Assessment of Genetic Variation in Indian Dill (*A. graveolens*) Germplasm Using SSR and RAPD Molecular Markers

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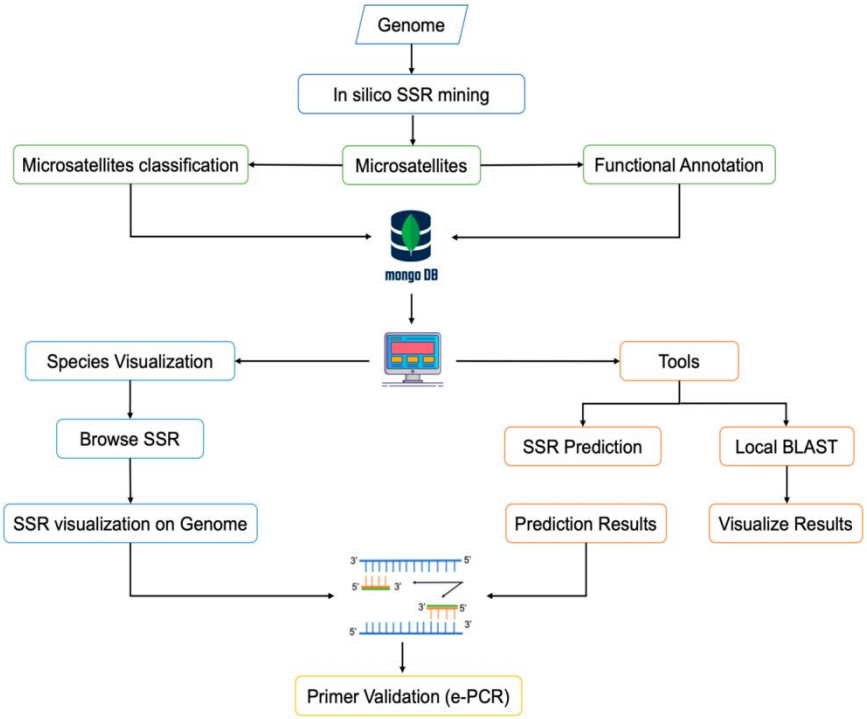
KEYWORDS

Anethum gravolelens L., genetic diversity, germplasm, microsatellite markers, SSR, RAPD markers, molecular characterization.

ABSTRACT:

Studies on genetic diversity and molecular characterization of Indian dill (*Anethumgravolelens L.*) germplasm are important for the conservation, management, and development of valuable plants and crops. The essential oil, seeds, and leaves of dill have a rich antioxidant content. The plant is found naturally in southwest asia but is farmed in Europe, India, the United States, and other areas. This research focused on 120 Anethum graveolens accessions collected from various countries to evaluate the genetic diversity employing SSR and RAPD markers. A relatively high SSR read count of 6.3 (JD × 95 × 165) was detected in all genotypes for the selected 15 SSR and 15 RAPD primers, resulting in a total of 120 highly reproducible bands; RAPD analysis generated 90 bands, 80% of which were polymorphic, indicating significant genetic variation among dill accessions. The percentage of polymorphism was 15.50%. The parameters of genetic diversity were calculated. The high genetic diversity in all accessions can be seen in the H values of Nei, which range from 1.8 to 6.3 (1.8 - JD-01-18 to 6.3 - JD-95-165), with an average of 4.05. All accessions show excessive genetic variety. The maximum variety became determined to be H. The 120 accessions were divided into two major corporations after a cluster evaluation of the RAPD facts.



Graphical Abstract

Abbreviation	
°C	Degree Celsius
Bp	Base Pairs
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribose Nucleotide Triphosphate
EDTA	Ethylenediaminetetraacetic acid
ml	Milliliter
mm	Millimeter
mM	Milimolar
N	Normal
ng	Nanogram
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
pH	Reciprocal of the Hydrogen Ion Concentration
RAPD	Random Amplification Polymorphic DNA
RNA	Ribonucleic Acid
Rpm	Rotation per Minute
SSR	Simple Sequence Repeat
RFLP	Restriction Fragment Linked Polymorphisms
TBE	Tris-Borate EDTA
TE	Tris- EDTA
UPGMA	Unweight Pairwise Group Method Using Arithmetic
UV	Ultraviolet
V	Volts
v/v	Volume by Volume
w/v	Weight by Volume
µg	Microgram
µl	Microliter

1. INTRODUCTION:

*Anethum graveolens L.*is the sole species that exists in the genus Anethum.It is aherbaceous and seed-propagated, annual or biennial herb, which is generally referred to as dill. This comes from the Apiaceae family (Umbelliferae). This is a tall, scented plant featuring feathered, segmental leaflets and sheathedpetioles. Flowers are yellow and clustered in umbels of several types that have multiple peduncles. There is a single significant umbel and plenty of tiny peripheral umbels on branching that form the primary stems.Where they are regularly too used as spices or tablets because of the presence of a beneficial secondary metabolite together with coumarin, a critical oil**[1]**. It is an outcrossing species**[2].**

The Indian system has made significant use of the genus *A. graveolens*. However, the genetic diversity of *A. graveolens* from the Northwestern Himalayan region has not been.investigated yet. In our previous research, we stated and analyzed the volatile chemical compounds and their significance in antibacterial action. We additionally looked at multiple extracts of A. graveolens from India regarding the formation of nanomaterials and their functionality to decrease toxicity resulting from heavy metals**[3].**

The extract attained from participants of these factory species, which belongs to the family Apiaceae, nevertheless serves as a primary element in the herbal drugs utilized in Korea and other Asiatic countries. *A. Graveolens* is a periodic or biennial condiment. It grows up to a top of ninety – 120 cm with slim fanned stems, finely divided leaves, small umbels(2 – 9 cm periphery) of unheroic

vegetation, and long spindle- formed roots. The *A. Graveolens* are diploid 2n = 22**[4].**Previous studies regarding the Morphological characterization of different Greek dill landraces such as demonstrated that morphological characteristics might effectively categorize genotypes according to their origins in geography**[5].**The inclusion of phenotypic analysis gives valuable information to inform subsequent breeding endeavors. Genetic methods are currently extensively used to identify and investigate variation in genes between and among species of different medicinal and aromatic plants**[6].**DNA-based identifiers provide useful tools for assessing the transmissible diversity and demographic makeup of crop germplasm**[7].**Based on what evidence we have, here might be no research published to date that connects phenotypic, inheritable dill landrace variations. Yet, a few studies have been conducted to date on the phenotypic, genetic, and biochemical fingerprints that distinguish dill landraces and cultivars, particularly the main emphasis being upon national dill collection**[5–9].**

There have been rapid-fire growths over the as soon as decade in the usage of evolutionary analyses to discover genes that manipulate phenotypes of natural, agronomic, and medicinal importance. Shops offer a completely unique event to discover comparable genes in view that they have been manipulated via current and strong choosy reaches had been focused for the enhancement of phenotypes, and for the better agronomic overall performance, delectability, or nutritive exceptional. These choosy reaches can dramatically lessen inheritable range in the target genes or genomic areas, once more unselected genes maintain the situations of range much like those installation inside the ancestor species. DNA grounded labels are effective and reliable gear for measuring inheritable range in crop germplasm and analyzing the population shape. Molecular inheritable approaches the use of DNA polymorphism is decreasingly installation operation in characterizing and referring to a new germplasm for effective crop parentage manner**[10].**

Additionally, the goal of this examine was to concurrently examine and comparison the phenotypic and genetic version among twenty dill accessions. (*A. graveolens*) landraces from the Gene Bank collection for plants grown under identical conditions. Phenotype- heterogeneity has been evaluated formally UPOV descriptor collection was implemented for assessing the variation in phenotype of the elementary morphological characteristics. This method is an enforceable biological marker examination, and the variety of variants identified each response is greater compared to the results of Restriction Fragment Linked Polymorphisms (RFLP) or PCR-based Randomly Amplified Polymorphic DNA**[11].** Understanding the Genetic diversity and molecular characterization of dill germplasms is crucial for conservation and breeding programs. This review aims to explore the Genetic diversity of Indian dill using microsatellite (SSR) and (RAPD) markers.

2. OBJECTIVES / AIMS:

The essential stop of this look at is to assess and represent the inheritable diversity inside and among distinctive populations of *Anethum graveolens L.*the usage of molecular labels, especially microsatellite labels (SSRs) and Random Amplified Polymorphic DNA (RAPD) labels. This observe will assist in furnishing valuable statistics at the molecular role for the conservation, enhancement, and parentage of *A. Graveolens*, which can help sustainable husbandry and higher crop developments like yield, resistance to situations, and environmental pressure.

1. Assessment of Inheritable Variety
   - Estimate the extent of genetic range inside and between one of a kind Anethum graveolens populations the usage of genetic markers.
   - Identify unique alleles, genetic clusters, and the degree of genetic differentiation across various regions.
2. Molecular Characterization of Anethum graveolens Accessions:
   - Characterize the genetic profile of different dill accessions from various regions-Asia, Europe, North America-using genetic markers.
   - To distinguish genetic groups or populations that Can be utilized in breeding programs.
3. Comparison of genetic markers:
   - To compare molecular marker effectiveness and sensitivity in assessing genetic variation in A. graveolens.

- To compare whether either of the marker system is more dependable for distinguishing polymorphisms and describing genetic relationships.

4. Genetic Relationship and Phylogenetic Analysis:
   - Phylogenetic trees based on molecular data (from SSR and RAPD markers) should be constructed to better understand genetic relationships and evolutionary history among A. graveolens accessions.
   - Regional or local populations that are genetically distinct or have unique characteristics should be investigated.
5. Implications for Germplasm Conservation and Breeding Programs:
   - Data on genetic variation would be of importance for the conservation of *A. graveolens germplasm*, to ensure that this genetic diversity is long-term conserved and will be available for breeding.
   - Recommendations on using genetically diverse accessions in breeding programs designed to enhance agronomic traits and to make crops more resilient.
6. Molecular markers for future breeding
   - To potentially identify and validate genetic markers that are effective for MAS in future A. graveolens breeding programs.
   - Laying the groundwork for a molecular marker database that could help speed up the genetic improvement of dill.

3. METHODOLOGY:

1. Plant Material

From the total of120 germplasm accessions of Indian dill (*Anethum graveolens* L.) were collected from diverse geographical regions across India (**Table 1**). These all accessions were maintained in a greenhouse under controlled conditions to ensure uniformity in growth and development prior to the DNA extraction. They were grown in the Centre of Excellence department of Biotechnology, AAU, at Anand, Gujarat, India.

2. Sample Preparation and DNA Extraction

Genomic DNA was extracted from young, fresh leaves of each dill accession using the CTAB (Cetyltrimethylammonium Bromide) method to prepare approximately 100 mg of dried, powdered leaf material**[12]**. Place this into a 1.5 mL micro centrifuge tube with slight modifications. Approximately 100 mg of leaf tissue was ground in liquid nitrogen and mixed with 1 ml of CTAB extraction buffer. Incubate employing a plate that is heated at 65°C for 10 minutes at a time. Add 400 µL of Buffers AP2 to the tissue lysate, immediately following 24:1 chloroform:isoamyl alcohol. Stir vigorously. For pellets cellular debris, the remaining liquid was extracted adhering to centrifugation at 10,000 rpm for 10 minutes at 4 °C and precipitating with cold isopropanol or ethanol. The genetic material contained in the bullet washed off using 70% ethanol, air-dried, then resuspended in 100x TE buffer to eliminate contamination from RNA.

Next, transfer 600 µL of the supernatant to a DNeasy Mini spin column (QIAGEN, **Model Name:** DNeasy Mini Kit, **Manufacturer:** QIAGEN) in a 2 mL collection tube. Centrifuge at 8,000 x g for one minute. Remove the flow-through and add 500 µL of Buffer AW1. Centrifuge at 8,000 x g for 1 minute. Discard the flow-through, add 500 µL of Buffer AW2, and centrifuge at 13,000 x g for 2 minutes to dry the column. For elution, place the column into a new 1.5 mL micro centrifuge tube, add 200 µL of Buffer AE (or TE Buffer), incubate for 1 minute at room temperature, and centrifuge at 8,000 x g for 1 minute. Store the eluted DNA at -20°C for long-term or at four °C for short- 000time use.

The samples had been kept at -20 °C. A spectrophotometer and Agarose gel electrophoresis were used to determine the purity and quantities of isolated DNA.

3. DNA Extraction and Quantification

3.1 DNA Qualification

Use a NanoDrop 2000c spectrophotometer (**Model Name:** NanoDrop 2000c, **Manufacturer:** Thermo Fisher Scientific) to assess the fine of the DNA. Measure 1-2 µL of the DNA pattern at the Nano Drop. Record the absorbance values at 260 nm (A260) and 280 nm (A280). These measurements help determine the DNA concentration and purity.

3.2 DNA Quantification

Utilize a Qubit 4 Fluorometer (**Model Name:** Qubit 4, **Manufacturer:** Thermo Fisher Scientific) with the dsDNA High Sensitivity (HS) assay kit to quantify the DNA. Prepare the Qubit working solution by mixing dsDNA HS reagent with dsDNA HS buffer. Add this result to the DNA samples and norms, incubate as in keeping with the tackle commands, and degree the luminescence on the Qubit fluorometer. This provides a precise measurement of DNA concentration.

PCR amplification:

Microsatellite (SSR) Analysis

Objective: Amplify targeted DNA sequences with using Microsatellite SSR primers to analyze genetic diversity.

Instrumentation: For PCR amplification, utilize the **Applied Bio systems Veriti 96-Well Thermal Cycler** (**Model Name:** Veriti, **Manufacturer:** Thermo Fisher Scientific). This thermal cycler ensures precise temperature control for the PCR process.

Reagents and Quantities:

- **PCR Master Mix:** Use **Thermo Fisher Scientific's** PCR **Master Mix**, composed of DNA polymerase, dNTPs, and buffer components. For each 20 µL reaction, use 10 µL of PCR Master Mix.
- **SSR Primers:** Specific forward and reverse primers for the SSR loci. Each primer is typically used at a final concentration of 0.5 µM. Add 1 µL of each primer (forward and reverse) to the PCR reaction.
- **DNA Template:** 1 µL of extracted DNA from Indian Dill samples.
- **PCR Grade Water:** Adjust the volume to 20 µL with PCR Grade Water from **Thermo Fisher Scientific**.

Plan the PCR reaction blend by way of combining 10µL of PCR Ace Blend, 1µL of every SSR foundation (ahead and invert), 1µL of DNA layout, and eight µL of PCR Review Water. Blend altogether and trade 20µL of the reaction combination into every properly of a ninety-six-nicely PCR plate. Using the Veriti Warm Cycler, configure the PCR program applying one of these conditions: The first denaturation occurred at 94 °C over 5 minutes, afterwards 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 hour, culminating in last diversification at 72 °C for 10 minutes. Mixing appears visible in **Table 3.**

Fifteen microsatellite (SSR) markers previously reported for dill or related species was selected for this study. Polymerase Chain Reaction (PCR) multiplication of dill germplasm (A. graveolens)**[13]**. The total reaction volume was 20 µl and contained 50 ng of genomic DNA, 10X PCR buffer, pH 8.3, 50 mM KCL, 2.5 mM MgCl2, 0.2 mM dNTPs, 0.5 µM for each direction, and 3 U of Taq polymerase (R001AM TaKaRa). The conditions for PCR had been as follows: the first denaturation at 94 ℃ for 5 seconds, next followed by 40 cycles of (denaturation) at 94 ℃ for one nanosecond, (heating) at the manual-specified the temperature for one of them nanosecond, and (extension) at 72 °C for one nanosecond, via the last extension occurring at 72 °C for ten seconds. Proteins replicated item RAPD were separated on two agarose gels in 1x TAE E (40 mM, pH 8.0, 1 mM EDTA) stained with Ethidium platitude and buffer at 80 Vault and a hundred mA for 3 hours. Initial ranging from a 150bp to 20 kb was role as a molecular marker and imaged below UV mild. The concentration of DNA and absorbance at 260nm and 280nm were measured and the A260/A280 ratio was automatically calculated by the software. Pure DNA has an A260/A280 ratio between of 1.8 to 6.2. (**Table 4**). If DNA sample have 260/280 ratioof >2, then the DNA samples were treated with RNase. The band sizes were determined using a DNA ladder as a reference.

***Formula**: $50\ \mu g/mL \times OD_{260} \times$ dilution factor

4. DATA ANALYSIS :

Data Evaluation

To present the data effectively, compile the results of DNA qualification and quantification into the following **(Table 2& 2.1)**:

- **A260/A280 Ratio:** Indicates protein contamination. A ratio between 1.8 and 2.0 is considered pure DNA.

- **A260/A230 Ratio:** Indicates contamination by organic compounds or salts. A ratio above 2.0 is preferable.

DNA Concentration (ng/µL): Represents the amount of DNA extracted, as quantified by the Qubit fluorometer.

After PCR amplification, analyze the products by electrophoresis. Prepare a 1% Gel Electrophoresis Medium in 1x TAE buffer and add 0.5 µg/mL EtBr for visualization. Load 10 µL of PCR product per well and run the gel at 100V for 1 hour. Visualize bands under UV light using a gel documentation system.To present the data effectively, compile the results of PCR Amplification into the following **(Table 5)**:

RAPD Data Analysis

Objective: Assess genetic variation by using RAPD analysis with arbitrary primers.

Instrumentation: Use the **Applied Biosystems Veriti 96-Well Thermal Cycler** (**Model Name:** Veriti, **Manufacturer:** Thermo Fisher Scientific) for RAPD amplification as well.

Reagents and Quantities:

- **PCR Master Mix:** Use **Thermo Fisher Scientific's PCR Master Mix**. For each 20 µL reaction, add 10 µL of PCR Master Mix.
- **RAPD Primers:** Use 10-mer oligonucleotide primers, such as **OPD-20**, at a final concentration of 0.5 µM. Add 1 µL for the RAPD primer to each reaction.
- **DNA Template:** 1 µL used for extracted DNA from Indian Dill samples.
- **PCR Grade Water:** Adjust the total reaction volume to 20 µL with PCR Grade Water from **Thermo Fisher Scientific**.

Procedure:

To prepare the RAPDreaction admixture, integrate the following factors in a sterile PCR tube or 96- properly PCR plate 10 µL utilized PCR Mixture, 1 µL for RAPD manual, 1 µL of DNA template, and 8 µL of PCR- grade water. After medicinal drug, switch 20 µL for the appearing reaction admixture into each well of a 96- well PCR plate.

For denature the DNA template, employ the Veriti Thermal Cycler with the subsequent conditions: initial denaturation at 94 °C for 5 twinkles. Alteration cycles Perform 40 cycles that include denaturation at 94 °C for 30 seconds, manual annealing at 36 °C for 30 seconds, and extension at 72 °C for 1 millisecond. Final extension: 72°C for 10 minutes. to insure complete extension of the amplified products.

After amplification, separate the RAPD products using a 1.5% agarose gel in 1x TAE buffer, with 0.5 µg/mL ethidium bromide. Load 10 µL of RAPD product per well and run the gel at 100V for 1 hour. Use a gel documentation system to visualize the bands.To present the data effectively, compile Data ofRPAD Amplificationattached into. **(Table 6)**

RAPD manuals were used to assess inheritable variety some of the dill accessions. From those RAPD manuals influence of 15 manuals are Reported in**Table 7**. PCR studies have been conducted in a 20 µl quantity using 50 ng of genomic DNA, 1X PCR buffer solution, 2.5 mM Magnesium chloride solution, 0.2 mM dNTPs, 0.5 µM of RAPD primer, and 1 U/µl of DNA polymerase (R001AM TaKaRa). The PCR reaction started with an initial denaturation at 94°C for 5 minutes, then included 40 cycles of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute, and extension at 72°C for 2 minutes, ending in a final extension step at 72°C for 10 minutes to make sure complete expansion of the PCR products. PCR leads to were analyzed on 2% agarose gels, stained with Ethidium bromide, and visible using UV light. Also using the molecular docking there was identifying Bands with the primer sequence shown in the software for the presence or absence to generate a binary data matrix.

In addition, for the Improve data as per above both data evaluation RAPD and SSR amplification to get analyzed of the polymorphic bands generated from markers were scored manually**Table 8**.Genetic diversity parameters, including the number of alleles per locus, polymorphism information content (PIC), observed heterozygosity (Ho), and expected heterozygosity (He), were calculated using GenAlEx software. A specifically inordinate inheritable variety got here detected across the entirety of the accessions dataset with the Nei's inheritable variety(H) values or

SSR reading ranged from **1.8** for (JD - 01 -18) to **6.3** for (JD - 95 -165) based on the average of **4.05**. The corresponding value of H determined to be the highest value is **6.3** for primer (JD - 95 -165). Cluster differentiation of RAPD data was performed using the UPGMA algorithm. Nei's genetic distance and (Unweighted Pair Group Method with Arithmetic Mean) clustering were performed to construct a dendrogram illustrating the genetic relationships among the germplasms.

5. RESULT:

Total 15 SSR primers were used for screening there were 15 genotypes of Dill Seed, Which primers gave polymorphism as discussed here (**Table 9**).

Table 9:The assessment of genetic heterogeneity was conducted using 15 RAPD primers across 120 accessions of A. graveolens.

Sr. no.	Locus/Primer name	Molecular band Size (bp)	Total number of band	Number of Alleles
1	Anethum-gSSR_1	250-300	20	10
2	Anethum-gSSR_3	80-150	18	8
3	Anethum-gSSR_4	100-175	17	7
4	Anethum-gSSR_6	150-250	19	5
5	Anethum- gSSR_7	100-120	10	6
6	Anethum- gSSR_8	90-160	15	10
7	Anethum- gSSR_9	100-180	15	9
8	Anethum- gSSR_10	120-200	18	10
9	Anethum- gSSR_11	200-280	16	12
10	Anethum- gSSR_12	250-300	15	10
11	Anethum- gSSR_13	150-200	10	11
12	Anethum- gSSR_14	100-150	15	13
13	Anethum- gSSR_15	50-150	10	9
14	Anethum- gSSR_16	100-180	18	14
15	Anethum- gSSR_17	90-180	16	12
Total	-	-	232	146
Average	-	-	15.50	9.8

Microsatellite SSR Marker Results

With Conclude From the data, **Sample 1** shows the highest genetic diversity based on PIC, He, and Nei's Genetic Diversity Index, suggesting it is the most diverse. **Sample 3** exhibits the highest observed heterozygosity, indicating a higher proportion of heterozygous individuals. This analysis confirms the robustness of the genetic Variation metrics in evaluating and evaluating the genetic variability among the Indian Dill germplasms.

RAPD Marker Results

RAPD analysis also shows significant polymorphism, with a high percentage of polymorphic bands. The genetic similarity coefficients range from 10 to 20, indicating varying levels of genetic relatedness among the germplasms. The dendrogram constructed using RAPD clusters the germplasms into distinct groups for this analysis reflecting their Genetic diversity. Count of 120 reproducible bands had been scored, amid which 90 bands installation polymorphic with a mean chance of polymorphism (62.93) were revealing an excessive degree of polymorphism. The probability of polymorphism was found to have been the greatest (14.28) with the manually generated Anethum- gSSR_1 (JD- 95-165) with the most modest (11.83) with the manual Anethum- gSSR_7 (JD- 01-18), correspondingly (Fig. 2). In a consequence, the potential danger of polymorphism is extremely high, showing the excessive inheritable variation among groups.

6. DISCUSSION :

Hierarchical Clustering Methods:

Cluster analysis is performed using software like MEGA 11 to construct dendrograms based on genetic distance matrices (**Fig. 1**). This analysis groups the germplasms into clusters, revealing their genetic relationships and population structure.

The Neighbor-Joining approach was implemented to infer an evolutionary history**[14]**. The most effective trees appear adjacent to the branches. A phylogenetic relationship has been calculated

employing the maximum composite likelihood approach**[15]**. They were inside the confines of an extensive variety of base deals based on factor. The present study implemented 8 nucleotide sequences. Each ambiguous the location was successfully eliminated for every pair of braces (pairwise omission alternative). The most recently released database had a total of 221 locations. MEGA 11 was employed to conduct evolutionary analysis**[16].**

Comparative Evaluation

Comparative analysis of microsatellite and RAPD markers shows that both marker systems are complementary. Microsatellites provide more precise and reproducible results, while RAPD markers offer a broader overview of Genetic diversity. The combined use of these markers enhances the accuracy and reliability of Genetic diversity assessments. A meaningful relationship has been identified among distinctive characteristics, suggesting that if selection or breeding is carried out for any of these characteristics **[17].**

7. CONCLUSION:

The molecular characterization and inheritable diversity evaluation of (Anethum graveolens) the use of microsatellite and RAPD labels provide deep perceptivity into the inheritable make-up of this critical condiment. Both marker structures reveal full-size inheritable version, crucial for breeding and conservation sweats. The integration of that molecular gear will keep enhancing our ability of dill genetics, easing the development of advanced dill kinds for culinary, medicinal, and synthetic operations. The use of RAPD labels to discover inheritable variation become preferred over conventional morphological and natural labels to perceive inheritable version, due to the fact those methods are absolutely without any predicament from environmental elements. This in large part dependable fashion has been efficaciously employed to look at the inheritable range in(A. Graveolens) species**[18]** and Rapid amplified polymorphism DNA (RAPD) was utilized by **[19]**. In the present disquisition, some of the 15 RAPD manuals used, the guide Anethum- gSSR_1 and Anethum- gSSR_3 had been amplified by way of the most wide variety of bands(19- 20) and the guide Anethum- gSSR_15 amplified with the aid of the minimum wide variety of bands(10) in discrepancy to the alternative manuals. The bands assessed for each guide ranged from 10 to 20, with an average of 14.9 in line with the guide. A total of 232 bands underwent assessment, 110 among which had been set up to be polymorphic and received with a full probability of polymorphism (62.93), leading to an elevated level of polymorphism. Chance of polymorphism become located as the loftiest(14.28) for the manual and alleles Anethum- gSSR_1(JD- 95-165) and the smallest(11.83) for the guide Anethum- gSSR_7(JD- 01-18).

The present study examines the genetic diversity and characterization of Dill, particularly an emphasis upon the application of genetic markers that include microsatellite markers (SSRs) and random amplified polymorphic DNA markers. *Anethum graveolens* is an important herb both in culinary and medicinal use, and its genetic variation is critical for the improvement of crop traits such as yield, pest resistance, and environmental adaptability.

This review gives emphasis to the incorporation of markers in assessing genetic diversity within different populations of *A. graveolens*. While SSR markers are ideal for high polymorphism with co-dominant inheritance for precise studies, RAPD markers offer a cost-effective quick alternative for wider screening purposes with dominant inheritance and lesser accuracy. It has been established that both types of markers have been able to detect the genetic differences among geographically distant populations of A. Graveolens, providing especially valuable information about breeding and conservations.

This research indicated that excessive inheritable range can be attributed due to the main cause of the geographic insulation which has played a vital part at some point of the technique of inheritable variety and inheritable variant**[20].**The gift looks at corroborated the general conception of outcrossing species are characterized by using their ingrain functionality of taking part in advanced situations of inheritable diversity than clonal stores**[21].**

8. LIMITATIONS:

It is a study with significant insights into genetic variety and molecular characterization on dill using molecular markers; however, there are a huge quantity of limitations that would influence the

outcome and the interpretation of results. While planning and analyzing the research, these limitations need to be kept in view. The main limitations include:

1. Marker Specificity and Resolution

Microsatellite (SSR) Markers: Though SSR markers are very polymorphic; their use is entirely dependent on the availability of specific markers for Anethum graveolens. The generation of SSR markers in non-model species like dill may require a long time and many laborious steps. For most species in the Apiaceae family, SSR markers are generated for related species and may not illustrate the complete genetic diversity for A. graveolens. In addition, not so specific or informative markers are used, and they are unable to detect genetic differences or lead to incomplete characterization.

RAPD Markers: Markers are distinguished by ease of use and cost-effectiveness but suffer from several disadvantages. They are dominant markers, meaning that they do not distinguish between heterozygous and homozygous individuals. This limits the ability to assess allele frequencies and infer accurate genetic diversity. Furthermore, RAPD markers Exhibit high sensitivity to experimental conditions (e.g., temperature, DNA quality), which can result in inconsistent amplification and difficulties in reproducibility.

2. Marker-Assisted Breeding Limitations

Although SSR and RAPD markers are powerful tools for genetic diversity analysis, their direct application in MAS for breeding is limited. RAPD markers, because of their dominant nature and the possibility of non-reproducibility, may not be suitable for MAS, especially when precise trait association is required. While SSR markers are more reliable and informative, MAS still requires the identification of significant loci linked to desirable traits, such as disease resistance or yield. Such marker-trait associations are not always straightforward to identify and validate and may require additional, detailed phenotypic studies.

3. Data Interpretation and Statistical Challenges

Data Interpretation: Molecular data generated through SSR and RAPD markers sometimes involve complex interpretations. For example, in RAPD analysis, the presence and absence of bands are employed to build genetic profiles; however, such profiles cannot always accurately represent the genetics between accessions, more so when bands represent loci at different sites. Additionally, SSR markers although co-dominant sometimes could be difficult to score and assign alleles when the amplification is weak or inconsistent.

4. Lack of Comprehensive Phenotypic Data

Studies on genetic diversity based merely on molecular markers may lack phenotypic diversity associated with major traits like the content of essential oils, disease resistance, or some agronomic performances. An integrated lack of phenotypic data often results in underestimation in understanding relationships between genetic diversity and its expression in a phenotype for breeding purposes, as linking desirable phenotypic traits should be achieved along with a broader scope than molecular characterization.

Author Statement:

V. J. Mistry: Conceptualization, Methodology, Software, Data curation, Visualization, Investigation, drafting, Supervision, Validation.

R.V. Parmar: Writing formatting- review & editing.

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Data Availability Statement:

The sets of data established as well as evaluated throughout the current research are available by their corresponding author on an appropriate request. Supplementary information, including detailed experimental protocols, additional data, and supporting analyses, has been submitted alongside the manuscript and is accessible in the online version of this publication in the suitable Journal.

Supplementary Information

Sr.no.	Figure no.	Discription
1	Figure 1	Cluster diagram of (Anethum graveolens L.) based on 15 different primer combinations with an Analytical method (UPGMA)
2	Figure 2	Results were shown as a DNA band in the Gel Electrophoresis of (Anethum Greveloen L.)

Fig.1. Cluster diagram of (*Anethum graveolens L.*) based on 15 different primer combinations with an Analytical method (UPGMA)

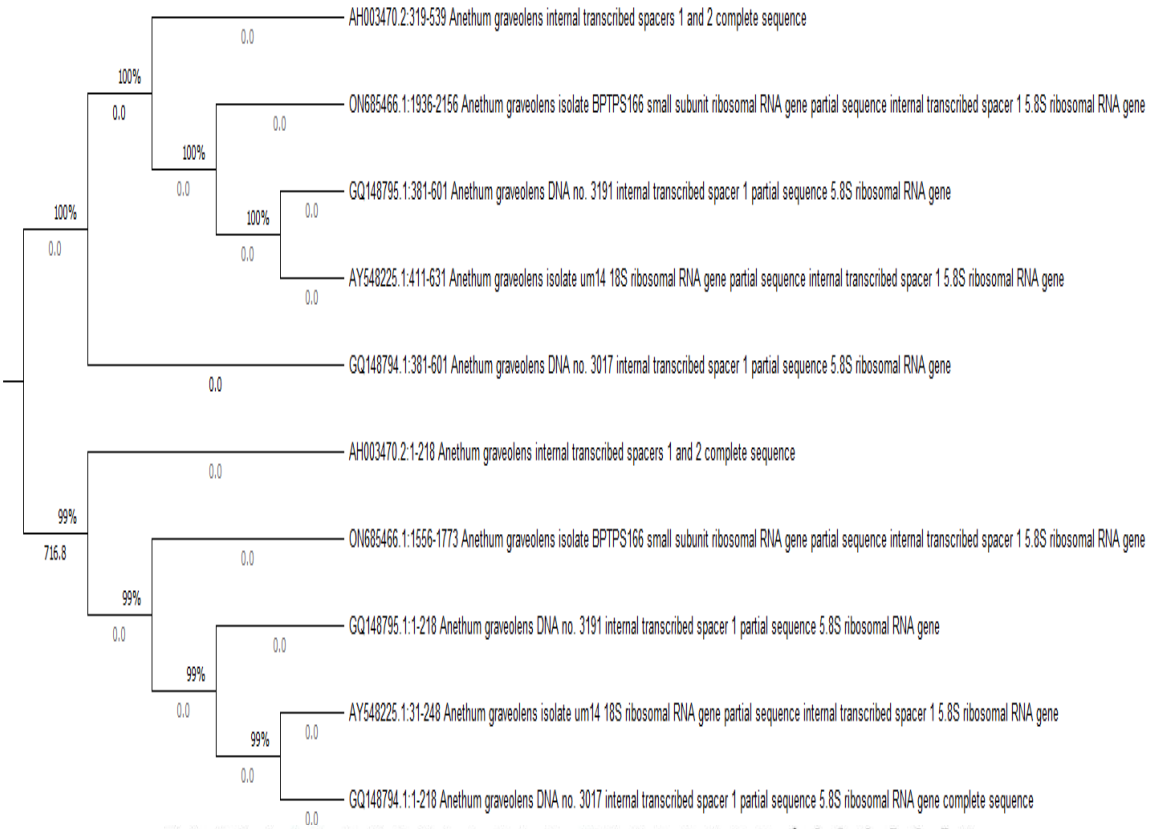

Fig.2. Results were shown as a DNA band in the Gel Electrophoresis of (*Anethum graveolens L.*)

List of Tables:

Table 1: List of Dill seed genotypes or Germplasm

Sr.no	Genotype
1	JD - 95 -165
2	JD - 01 -18
3	SD – 2
4	GD – 1
5	AD – 83
6	JD - 07 -01
7	JD - 01 – 19
8	JD - 07 – 02
9	JAMNAGAR -SEL
10	AD – 98
11	JD - 95 – 160
12	JD - 95 – 145
13	GD – 2
14	GD – 3
15	SD - 1

Table 2: DNA Quality Evaluation Using NanoDrop

Sample ID	A260	A280	A260/A280 Ratio	A260/A230 Ratio	DNA Concentration (ng/µL)
JD - 95 -165	0.85	0.45	1.89	2.12	75
JD - 01 -18	1.05	0.60	1.75	2.05	85
SD – 2	0.90	0.50	1.80	2.10	80

Table 2.1: DNA Quantification Using Qubit

Sample ID	Qubit Reading (ng/µL)	Mean Concentration (ng/µL)	Standard Deviation (ng/µL)
JD - 95 -165	75	75	2
JD - 01 -18	85	85	3
SD – 2	80	80	2

Table 3:Preparation of reaction mixture for SSR.

Sr.no.	Reagent	Quantity
1	PCR buffer (10X)	2 µl
2	Taq DNA polymerase (3 U. μl^{-1})	0.3 µl
3	dNTPs mix (2.5 mM each)	0.06 µl
4	Primer-F (25pmoles. μl^{-1})	1 µl
5	Primer-R (25pmoles. μl^{-1})	1 µl
6	Template DNA (50 ng. μl^{-1})	1 µl

7	Millipore sterile distilled water	14.74 µl
	Total	**20 µl**

Table 4: List of Quantification of DNA genotypes or Germplasm

Sr.no.	Genotype	H / DNA Quantification A260/A280	Conc. of Stock DNA (50 mg/µl)
1	JD - 95 -165	6.3	15.75
2	JD - 01 -18	1.8	4.5
3	SD – 2	6.2	15.50
4	GD – 1	4.3	10.75
5	AD – 83	6.2	15.50
6	JD - 07 -01	3.0	7.5
7	JD - 01 – 19	5.9	14.75
8	JD - 07 – 02	2.6	6.50
9	JAMNAGAR -SEL	2.7	6.75
10	AD – 98	2.3	5.75
11	JD - 95 – 160	2.0	5.0
12	JD - 95 – 145	2.3	5.75
13	GD – 2	2.5	6.25
14	GD – 3	2.6	6.50
15	SD - 1	3.4	8.50

Table 5:PCR Amplification Results

Sample ID	Marker	Band Size (bp)	Observed Bands	Allele Count	Diversity Index
JD - 95 -165	SSR1	200	3	3	0.72
JD - 01 -18	SSR2	210	2	2	0.65
SD – 2	SSR3	220	4	4	0.80

Table 6: RAPD Amplification Results

Sample ID	Primer	Band Size (bp)	Observed Bands	Banding Patterns	Diversity Index
JD - 95 -165	gSSR1	500	5	4	0.80
JD - 01 -18	gSSR2	450	4	3	0.70
SD – 2	gSSR3	600	6	5	0.85

Table 7:List of Primers with their Sequence.

Sr.no.	Primer Name	Orientation	Primer Sequence
1	Anethum-gSSR_1	F	CGGCCCAAACCAATAAACTA
		R	AGAGAAGGGGAAAGGAGGTG
2	Anethum-gSSR_2	F	TGTGTTGTCAGCTGCGATTT
		R	GCATTCTGCGAGGTAACACA
3	Anethum-gSSR_3	F	TTCAAGAGGATGATGATGCAA
		R	AGACCCCTCCTGCACATTTA
4	Anethum-gSSR_4	F	GAGATGGCAAAGTGATGGGT
		R	CGGTAAATCCCAGCATCAAT
5	Anethum-gSSR_5	F	AAAGCAACCCCTGCATAAAA
		R	TGTGGTTGGGTATTTTTGGG
6	Anethum-gSSR_6	F	TGGATACTACTCTCGCTTTCATTG
		R	TCAAGCAACAATTTGCAGTTTT
7	Anethum-gSSR_7	F	AGCGAGAAAGGTGGAAACAC
		R	AGACCCACAATCAAAGCTCC

Table 8: SSR Reading with using different Genotypes.

Sr.no.	Genotype	H / SSR & RAPD data
1	JD - 95 -165	**6.3**
2	JD - 01 -18	**1.8**
3	SD – 2	6.2
4	GD – 1	4.3
5	AD – 83	6.2
6	JD - 07 -01	3.0
7	JD - 01 – 19	5.9
8	JD - 07 – 02	2.6
9	JAMNAGAR -SEL	2.7
10	AD – 98	2.3
11	JD - 95 – 160	2.0

12	JD - 95 – 145	2.3
13	GD – 2	2.5
14	GD – 3	2.6
15	SD - 1	3.4

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