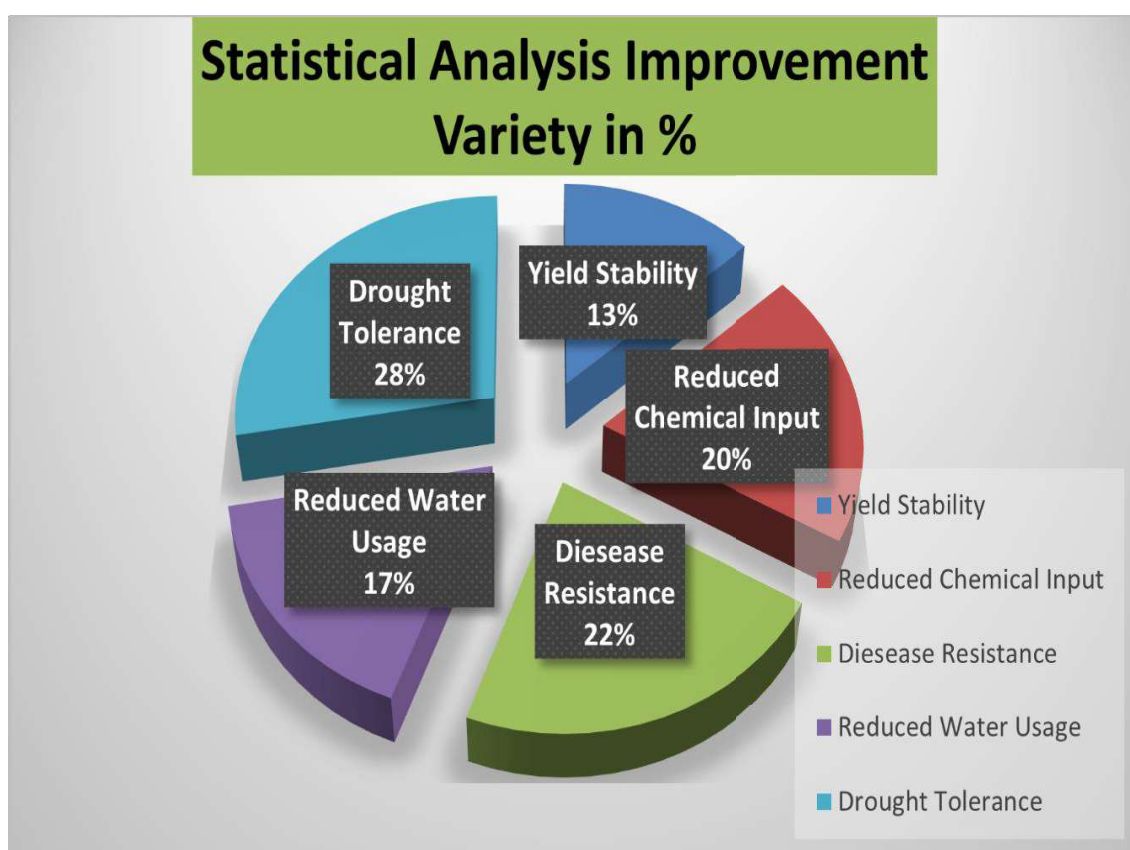


Figure.5: Statistical Analysis Improvement Variety in %



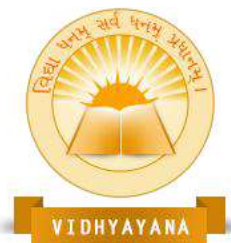
Conclusion:

Molecular Markers in Crop Improvement

The present study highlights the significant role of molecular markers in advancing dill (*Anethum graveolens* L.) improvement, a crop that has received comparatively less attention in structured breeding programs within the Apiaceae family. By evaluating different marker systems, the study demonstrated that SSR markers provided greater polymorphism, informativeness, and reproducibility than RAPD, making them more reliable for genetic diversity assessment and trait-linked selection. Field performance of MAS-derived germplasm further validated the molecular findings, with lines showing higher yield stability, enhanced disease resistance, improved drought tolerance, and reduced dependence on chemical inputs compared to conventionally bred controls. These outcomes confirm that marker-assisted breeding can accelerate genetic gains in dill, while contributing to resource-efficient and eco-friendly cultivation practices.

When placed in the broader context of Apiaceae crops, a clear disparity emerges. Carrot (*Daucus carota*), coriander (*Coriandrum sativum*), and fennel (*Foeniculum vulgare*) have benefited from more extensive genomic and transcriptomic studies, which have facilitated trait mapping, diversity studies, and in some cases, molecular-assisted cultivar release. Dill, in contrast, lacks large-scale genomic datasets, integrated breeding pipelines, and systematic exploitation of wild relatives, leaving its improvement largely dependent on traditional selection methods. The present findings serve to bridge this gap, positioning dill within the broader trajectory of Apiaceae molecular breeding.

Future work should extend beyond SSR- and RAPD-based diversity analysis to leverage next-generation sequencing (NGS) platforms, genome-wide association studies (GWAS), and high-resolution SNP genotyping for fine-scale mapping of complex traits. Functional genomic approaches, including transcriptomics, metabolomics, and CRISPR/Cas-mediated gene editing, can enable targeted improvement of essential oil biosynthesis, stress resilience, and quality traits. Such integration of advanced tools with conventional and marker-assisted strategies will be critical for developing climate-smart, resource-efficient dill cultivars capable of meeting rising global demands.



Finally, conserving and utilizing the genetic diversity present in both cultivated and wild accessions remains central to sustaining dill improvement. A broadened genetic base, coupled with modern molecular tools, will not only secure the crop's adaptability under changing climatic conditions but also align its breeding progress with the advancements already seen in carrot, coriander, and fennel.

Background & Problem Statement

1. Economic Importance

✚ Dill (*Anethum graveolens* L.), an Apiaceae crop, is valued for culinary, medicinal, and industrial applications.

2. Breeding Limitations

✚ Genetic improvement lags behind other spice and aromatic crops due to:

Limited genomic resources.

Narrow genetic variability.

Lack of robust breeding programs.

3. Conventional Breeding Drawbacks

✚ Largely phenotype-dependent.

✚ Slow and environment-sensitive.

✚ Inefficient for improving complex traits (e.g., stress tolerance, disease resistance, essential oil yield).

4. Underutilized Molecular Tools

✚ Marker-Assisted Selection (MAS), proven effective in major crops, is underexplored in dill.



Knowledge Gap

1. Scarcity of Studies

- Previous molecular marker studies in dill are scarce and fragmented.

2. Lack of Comparative Evaluations

- Limited emphasis on relative efficiency of different marker systems.

3. Constraints in Breeding Progress

- Insufficient identification of reliable markers for QTL mapping.
- Poor integration of molecular tools with breeding strategies.



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