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Genetic Variation Assessment of Coriander (*Coriandrum sativum* L.) Genotypes Using Randomly Amplified Polymorphic DNA (RAPD) Analysis

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ABSTRACT

The identification of genetic relationships between individuals and the conservation of germplasm has benefited greatly from molecular characterization using different kinds of markers including RAPD. The main aim of the study to find out the genetic diversity of coriander genotypes through molecular characterization. Based on the results obtained from genotyping 75 coriander accessions (Coriandrum sativum L.) that were cultivated in Tamil Nadu, India using ten RAPD primers, it was found that investigated primers revealed 38 to 71% polymorphisms by producing 106 amplicons. A dendrogram was created to show the genetic relationships among the 75 genotypes. It was evident from this and D^2 analyses that the genotypes UD 685 and CS 101 had greater variation. Thus, this study has identified two genetically diverged Coriandrum sativum genotypes for further breeding programs that focused on the genetic improvement of coriander for high-yielding, resistant with high-quality features

Keywords: *Coriander genotypes, RAPD, molecular diversity, dendrogram analysis, variability, genetic improvement, yield, quality*

INTRODUCTION

Coriander (*Coriandrum sativum* L.) belongs to the family Apiaceae is an annual herb and, spice. It is native to the Mediterranean region, a diploid, and cross-pollinated plant [1]. Globally, coriander is chiefly produced by Morocco, Canada, India, Pakistan, Romania, and Russia. Besides, it is also cultivated in Iran, Turkey, Egypt, Israel, China, Thailand, Myanmar, Poland, Bulgaria, Hungary, France, Netherlands, USA, Argentina, and Mexico [2]. In India, *Coriandrum sativum* is cultivated in the states of Rajasthan and Gujarat, and maximum sizeable cultivation is practiced in the states of Andhra Pradesh, Bihar, Haryana, Madhya Pradesh, Punjab, Tamil Nadu and Uttar Pradesh. It is estimated that coriander is cultivated in India on an area of 14,59,992 acres with coriander production of 3,38,260 tonnes [3].

Coriander seeds and dry coriander are known for diarrhea and chronic dysentery remedial actions [4]. Coriander fruits have antifungal, antibacterial, stomachic, anticancer, spasmolytic, carminative, and antioxidant properties thus making it a valuable plant in Siddha, Allopathic, and Ayurvedic industries [5]. Coriander oil has huge value and more importance in aroma industries [6].

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To improve the yield, productivity, and coriander seed quality components of this important spice-cum-leaf yielding medicinal crop, high-yield coriander varieties through breeding programs becomes inevitable. Coriander is an umbelliferous spice crop with tiny flowers in the umbel, which are protandrous. It is also noticed that about 50 per-cent self-incompatibility exists and thus pollination by insects is the rule. The process of coriander hybridization program by artificial crossing is difficult due to the smaller flower buds [7].

Hence, germplasm collection and characterization through systematic and recent tools is essential to identify promising types from the gene pool for an effective hybridization program. Among the conventional and advanced strategies, RAPD is a simple and cost-effective method to catalog genetic variation that exists in crop plants [8].

RAPD was widely used for genetical mapping studies [9], genetic diversity analysis [10], genetic habits [11] as well as - evolutionary investigations [12]. RAPD was also employed in coriander to explore genetic diversity by [13-16]. Internal Transcribed Spacer (ITS) markers together with RAPD markers to reveal genetic diversity in most of the coriander cultivars [17]. They mentioned that genetic distances among the coriander varieties and their geographical location did not correlate and hence, genetic variability with molecular markers is essential for the conservation of germplasm Though coriander is widely cultivated in Tamil Nadu, India with different ecotypes, little information on its genetic diversity available. This greatly limits the progress of the genetic improvement programs and hence this study was conducted to characterize the coriander germplasm cultivated in Tamil Nadu using RAPD markers.

Such effort has helped to identify genetically diverged lines that have potential applications in future coriander breeding programs.

MATERIALS And METHODS

The trials were laid out at Tamil Nadu Agricultural University, 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 (season I (June 2022 – August 2022), season II (October 2022-December 2022) and season III (June 2023 – August 2023). Seventy-five genotypes (Table 1) were raised in a randomized block design with two replications. Each genotype was raised in flat beds of 4 x 3.0 m and seeds were sown @ 40 grams per bed in rows spaced 30 cm apart. Five plants were selected randomly in each genotype and they were tagged for recording observations on plant characters. The mean values were used for statistical analysis.

Genetic diversity studies of coriander genotypes based on molecular markers

DNA from all 75 genotypes was extracted using the protocol and molecular profiling of the selected genotypes was done using RAPD markers [18]. RAPD markers OPZ (Operon Technologies, Zenica) 01, 03, 05, 06, 08, 09, 011, 012, 013 and 016 (Operon Technologies, Alameda, California, USA; Table 2) were employed in this study 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 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 DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore) and 8.12 µl of sterile water. Amplifications were performed in PTC Thermal Cycler (MJ Research Inc.,) that was 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 then a final extension of 10 minutes at 72°C. PCR amplified products were detected by electrophoresis in a 1.2 per cent 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 (Alpha Innotech Corporation, USA). Sizes of the PCR amplicons were identified using a 100 bp ladder (Bangalore Genei, Bangalore) that was run simultaneously on the same gel.

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

All possible {n (n-1) 1/2} D^2 values between 75 genotypes were calculated utilizing the replicated values. 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.

RESULTS AND DISCUSSION

Genetically diversity Information and genetic relationships among the individual population, crop cultivar, and species are very important to breeders for producing 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 enabled to survive in more diverse characters. This information is valuable for germplasm conservation, population individuals, cultivars, and molecular improvement [19]. Various types of markers such as morphological, biochemical, and molecular are used for this purpose [20].

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

Table 1. List of seventy-five coriander genotypes employed in this study

S. No.	Geno types	S. No.	Geno types	S. No.	Geno types	S. No.	Geno types	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

Among these, 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. 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.

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

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

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

More amplified fragments were produced for OPZ-01, 05, 06, and 11 and the primers OPZ-06 and OPZ-11 resulted in 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 was developed. From the scientific reports of D^2 analysis (Fig. 1) (Table 4) and RAPD analysis it was mentioned that the coriander genotypes of UD 685 and CS 101 were more differentiation and the analytical reports inferred that these two coriander genotypes would be helpful in future high yield varieties through the breeding program for new variety emerging with high biomass yield.

The RAPD finger-printing in *Piper longum* randomly using forty decamer oligonucleotide primers [21], vanilla [22], and RAPD markers are in concordance with the present view in turmeric [23]. The result assessed in the present investigation was in agreement with previous scientific works on various crops [24-25]. The RAPD techniques are quite sensitive because different DNA profiles were generated by each primer for each variety, cultivar, and species. RAPD techniques using, unique DNA profiles were obtained in different crop species and cultivars. 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 [26].

Plate 1. RAPD marker profiles of seventy five coriander genotypes

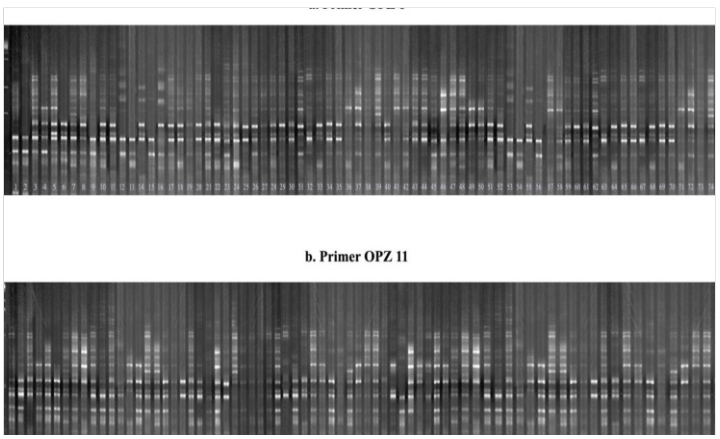

Figure 1. Dendrogram for seventy five coriander genotypes using RAPD markers

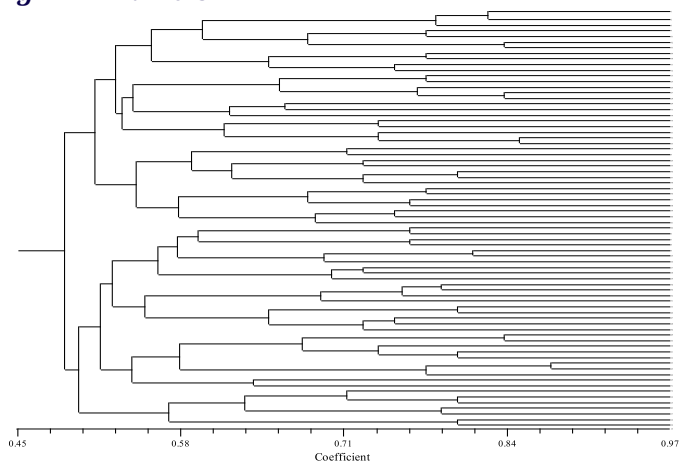

Table 4. Constitution of D2 cluster of 75 genotypes of coriander

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

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.

In the present study, the coriander genotypes viz., UD 685 and CS 101 were grouped in to one cluster. Certain genotypes having the same geographical origin and higher levels 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 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 levels. The molecular diversity data-base can indication to be directly helpful to the development and investigation 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^2 analysis also concluded that, the genotypes CS 101 and UD 685 were found to be more varied. A dendrogram analysis representing the genetical relationship among the seventy-five coriander genotypes based on RAPD techniques were established.

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 genetic relationship amongst the seventy-five genotypes of coriander based on RAPDs was adopted.

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Authors' Contribution Statements

Palanikumar Muniyandi, Manikanda Boopathi Narayanan, Arularasu Palanichamy, and Rajarathinam Palanivel designed the study, executed the field research, data collection and in writing and improving paper critically, Jaiganesh Vaikundaperumal carried out few laboratory analysis, literature collection, improving paper critically and finalize the paper, Sundharaiya Kalangiyam was involved in the basic idea of the paper, statistical analysis of data and critical review of the paper. All these authors have substantial contributions to the finalized manuscript and approved this submission.

Feature scope of scope of the study

From the scientific reports of RAPD analysis and D^2 analysis, it was stated that the coriander genotypes CS 101 and UD 685 were more differentiated 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.

Conflict of Interest

The authors declare that there is no conflict of interest

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