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Molecular characterization of coriander (*Coriandrum sativum* L.) genotypes using random amplified polymorphic DNA (RAPD) markers

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Abstract
Coriander (*Coriandrum sativum* L.) is an important seed spice crop grown in india and throughtout the world. Twenty–three genotypes of coriander were selected with an aim to find out genetic variability using random amplified polymorphic DNA (RAPD) marker. A total of 72 amplified bands were obtained using 14 RAPD primers out of which 48 were polymorphic. RAPD analysis showed 64.34% of polymorphism. The total number of amplified bands varied between 3 (primer S-34) and 11 (primer OPB-06) with an average of 7 bands per primer. The overall size of PCR amplified products ranged between 300 bp to 3000 bp. The average PIC was 0.229 ranging from 0.041 to 0.695. 3 unique bands were detected in 3 genotypes with 3 RAPD primers. The size of these unique bands ranged from 300 to 1400 bp. The Jaccard's similarity coefficient values ranged from 0.63 to 0.93 with an average of 0.78. The dendrogram generated through UPGMA clustering method, genotypes divided into 2 main clusters. Cluster I is a major cluster including 22 genotypes and it could be further divided into several subclusters respectively. Cluster II included single genotype only. Hence, the overall grouping pattern of cluster analysis based on RAPD showed polymorphism among most of the genotypes.

Keywords: Coriander, molecular characterization, molecular marker, RAPD

Introduction
Coriander (*Coriandrum sativum* Linn.) is an important annual seed spice crop which belongs to the family Umbelliferae/Apiaceae and is diploid cross pollinated crop. The plant is originated from the Mediterranean and near eastern region (Bhandari and Gupta, 1991) [2] and is broadly cultivated in North Africa, Europe, India, China and Thailand. In recent years, principal commercial coriander producers countries in the world included members of the former Soviet Union, Hungary, Poland, Romania, Czech Republic, Slovakia, Morocco, Canada, India, Pakistan, Iran, Turkey, Guatemala, Mexico and Argentina (Kiehn and Reimer, 1992) [15]. India comes first in terms of production and area in the world (Datta *et al*., 2006) [6] and here it is commonly called as "dhanya". In India, it is mainly grown in Rajasthan and Gujarat with sizeable acreage in Madhya Pradesh, Haryana, Punjab, Uttar Pradesh, Andhra Pradesh, Tamil Nadu and Bihar. Rajasthan is the major coriander producing state, Here zone V (districts of Kota, Bundi, Baran, Jhalawar) covers approximately 98 percent coriander area of the state. The fresh green herb and a dry spice are the two main products obtained from coriander plants besides steam distilled essential oil and solvent extracted oleo-resin (Islam *et al*., 2009) [13]. The green leaves and seeds of coriander contained major phytochemicals *viz*., carotenoids, chlorophylls, sugar, ascorbic acid, tocopherols, phenolics, flavonoids, tannins and anthocyanins (Dias *et al*., 2011) [8]. Coriander plant is mainly used as spice, in perfumery, food, beverage and pharmaceutical industry, medicine such as antioxidant, treatment of nervous disorder, gut modulatory, blood pressure lowering agent and diuretic activities, anti diabetic and antimicrobial agent (Isabelle *et al*., 2010; Qaiser *et al*., 2009) [12, 20]. Due to cross-pollination, this crop has high level chances of molecular variablility and scope for the development of improved varieties/genotypes and characterization of germplasm. Its rapid life cycle allow it to fit into different growing seasons and making it possible to grow the crop under a wide range of conditions (Diederichsen, 1996) [9].

The knowledge, extent and magnitude of genetic variability/diversity of agronomic and quality trait is limited. With the innovations in molecular biology and biotechnology it is now possible to analyze large number of loci distributed throughout the genome of a plant. Molecular tools developed in the past few years provide easy, less laborious means to characterize known and unknown plant taxa. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity assessment within and between species and

populations by revealing different classes of variation (Powell *et al*., 1996)[19]. The techniques include either or both DNA and enzyme based system (Packia *et al*., 2000)[17]. It is correlated with the genome fraction surveyed by each kind of molecular marker, their distribution throughout the genome and the extent of DNA target which is analyzed by each specific assay (Davila *et al*., 1999b)[7]. RAPD (Random amplified polymorphic DNA) is a PCR based technique developed by Williams *et al*., (1990)[26] and detect nucleotide polymorphism in a DNA amplification based assay using a short single synthetic primer of arbitrary nucleotide sequence. The advantages of using RAPD in genetic analysis are that it is sequence independent, easy, fast, cost-effective, efficient and requires small amount of DNA for cultivar identification and diversity analysis (Haque *et al*., 2007) [11]. RAPD marker have been efficiently used for the study of molecular diversity in coriander genotypes (Tomar *et al*., 2014, Singh *et al*., 2013, Singh *et al*., 2012, pareek *et al*., 2011, melo *et al*., 2010 and Al-kordy *et al*., 2013) and in various seed spice crops like cumin, fenugreek and fennel (Choudhary *et al*., 2013; 2015, Choudhary *et al*., 2018) [3, 4, 5]. Therefore, the present study was undertaken with an aim to characterize pattern and extent of genetic variability and relatedness among 23 diverse genotypes of *Coriandrum sativum* L. using RAPD marker which could further facilitate the analysis of phylogenetic relationship for crop improvement and breeding programs.

Materials and Methods

Plant material: In the present investigation the seeds of 23 diverse genotypes of *Coriandrum sativum* L. were procured from ARS, Kota and planted at Molecular biology and biotechnology department, Rajasthan college of agriculture, MPUAT, Udaipur, Rajasthan, India during 2016-17 in earthen pots for molecular analysis using RAPD marker. List of the 23 genotypes of *C. sativum* L. are presented in Table 1.

Table 1: List of 23 genotypes of *C. sativum* L.

S. No.	Code	Genotypes
1.	G_1	RCr-436
2.	G_2	C0R-49
3.	G_3	RKC-39 I
4.	G_4	RKC- 44 I
5.	G_5	RKD –18
6.	G_6	RKC- 20
7.	G_7	RKC- 53 I
8.	G_8	RKC-28 I
9.	G_9	RKC-57 I
10.	G_{10}	UD– 509 I
11.	G_{11}	RKC – 26 I
12.	G_{12}	RKC – 54 I
13.	G_{13}	RKC– 42 I
14.	G_{14}	RKC– 53 II
15.	G_{15}	RKC- 17 I
16.	G_{16}	COR– 40
17.	G_{17}	COR– 56
18.	G_{18}	COR – 38
19.	G_{19}	Hisar Anand
20.	G_{20}	UD – 507 I
21.	G_{21}	RKC-55 I
22.	G_{22}	UD-503 I
`23.	G^{23}	RKS-45 I

DNA extraction: The DNA extracted from young leaves of 21-28 days old seedling of each genotypes using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990) [10]. Two gram of fresh leaves samples of each genotypes was homogenized in liquid nitrogen with the help of pestle mortar. The homogenized material was then transferred to 20 ml prewarmed extraction buffer (2 X CTAB, 100 mM Tris, 20 mM EDTA, 1.4 M NaCl, 2% CTAB (w/v) and 2 µl/ml β-mercaptoethanol) and then incubated for 1 hr at 65^0C with occasional mixing by gentle swirling in water bath. After removing from water bath equal volume of chloroform: isoamylalcohol (24:1) mixture was added and mixed by inversion for 15 minutes to ensure emulsification of the phase. Spun at 15000 rpm for 10 minutes. After centrifugation, the aqueous phase was recovered and phenol traces removed by addition of equal volume of chloroform again. Spun at 10,000 rpm for 10 minutes. Now equal volume of ice-cold isopropanol was added to aqueous phase to precipitate DNA. DNA-CTAB complex was precipitated as a fibrous network. The pellet was washed with 70% ethanol, air dried over night and resuspended in 500 µl TE buffer. RNA was digested by the addition of 25 µg RNase A per 500 µl DNA solution and incubated at 37ºC for 1 hr. The DNA samples were diluted to 25 ng/µl and kept at -20ºC until use. DNA integrity of the samples was determined by comparision with markers of known concentration by electrophoresis on 0.8% agarose gel. The quality and quantity of DNA was measured by a spectrophotometer (UV visible-UNICAM).

PCR (Polymerase chain reaction) amplification and gel analysis: A set of 20 RAPD primers were chosen for PCR amplification. The sequences of these primers were selected from literature and purchased from Bangalore Genei Pvt. Ltd., Banglore. After screening only 14 primers scorably amplified and used for this study (Table. 2). Quantity of DNA was diluted to final concentration of 12.5 ng/µl using TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The amplification was carried out in 20 µl of reaction mixture (3U *Taq* DNA polymerase, 200 µM each of dNTPs, 10 pmols/reaction of primer's, 10X assay buffer and 25 ng of template DNA). The amplification was performed in PCR machine (Thermocycler) from Eppendorf AG, Germany using the following cycling parameters: 1 initial denaturation step at 94ºC for 5 min., followed by 34 cycles at 94ºC for 1 min., annealing step at 40ºC for 1 min. and extension step at 72ºC for 1 min. and a final extension step at 72ºC for 7 min. The amplified products were then separated by gel electrophoresis on 1.2% agarose gel which was prepared with 1X TAE (Tris, Glacial acetic acid, 0.5 M EDTA, pH 8.0) buffer containing 0.5 µg/ml of ethidium bromide (EtBr). The Amplified products were electrophoresed for 3-4 hrs at 50 V with cooling. After separation, the gel was viewed under UV trans-illuminator and photographed with the help of gel documentation system (Alpha DG DOC) in order to score and preserve the banding pattern.

Scoring the PCR products: The bands were designated on the basis of their molecular size. A DNA ladder loaded simultaneously, with primer products in the gel, to estimate the molecular size. The distance run by amplified fragments from the well was translated to molecular size with reference to molecular weight markers (100bp DNA ladder and low range DNA ruler from Banglore Genei Pvt. Ltd., Banglore). The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored, but a major band corresponding to faint bands was considered for scoring.

Statistical analysis: The dendrogram generated using computer program NTSYSpc version 2.02 (Rohlf, 2004)[21] on

the basis of cluster analysis using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on Jaccard's Similarity Coefficient (Jaccard, 1908) [14]. The PIC (Polymorphic information content) value for each locus was calculated using formula (smith *et al*., 1997) [24]; PICi = 2fi (1 - fi), Where PICi is the polymorphic information content of the locus i, fi is the frequency of the amplified fragments and 1-fi is the frequency of non-amplified fragments. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions (excluding missing data). The PIC of each primer was calculated using average PIC value from all loci of each primer.

Results and Discussion

Polymorphism in *C. sativum* L. genotypes using RAPD primers

20 RAPD primers having 60% or more GC content were screened for the present investigation. Out of 20 primers, only 14 primers were amplified. The non-amplication of decamer primers were also reported Singh *et al*., (2012) [22] and Melo *et al*., (2010) [16] in different coriander genotypes. A total of 72 amplified bands were obtained out of which 48 were polymorphic. The percent polymorphism ranged from as low as 25% with primer OPB-05 to as high as 100% with primers OPA-06 and OPB-01. The average polymorphism was 64.34%. The total number of amplified bands varied between 3 (primer S-34) and 11 (primer OPB-06) with an average of 7 bands per primer. Similar results have been reported by Singh *et al.*, (2012) [22]. The overall size of PCR amplified products ranged between 300 bp to 3000 bp. Similar to present finding Singh *et al*., (2013) [23] obtained band size after amplification by RAPD varied in size from 250 to 3000 bp. The discrimination power of each locus was determined by the PIC value. It ranged from 0.041 with primer OPA-05 to 0.695 with primer OPA-08 with a mean of 0.229. The primer sequences with GC content (%), molecular weight range (bp), total number of bands amplified, polymorphic bands, percent of polymorphism (frequency %) and PIC value generated among 23 genotypes of *C. sativum* L. using RAPD primers are presented in Table 2. Three unique bands/genotype specific bands (band which is present in a particular genotype but absent in rest of the genotypes) were detected in 3 genotypes *viz*., RKD-18, RKC-20 and RCr-436 with 3 RAPD primers OPA-05, OPB-01 and OPB-07. The sizes of these unique bands ranged from 300 to 1400 bp (Table. 3). Fig. 1 display a representative picture of RAPD profile generated through RAPD primers OPB-01, OPB-06 and OPB-07.

Genetic relationship and cluster tree analysis:

The genetic similarity based on RAPD analysis was calculated using method of Jaccard's similarity coefficient analysis. The similarity coefficient matrix generated for the primers was subjected to algorithm UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and cluster was generated using NTSYSpc 2.02 program (Rohlf, 2004) [21]. Table. 4 presented the jaccard's similarity coefficient among 23 genotypes of *C. sativum* L. Similarity coefficient for all the 23 genotypes ranged from 0.63 to 0.93 with a mean of 0.78. Maximum similarity value of 0.93 was observed between genotypes RKD-18 and RKC-39 I, UD-507 I and Hisar Anand and COR-56 and RKC-28 I. Similarly, minimum similarity value of 0.63 was observed between genotypes RKC-42 I and RKC-20, RKC-42 I and RKC-53 I and RKS-45 I and COR-56. Thus the dendrogram generated on the basis of jaccard's similarity coefficient, clearly indicated distinct clusters from 23 genotypes (Fig 2). The resultant dendrogram was divided into 2 main clusters I and II at the similarity coefficient of 0.75 and 0.71 respectively. cluster I is a major cluster including 22 genotypes and it could be further divided into 2 subclusters I and II at the similarity coefficient of 0.77 and 0.85 respectively. Subcluster II included 2 genotypes *viz*., RKC-54 I and RKS-45 I. Subcluster I could be further classified into 2 subgroups I and II at the similarity coefficient of 0.78 and 0.83. Subgroup II included 2 genotypes RKC-42 I and UD-503 I. Subgroup I could be further divided into 2 subgroups A and B at the similarity coefficient of 0.79 and 0.78. Subgroup B included single genotype RKC-20. Subgroup A could be further divided into 2 subgroups C and D at the similarity coefficient of 0.81 and 0.80. Subgroup C included 8 genotypes could be further divided into 2 minor subgroups E and F at the similarity coefficient of 0.83 and 0.86. Subgroup E included 5 genotypes *viz*., RCr-436, RKC-39 I, RKD-18, RKC-44 I and COR-40. Genotype RKC-39 I and RKD-18 are closely related to each other at the similarity coefficient of 0.93 and related to RKC-44 I at the similarity coefficient of 0.87. Genotype COR-40 is related to RKC-39 I, RKD-18 and RKC-44 I at the similarity coefficient of 0.87. Genotype RCr-436 is outgrouped from rest of the genotypes at the similarity coefficient of 0.83. Subgroup F included 3 genotypes *viz*., UD-509 I, RKC-26 I and RKC-17 I. Genotype UD-509 I and RKC-26 I are related to each other at the similarity coefficient of 0.88 and related to genotype RKC-17 I at the similarity coefficient of 0.86. Subgroup D could be further classified into 2 minor subgroups G and H at the similarity coefficient of 0.81 and 0.80. Subgroup H included single genotype C0R-38. Subgroup G could be further divided into 2 minor subgroups I and J at the similarity coefficient of 0.84 and 0.81. Subgroup I included 7 genotypes *viz*., C0R-49, Hisar Anand, UD-507 I, RKC-28 I, COR-56, RKC-57 I and RKC-55 I. This subgroup could be further subdivided into 2 subgroups K and L at the similarity coefficient of 0.87. In subgroup K, genotype Hisar Anand and UD-507 I are closely related to each other at the similarity coefficient of 0.93 and related to COR-49 at the similarity coefficient of 0.87. Similarly in subgroup L genotype RKC-28 I and COR-56 are closely related to each other at the similarity coefficient of 0.93 and related to RKC-57 I at the similarity coefficient of 0.90 whereas genotype RKC-55 I is related to RKC-28 I, COR-56 and RKC-57 I at the similarity coefficient of 0.87. Genotype RKC-53-2 is outgrouped as J subgroup at the similarity coefficient of 0.81.

Genotype RKC-53 I is outgrouped from the cluster I at the similarity coefficient of 0.71 as cluster II. On the basis of similarity coefficient value the cluster tree analysis finally revealed that those genotypes which lay nearer to each other were more similar than those which were lay far apart.

Table 2: Performance of 14 RAPD primers used in molecular characterization of 23 genotypes of *C. sativum* L.

S. No.	Primer Code	Sequence 5' to 3'	GC Content %	Molecular weight range (bp)	Total no. of bands amplified (x)	Polymorphic bands		PIC
						Number	Frequency (%)	
1	OPA-02	TGCCGAGCTG	70	350-1100	4	2	50	0.156
2	OPA-03	AGTCAGCCAC	60	300-1500	5	3	60	0.176
3	OPA-04	AATCGGGCTG	60	300-1400	5	0	0	0
4	OPA-05	AGGGGTCTTG	60	300-1000	4	2	50	0.041
5	OPA-06	GGTCCCTGAC	70	300-1100	5	5	100	0.281
6	OPA-08	GTGACGTAGG	60	450-1300	4	3	75	0.695
7	OPA-09	GGGTAACGCC	70	300-3000	5	3	60	0.145
8	OPA-10	GTGATCGCAG	60	300-2750	6	4	66.66	0.216
9	OPC-05	GATGACCGCC	70	400-900	5	4	80	0.341
10	OPB-01	GTTTCGCTCC	60	400-1250	4	4	100	0.187
11	OPB-05	TGCGCCCTTC	70	600-1100	4	1	25	0.105
12	OPB-06	TGCTCTGCCC	70	450-3000	11	9	81.81	0.25
13	OPB-07	GGTGACGCAG	70	400-2000	7	6	85.71	0.308
14	S-34	TCTGTGCTGC	60	350-500	3	2	66.66	0.299
	Total				72	48	64.34	0.228

Table 3: List of unique bands as detected by 3 RAPD primers.

S. No.	Primer code	Total no. of unique bands	Genotype	Size of bands (bp)
1.	OPA-05	1	RKD-18	300
2.	OPB-01	1	RKC-20	400
3.	OPB-07	1	RCr-436	1400
	Total	3		

Table 4: Jaccard's similarity coefficient among 23 genotypes of C. *sativum* L. based on RAPD analysis.

Genotypes	RCr-436	COR-49	RKC-39 I	RKC-44 I	RKD-18	RKC-20	RKC-53 I	RKC-28 I	RKC-57 I	UD-509 I	RKC-26 I	RKC-54 I	RKC-42 I	RKC-53 II	RKC-17 I	COR-40	COR-56	COR-38	Hisar Aanand	UD-507 I	RKC-55 I	UD-503 I	RKS-45 I
RCr-436	1.00																						
COR-49	0.82	1.00																					
RKC-39 I	0.84	0.80	1.00																				
RKC-44 I	0.84	0.86	0.86	1.00																			
RKD-18	0.83	0.79	0.93	0.87	1.00																		
RKC-20	0.73	0.77	0.80	0.83	0.79	1.00																	
RKC-53 I	0.68	0.69	0.72	0.77	0.70	0.75	1.00																
RKC-28 I	0.70	0.80	0.80	0.83	0.82	0.80	0.75	1.00															
RKC-57 I	0.76	0.80	0.80	0.83	0.82	0.77	0.66	0.89	1.00														
UD-509 I	0.77	0.79	0.84	0.82	0.86	0.76	0.70	0.87	0.79	1.00													
RKC-26 I	0.76	0.71	0.80	0.77	0.79	0.69	0.69	0.77	0.80	0.87	1.00												
RKC-54 I	0.73	0.80	0.75	0.77	0.73	0.72	0.66	0.80	0.80	0.82	0.83	1.00											
RKC-42 I	0.73	0.75	0.72	0.72	0.73	0.63	0.63	0.80	0.77	0.79	0.75	0.80	1.00										
RKC-53 II	0.76	0.77	0.77	0.86	0.76	0.77	0.80	0.83	0.80	0.79	0.80	0.80	0.77	1.00									
RKC-17 I	0.79	0.83	0.83	0.83	0.82	0.77	0.66	0.77	0.77	0.87	0.83	0.80	0.69	0.75	1.00								
COR-40	0.79	0.77	0.86	0.86	0.87	0.77	0.72	0.86	0.80	0.87	0.80	0.75	0.80	0.77	0.83	1.00							
COR-56	0.75	0.79	0.79	0.84	0.83	0.79	0.70	0.93	0.90	0.80	0.73	0.76	0.76	0.79	0.76	0.87	1.00						
COR-38	0.70	0.86	0.77	0.80	0.79	0.75	0.72	0.80	0.80	0.70	0.66	0.75	0.77	0.77	0.72	0.77	0.82	1.00					
Hisar Anand	0.79	0.89	0.86	0.86	0.82	0.80	0.77	0.89	0.83	0.81	0.77	0.80	0.77	0.83	0.80	0.83	0.84	0.83	1.00				
UD-507 I	0.77	0.84	0.79	0.84	0.77	0.76	0.79	0.87	0.79	0.80	0.76	0.79	0.79	0.84	0.73	0.82	0.86	0.76	0.93	1.00			
RKC-55 I	0.79	0.80	0.77	0.83	0.79	0.77	0.69	0.86	0.86	0.79	0.80	0.80	0.77	0.83	0.80	0.83	0.87	0.77	0.89	0.84	1.00		
UD-503 I	0.73	0.77	0.75	0.77	0.79	0.69	0.75	0.83	0.83	0.79	0.72	0.72	0.83	0.77	0.75	0.83	0.82	0.83	0.80	0.79	0.80	1.00	
RKS-45 I	0.75	0.70	0.79	0.70	0.75	0.68	0.70	0.70	0.70	0.77	0.84	0.84	0.79	0.79	0.73	0.70	0.63	0.68	0.76	0.72	0.73	0.70	1.00

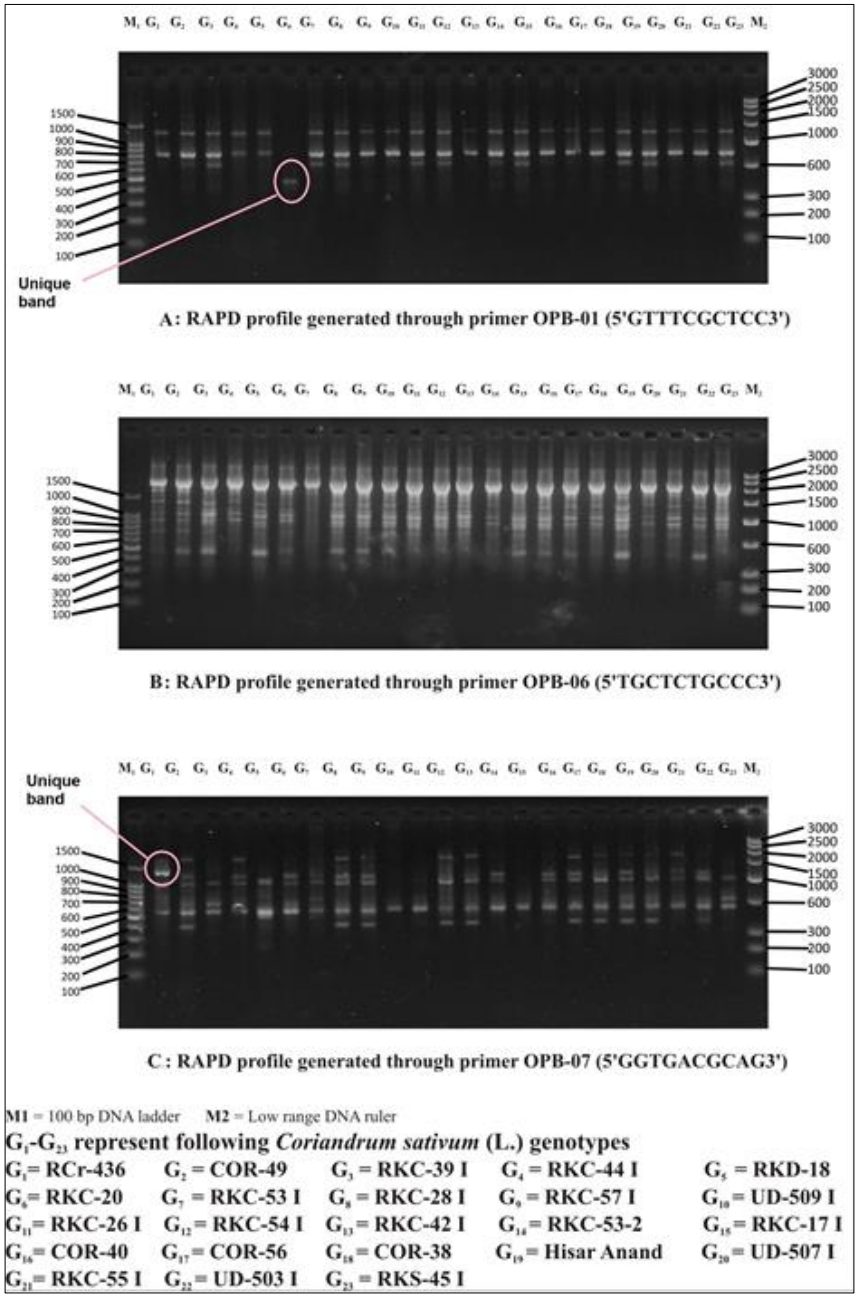

Fig 1: G1- G23 represent following Coriandrum sativum (L.) genotypes

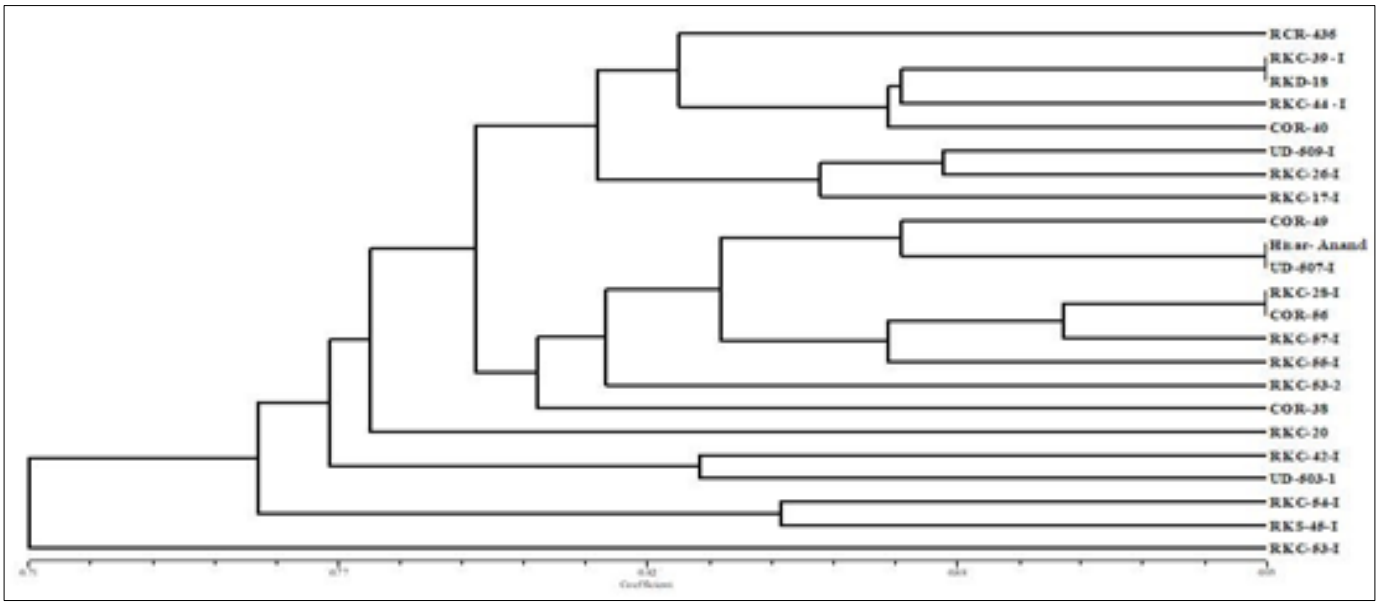

Fig 2: UPGMA dendrogram of 23 genotypes of *C. sativum* L. based on 14 RPAD primers.

Conclusion

In conclusion, based on similarity matrix dendrogram, results indicate polymorphism among most of the coriander genotypes. Hence, RAPD marker is suitably proven in characterization of coriander genotypes. 3 unique bands were detected in 3 genotypes *viz.*, RKD-18, RKC-20 and RCr-436 in our study. These unique sequences further can be cloned to get the nucleotide sequences linked to a trait of interest. These genotypes could be efficiently utilized in crop genetic improvement and breeding programs. Thus, the result of present study could be useful to facilitate the phylogenetic relationship for crop improvement and breeding programs.

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