MOLECULAR CHARACTERIZATION IN CORIANDER (*Coriandrum sativum* L.) GENOTYPES THROUGH RANDOM AMPLIFIED POLYMORPHIC DNA

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Abstract. The present studies were undertaken to investigate the different genotypes of coriander through molecular characterization. From the study on mean performance of genotypes of Coriander, the polymorphism shown by ten primers varied from 38 to 71%. A total of 106 bands were observed with ten primers and were used for the genetic variability analysis in Coriander. All the genotypes of coriander exhibited different characteristic profile amplification, which can be used for genotypes documentation. The genotypes CS 101 and UD 685 were found to be more diverse. A dendrogram representing the genetic relationship among the seventy-five genotypes based on RAPD techniques was developed. From the scientific analysis of RAPD and dendrogram analysis clearly mentioned that the CS 101 and UD 685 *Coriandrum sativum* genotypes were more varied. The reports inferred that the genotypes of two *C. sativum* would be valuable in the future high yielding and resistance breeding programme with high quality traits.
Keywords: *coriander, molecular characterization, molecular marker, dendrogram analysis, variability*

Abbreviations. RAPD - Random Amplified Polymorphic DNA; ISSR - Inter Simple Sequence Repeat; PCR - Polymerase Chain Reaction; ITS - Internal Transcribed Spacer; TNAU - Tamil Nadu Agricultural University

Introduction

Coriander (*Coriandrum sativum* L.) is an annual herb, spice plant which belongs to the family Apiaceae. It is native to the Mediterranean region, diploid, cross pollinated and medicinal plant (Palanikumar et al., 2012; Tulsani et al., 2020). The major producers are Morocco, Canada, India, Pakistan, Romania and Russia. Other producers

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include Iran, Turkey, Egypt, Israel, China, Thailand, Myanmar, Poland, Bulgaria, Hungary, France, Netherlands, USA, Argentina and Mexico (Sriti et al., 2014). In the Republic of India, *Coriandrum sativum* is cultivated over a large area of Rajasthan and Gujarat with a maximum sizeable cultivation in the states of Andhra Pradesh, Bihar, Haryana, Madhya Pradesh, Punjab, Tamil Nadu and Uttar Pradesh. It is cultivated on an area of 14,59,992 acres with the coriander production of 3,38,260 tonnes (Kallupurackal and Ravindran, 2003). Also, coriander seeds and dry coriander are highly useful for diarrhea and chronic dysentery remedial actions (Maroufi et al., 2010). coriander fruits have various antistress activities such as antifungal, antibacterial, stomachic, anticancer, spasmolytic, carminative and antioxidant properties, making it a valuable plant in Siddha, Allopathic and Ayurvedic industries (Lopez et al., 2008; Palanikumar et al., 2012; Duarte et al., 2016; Sahoo and Brijesh, 2020). Coriander oil has huge value and more importance in aroma industries (Lopez et al., 2008). To improve the yield, productivity, and seed quality of this important spice-cum-leaf yielding medicinal crop, developing high-yield coriander varieties through breeding programs is essential. Coriander is an umbelliferous spice crop with tiny flowers in the umbel, which are protandrous in nature. It has also been observed that about 50 percent self-incompatibility exists, making cross-pollination by insects necessary. The process of coriander hybridization through artificial crossing is challenging due to the small size of the flower buds. Also, mechanical selfing and / or bagging of the coriander buds, which invariably outcomes are shedding of flowers (Hore, 1979).

Hence, the breeding crop improvement programme in coriander through germplasm collection is a systematic manner and selection of promising types from the gene pool is the most useful method. The scientific information received from RAPD analysis has indicated huge diversification of genetical sources than allozymes in plant species (Choudhary et al., 2019). RAPD technique uses 10-base pair primer to amplify the random portion of genome with the genetical variability, marvelous scope for new high yielding varieties selection. Morphological methods are commonly used but they not given the exact information due to the environmental conditions and now a days genetical and molecular methods overcome these issues. Molecular markers are a more recent development and possess many advantages, which make them superior to morphological and biochemical markers. Molecular markers can be used to examine the DNA directly in order to find polymorphisms in the sequence. This offers the possibility of finding large number of polymorphisms which are not subject to environmental influence. Each molecular marker technique varies from each other in the number of loci examined, dominance or co-dominance and ease of application. Genetic relationships and diversity show a huge role in high yielding varieties programme to provide raw materials for developing new varieties with different traits, pest, disease resistance and abiotic stresses tolerance. It also shows an aim for recognizing the state of genotypic genetic improvement, various DNA profiling techniques and germplasm management. Also, RAPD and ISSR markers extensively used with successfully to identify the species characters differentiation, genetical population and varietal levels in many medicinal and aromatic crops (Haouari and Ferchichi, 2008).

The genetic variability technique namely RAPD has been moderately suitable. This technique broadly applicable for detection purpose which is an easy and quick manner. This RAPD marker has the merit of neither DNA probes necessitating nor prior primer sequence information for the purpose of primer designing (Amiteye, 2021). Such the entire data sequence is not an essential because commercially random primers are

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already available in market. Besides, because PCR is applicable in genetic variability analysis of RAPD, only high molecular weight purified DNA with a small amount is required. Very importantly, sample DNA template contamination must be eliminated. This defensive measure is an essential RAPD techniques point of view only the short random primers used and it can rapidly multiply with fragments of DNA of microbes (Amiteye, 2021). Mostly, a strict observance to the variability techniques such as RAPD protocols are dynamic due to the higher sensitivity in profile generation of RAPD to results of PCR reaction (Vekariya et al., 2017). Consistently, the low amplification of RAPDs marker profiles are ineffective to comparison or usage between or the scientific laboratories were working with similar research objectives.

Moreover, the same DNA sequences constituted by the RAPD and PCR fragments with the similar lengths and it may be non-homologous. Additionally, RAPD is a dominant marker due to the data quality is limited. Molecular markers of RAPD are most widely used for genetical mapping studies (Reiter et al., 1992), genetic diversity analysis (Shidfar et al., 2018), molecular markers identification linked to genetic habits (Bhutta and Hanif, 2013), evolutionary genetic population (Jordano and Godoy, 2000) because of its low simplicity and cost, technically usefulness does not require sequence knowledge. Molecular markers particular RAPD were applied in coriander to explore genetic diversity by Yilmaz et al. (2022), Omidbaigi et al. (2009), Nisha et al. (2013), Tomar-Rukam et al. (2014) and Choudhary et al. (2019). Singh et al. (2012) used ITS markers together with RAPD markers to reveal genetic diversity in most of the coriander cultivars. They mentioned that genetic distances among the coriander varieties and their geographical location did not correlate. Genetic variability with molecular markers are essential tool for conservation of plant germplasm, detection of genetical relationship among the individuals. Germplasm collection means collection of genetic resources from different areas and storing them in one place. Germplasm collections can include wild species, landraces, and developed varieties. Germplasm conservation means preservation of genetic traits of economically important plants. Molecular variability through RAPD techniques has been exploited in agricultural and horticultural crops such as *Triticum* species (Bhutta, 2007), *Oryza sativa* (Chen et al., 2017), *Zea mays* (Dev et al., 2007), *Jasminum* species (Ghosh et al., 2020), *Malus domestica* (Kaya et al., 2015) and *Helianthus* species (Raza et al., 2018). In case of Coriander seeds, the scientific reports mentioned by Singh et al. (2013) proved that the RAPD molecular markers were extremely efficient in the genetical characterization of coriander varieties. The main objective of the research work is to investigate the different genotypes of coriander through molecular characterization. Hence, this scientific study was undertaken for new coriander varieties development.

Materials and methods

The trials were laid out at Horticultural College and Research Institute, TNAU, Coimbatore, which is located at 11°N latitude, 77°E longitude and at an altitude of 426.26 m above MSL. The present investigation was carried out during three seasons viz., season I (June 2022 – August 2022), season II (October 2022-December 2022) and season III (June 2023 – August 2023). Seventy-five genotypes were raised in randomized block design with two replications. Each genotype was raised in flat beds of 4 x 3.0 m and seeds were sown at 40 grams per bed in rows spaced 30 cm apart. Five plants were selected randomly manner and the plants in each replication purpose in an

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each genotype and they were tagged for recording observations on plant characters and the mean values were limited to statistical scrutiny. For each replication purpose five plants were selected randomly manner in each genotype and tagged for analyses the results. The mean values were used for statistical analysis.

Data scoring and similarity matrix of biochemical markers and molecular marker analysis were subjected to using NTsys packages available at Plant Breeding and Genetics, TNAU, Coimbatore.

Genetic diversity studies of coriander genotypes based on molecular markers

The following steps were involved in the molecular marker analysis
a. DNA extraction
b. DNA purification
c. DNA quantification
d. Screening of primers
e. Marker analysis

DNA extraction

DNA from all the 75 genotypes was extracted following the protocol described by Shyamkumar et al. (2003) from leaves frozen at -20^{o}C. Molecular profiling of the selected genotypes were done using RAPD markers.

Reagents and chemicals:
1M Tris - HCl (pH 8.0) (HiMedia)
0.5 M EDTA (pH 8.0) (HiMedia)
5 M NaCl solution (HiMedia)
Chloroform - isoamylalcohol (24:1 [(v/v)] mixture (HiMedia)
β-Mercaptoethanol solution (HiMedia)
N-Cetyl N, N, N - Trimethylammonium bromide (CTAB) (HiMedia)
Phenol – chloroform – isoamyl alcohol [25:24:1 (V/V)] (HiMedia)
Polyvinyl pyrrolidone (PVP) (HiMedia)

Preparation of the reagents:

1M Tris-HCl (pH 8.0): 157.6 g of Tris HCl was dissolved in 800 ml of distilled water and the pH was adjusted to the desired value by adding concentrated HCl. The volume was made up to 1000 ml with distilled water and the contents were sterilized.

0.5 M EDTA (pH 8.0): 186.1 g of Di sodium ethylene diamine tetra acetic acid was dissolved in 800 ml of water. The contents were stirred vigorously on a magnetic stirrer. Then the pH was adjusted in to 8.0 with NaOH and autoclaved.

5M NaCl: 292.2g of NaCl was dissolved in 800 ml of water. Then the volume was made up to 1000ml with distilled water and autoclaved.

*4% CTAB:*40 g of N-Cetyl N, N, N – tri methyl ammonium bromide (CTAB) was dissolved in 1000 ml of distilled water.

Chloroform- isoamyl alcohol (24:1): 24 ml of Chloroform was added with isoamyl alcohol (1 ml).

1% Poly vinyl pyrrolidone: 1 g Polyvinyl pyrrolidone was dissolved in 99 ml of water to make 1% PVP solution.

β-Mercaptoethanol: 0.3 ml of β-mercaptoethanol was dissolved in 97 ml of water to make 0.3% solution.

Phenol – chloroform – isoamylalcohol [25: 24: 1 (V/V)]: 25 ml of Phenol, 24 ml of chloroform and isoamyl alcohol (1 ml) were mixed to get the 25: 24: 1 concentration.

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*Extraction buffer:*Tris - HCl 100 mM (pH 8.0), EDTA 20 mM, 2M NaCl and 4% CTAB were added. 0.3 % of β- mercaptoethanol was mixed in 1% PVP immediately before the use of solution.

DNA isolation protocol: Six grams of fresh leaf sample were ground using liquid nitrogen. The materials were transferred into a 50 ml polypropylene tube. In the poly propylene tubes 3 ml of freshly prepared pre-heated extraction buffer was added per gram of tissue. While adding the CTAB buffer, care was taken for maintaining viscous nature. These poly propylene tubes were incubated at 60^{o}C in a water bath for 45-60 minutes with occasional mixing. Equal volume of chloroform - isoamylalcohol (24:1) was added to the contents of poly propylene tubes and mixed by inversion for 15 min. The poly propylene tubes were centrifuged (@ 8400 rpm) at 25-30^{o}C for 600 seconds and the clear upper aqueous layer was carefully transferred to another 50 ml poly propylene tube. Later 0.6 ml volume of ice-cold ethanol was added in the tube and incubated at -20^{o}C for 2 hours. The ice-cold ethanol should be carefully mixed for the isolation of fibrous DNA from the floating contents. This DNA was separated by centrifugation at 10000 rpm at 25-30^{o}C for 30 minutes. The supernatant materials were discarded and the pellet was washed with 80% ethanol.

DNA purification

The DNA pellet was dried for 15 minutes and dissolved in sterile double distilled water. To this 10 µg/ml of RNA ase was added and incubated at 37^{o}C for 30 minutes. This was extracted with an equal volume of phenol-chloroform – isoamyl alcohol (25:24:1) and mixed by inversion for 15 minutes. The contents were centrifuged at 10000 rpm at 25-30^{o}C for 15 minutes. The aqueous layers were moved into a new 2-ml Eppendorf tube. To this an equivalent volume of ice-cold 100% ethanol was added to precipitate the DNA. The tube was then centrifuged at 8400 rpm at 25-30^{o}C for 5 minutes. The supernatant was discarded and the pellet was washed with 80% ethanol. The pellet was dried and dissolved in nuclease free water.

Quantity of DNA and quality check

Agarose gel electrophoresis on 0.8 per cent gel was achieved to check the quantity of DNA and quality. The concentration of DNA for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions that have good amplification.

Screening of primers

RAPD (Random Amplified Polymorphic DNA) Primers: Primer numbers of OPZ (Operon Technologies Zenica)-TNAU-01, 03, 05, 06, 08, 09, 011, 012, 013 and 016 have produced good amplifications. These primers were supplied by Sigma from Genosys, India.

Random amplified polymorphic DNA analysis (RAPD)

DNA from each of 75 coriander genotypes were amplified using a set of 15 arbitary oligonucleotide decamer primers (as described by Operon technologies, Alameda, California, USA). The RAPD primers were produced by M/s Sigma – Aldrich (USA) at Bangalore, India. Amplification reactions were in the volumes of 15 µl reactions containing 10-20 ng of genomic DNA, 1.5 µl of 1.5 mM of assay buffer 1.0 µl of

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10.0 mM o d NTPs, 1.0 µl of 10 µM primer, 0.18 µl of 15 mM $MgCl_2$, 0.30 µl (1 unit) of Taq (*Thermatus* aquaticus) DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore) and 8.12 µl of sterile water. Amplifications were performed in PTC Thermal Cycler (MJ Research Inc.,) programmed for 44 cycles of denaturation (60 seconds) at 94°C, 1 min annealing at 37°C and 2 min. and 72°C for extension than a final extension of 10 minutes at 72°C. PCR amplified products were detected by electrophoresis in a 1.2 percent agarose gel in 1X TAE buffer at 100 volts for 2 hours using a horizontal gel electrophoresis unit (Bangalore Genei, Bangalore). The Ethidium bromide stained gels were documented using the Alpha Imager TM 1200-Documentation and Analysis system of the Alpha Innotech Corporation, USA. Sizes of the bands were identified relative to a 100 bp ladder (Bangalore Genei, Bangalore) run simultaneously on the gel (Palanikumar et al., 2012).

List of seventy-five coriander genotypes for RAPD analysis in *Table 1* and the RAPD primers used in this study are listed in *Table 2*.

Table 1. *List of seventy-five coriander genotypes for RAPD analysis*

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	UD 685	16	UD 209	31	CS 156	46	CS 108	61	CS 27
2	CS 71	17	CS 198	32	UD 120	47	CIMPO-S-33	62	CS 200
3	CS 180	18	CS 136	33	CS 83	48	DH 221	63	CS 152
4	CS 177	19	CS 187	34	CS 37	49	DH 266	64	CS 32
5	CS 119	20	CS 3	35	CS 70	50	DH 226	65	ND Cor-2
6	CS 88	21	CS 110	36	CS 91	51	UD 744	66	CS 36
7	CS 101	22	CS 25	37	CS 65	52	DH 208	67	CS 845
8	CS 18	23	CS 33	38	CS 68	53	UD 273	68	CS 74
9	CS 63	24	CS 142	39	CS 26	54	J Co-387	69	CS 13
10	UD 686	25	CS 10	40	CS 40	55	Velachikulam (local)	70	ATP 72
11	CS 66	26	CS 170	41	CS 194	56	DH 259	71	CS 49
12	CS 144	27	CS 146	42	CS 39	57	CS 745	72	CS 20
13	CS 169	28	CS 131	43	DH 230	58	UD 158	73	CS 142
14	CS 52	29	CS 62	44	DH 232	59	RCR 144	74	CS 45
15	CS 178	30	CS 106	45	CS 497	60	CS 89	75	CS 176

Table 2. *List of random primers used for RAPD analysis*

S. No.	Primer Details	Sequence information (5’ – 3’)
1.	OPZ-TNAU- 01	**TCTGTGCCAC**
2.	OPZ-TNAU- 03	CAGCACCGCA
3.	OPZ- TNAU-05	AGGCTGTGCT
4.	OPZ- TNAU-06	TCCCATGCTG
5.	OPZ- TNAU- 08	GGGTGGGTAA
6.	OPZ- TNAU- 09	CACCCCAGTC
7.	OPZ- TNAU- 11	CTCAGTCGCA
8.	OPZ- TNAU- 12	TCAACGGGAC
9.	OPZ- TNAU- 13	GACTAAGCCC
10.	OPZ- TNAU- 16	TCCCCATCAC

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Statistical data analysis

Scoring: Amplified DNA fragments were detected after electrophoretic separation in each genotype was scored for the presence (1) or absence (0) of clear and unambiguous bands. A comprising of data matrix '1' and '0' was formed and this data matrix was subjected to the conditions for further analysis.

Computation of D^2 values

All possible {n (n-1) 1/2} D^2 values between 75 genotypes were calculated utilising the replicated values. Packages available at Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

The replicated data of the genotypes for the characters were subjected to analysis of variance using AGRES and D^2 statistic was employed using INDOSTAT and WINDOSTAT packages. Data scoring and similarity matrix of biochemical markers and molecular marker analysis were subjected to using NTsys packages available at Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. A dendrogram mentioned the clustering of genes or samples and is used for represent the arrangement of clusters produced by hierarchical clustering. Hence, dendrograms can be used for genetic variability analysis particularly food staple crops and horticultural crops.

Results

RAPD profiles among seventy-five genotypes were generated from eighteen decamer primers used for genetic characterization analysis (*Table 1*).

Among this, 10 primers yielded scorable, unambiguous markers and 8 primers failed to amplify any fragment. The amplification of the template DNA produced a total of 51 markers, of which 26 markers as a polymorphic nature (38 to 71%) and the rest (25) were monomorphic markers. The polymorphism exploited by ten primers differentiated from 38 to 71%. (*Table 2*). Totally one hundred and six bands were observed with the primers of ten and were used for the genetic diversification of analysis.

All the coriander genotypes shown a distinguishing profile amplification, which can be used for genotypes documentation (*Table 3*).

More amplified fragments were produced for OPZ-01, 05, 06 and 11 and the primers OPZ-06 and OPZ-11 resulted high polymorphism in RAPD analysis (*Plate 1*). The genetic diversity assessed through RAPD profiles expressed 38 to 71% per cent of polymorphism. The genotypes were classified into two major groups. The genotype CS 70 was found to be more varied. Analysis of dendrogram representing the genetical relationship among the seventy-five coriander genotypes based on RAPD technique were developed. The square of the distance (D^2 values) between the genotypes, calculated as the sum of squares of the difference between the mean values of all the variables were used for final grouping of the genotypes. By the application of clustering technique, the seventy-five genotypes were grouped into different clusters during different seasons. The second, fourth, fifth, seventh, eighth, ninth and tenth clusters consisted one genotype each. Cluster three included five genotypes and sixth included two genotypes.

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Table 3. *Percentage polymorphism shown by different primers*

S.No	Primers	Total number of bands	Total number of polymorphic bands	Per cent polymorphism P/T x 100
1.	OPZ-TNAU- 01	8	5	62.50
2.	OPZ-TNAU- 03	12	7	58.33
3.	OPZ- TNAU-05	13	9	69.23
4.	OPZ- TNAU-06	15	12	80.00
5.	OPZ- TNAU- 08	11	5	45.45
6.	OPZ- TNAU- 09	6	3	50.00
7.	OPZ- TNAU- 11	7	5	71.42
8.	OPZ- TNAU- 12	8	4	50.00
9.	OPZ- TNAU- 13	13	5	38.46
10.	OPZ- TNAU- 16	13	6	46.15

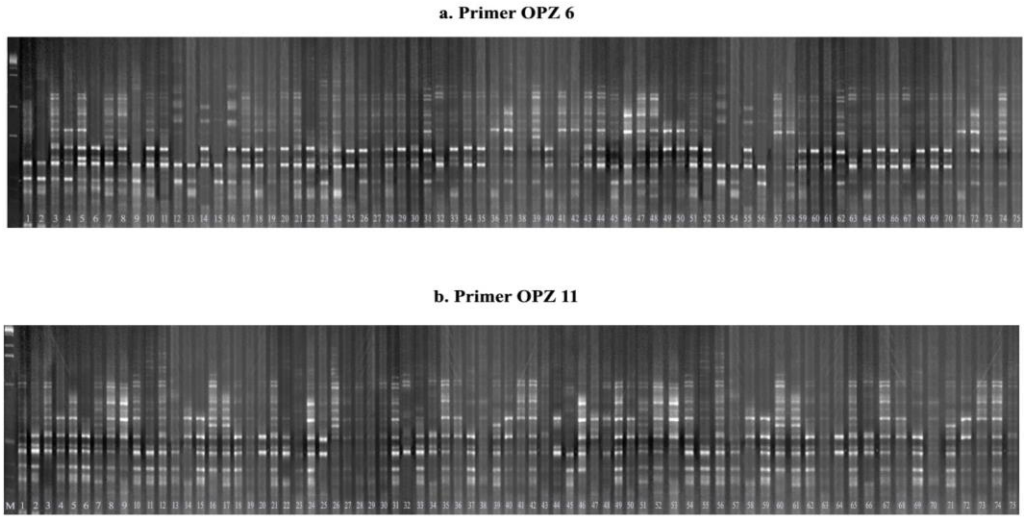

Plate 1. *RAPD marker Profiles of seventy-five coriander genotypes*

From the scientific reports of D^2 analysis (*Fig. 1, Table 4*) and RAPD analysis it was mentioned that the coriander genotypes of UD 685and CS 101 were more differentiation and the analytical reports inferred that these two coriander genotypes would be helpful in future high yield varieties through breeding programme for new variety emerging with high biomass yield.

Primers were carefully selected and RAPD protocols were optimized to get more reproducibility in the present research work. Among the 20 random primers used for the initial screening, six provided optimal and reproducible RAPD profiles for all the genotypes studied. The differences obtained via traditional morphological classification and RAPD data could be due to morphological modifications by regional and environmental changes. The advantages of this approach are the limited amount of DNA required, procedure simplicity and the lack of isotopes or prior genetic information. Low polysaccharide and polyphenolic content in the sample was thought to be suitable for direct PCR and further it is suggested that genetic diversity analysis in coriander with more number of primers for RAPD producing a large number of informative polymorphic markers per primer pair that are highly reliable and reproducible.

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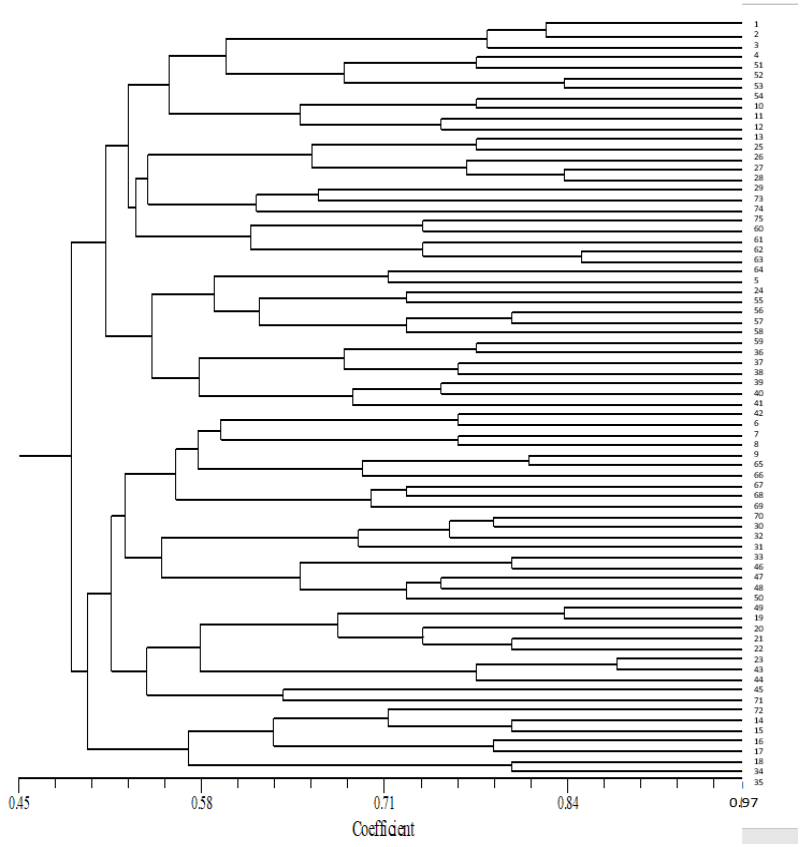

Figure 1. *Dendrogram for seventy-five coriander genotypes using RAPD markers*

Table 4. *Constitution of D^2 cluster of 75 genotypes of coriander*

Clusters number	Number of genotypes	Name of the genotypes
1	68	CS 497, CS 108, CS 177, DH 266, CS 36, UD 158, CS 131, CS 146, CS 45, CS 68, CS 152, DH 221, CS 13, CS 49, CS 74, ND Cor-2, CS 20, CIMPO-S-33, CS 200, DH 259, CS 10, CS 198, J Co-387, CS 142, CS 91, Velachikulam (local), DH 208, CS 27, CS 187, DH 230, CS 32, CS 106, ATP 72, UD 744, CS 83, DH 226, RCR 144, CS 180, CS 170, CS 142, CS 3, CS 119, CS 52, CS 88, UD 209, CS 33, CS 37, CS 65, UD 273, CS 25, CS 71, CS 110, CS 26, CS 845, CS 144, CS 18, CS 39, CS 156, UD 686, CS 194, CS 176, CS 745, CS 169, CS 66, CS 63, CS 89, CS 40, UD 120
2	3	CS 70, DH 232, CS 177
3	1	CS 62
4	1	CS 136
5	2	CS 101, UD 685

In the present study, the coriander genotypes viz., UD 685 and CS 101 and were grouped in to one cluster. Certain genotypes having same geographical origin and higher level of similarity based on the quantitative data were observed to be in different clusters. At the same time, some genotypes formed separate clusters. The clustering

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pattern revealed a poor level of similarity between the quantitative data and RAPD marker data. However, the clustering of some genotypes of diverse origin was similar at both at morphological and molecular level. The molecular diversity data base can indication to be directly helpful to the development and investigates an inside as well as interspecific diversity as the morphology informational data alone may be misleading since the genotypes appearing in the same group morphologically, several times exploit the different molecular groupings. To conclude, the genotypes CS 101 and UD 685 were identified for higher biomass yield. The RAPD marker and D^2analysis also concluded that, the genotype CS 101 and UD 685 was found to be more varied. A dendrogram analysis representing the genetical relationship among the seventy-five coriander genotypes based on RAPD techniques were established.

Discussion

Genetically diversity Information and genetically relationship among the individual population, crop cultivar and species are very important to breeders for producing the new spices and medicinal crop varieties. Genetical diversity studies can identify the allele population that might affect the ability of the organism to survive in its existing habitat, or it might be enable to survive in more diverse characters. This information is valuable for germplasm conservation, population individuals, cultivar and molecular improvement (Duran et al., 2009). Various types of markers such as morphological, biochemical and molecular are used for this purpose (Barwar et al., 2008).

The RAPD finger printing of Banerjee et al. (1999) in *Piper longum* using forty decamer oligonucleotide primers as a randomly manner, Besse et al. (2004) in vanilla and Sasikumar (2001) in turmeric with RAPD markers are in concordance with present view. The result assessed in the present investigation was in agreement with previous scientific works on various crops (Vladova et al., 2004; Nair and Keshavandran, 2006). The RAPD techniques are quite sensitive because different DNA profiles were generated by each primer for each of the variety, cultivar and species. RAPD techniques using unique DNA profiles were obtained in different crop species and cultivars. Zhang et al. (2024) mentioned that a total of 8 different *Asparagus* species and six *A. officinalis* cultivars were used using eight RAPD primers. A collection of fifty amplification fragments were scored, among these, 36 (72%) were polymorphic, and 14 (28%) were monomorphic bands. Now a days, Plant genotype(s) with significant mean values were identified to assess relative of genetic performance for biometric and yield attributes with molecular protein marker-based clustering with the help of dendrogram. The cluster-wise mean values allow a simultaneous comparison of many quantitative traits of the testing genotypic characters with reference to the position of each genotype in the dendrogram analysis. It is also observed that the genotypes in the same cluster based on protein profiling have some common phenotypic performance and such clusters with distinctive phenotypic performance could be identified for genetic improvement (Tripathy et al., 2015). D2 analytical reports were also confirmed with earlier works Singh et al. (2000) in ginger.

The importance of the choice of characters has been stressed since they reflect the usefulness of D^2 analysis. In the present study, Mahalanobis D^2 analysis was applied for assess the genetic divergence among the genotypes and identify the promising genotypes with more divergence to initiate crossing programme. Also, its applicable for assess the contribution of different characters to genetic diversity. With regard to the

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contribution of different characters towards the genetic divergence, it was found that the maximum contribution to the genetic divergence was accounted by fresh weight of root, fresh weight of stem and weight of leaves. The low contribution to genetic divergence by other characters may be due to the fact that selection towards uniformity in these characters could have caused an eroding effect of genetic diversity. This is in conformation with earlier works by Reddy (1987) and Shanmugasundaram (1998) in turmeric and Singh et al. (2000) in ginger.

A dendrogram representing the genetic relationship among the seventy-five genotypes based on RAPDs was developed. From the foregoing, it is clear that, genetic diversity is a ubiquitous property of all species in nature. The distribution and organization of genetic variation with in and among the populations of a species are the consequence of its evolution. Further, the various multivariate tools used viz., D^2 analysis, k means on phenotypic data as well as RAPD marker data elucidated the same results on genetic divergence, though these were minor differences.

In coriander spice crop, most of the research works carried out on variability so far based on single environment conditions only, but their performance is known to vary from season to season for different characters. An effective and viable breeding programme has to be experimented in the knowledge of genetic variability and association of different characters for effective selection of suitable parents. These genotypes could be efficiently utilized in coriander genetic improvement and high yielding, pest and disease resistance programs. Hence, the scientific reports of the present work could be highly useful to facilitate the phylogenetic relationship for coriander spice improvement and high yielding programs in future.

Conclusion

The polymorphism exploited by ten primers differentiated from 38 to 71%. A collection of one hundred and six bands were observed with ten primers and were applicable for the analysis of genetic diversity. All the coriander genotypes exhibited a characteristic profile amplification, which can be used for genotypic documentation. The genotypes of UD 685 and CS 101 were found to be more diverse. A dendrogram analysis representing the genetical relationship amongst the seventy-five genotypes of coriander based on RAPDs was adopted. From the scientific reports of D^2 analysis and RAPD analysis it was stated that the coriander genotypes CS 101 and UD 685 were more differentiate and the reports contingent that these two genotypes would be highly helpful in further coriander breeding method for new variety identification with high production quality and productivity.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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