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Research Articles

Molecular diversity in coriander (*Coriandrum sativum*) using RAPD and ISSR markers

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ABSTRACT

Coriander (*Coriandrum sativum* L.) is an annual aromatic herb that belongs to Apiaceae family. Present investigation was carried out to explore the molecular characterization in 24 coriander genotypes. For molecular characterization two DNA based molecular marker techniques, viz. random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), were used. A total of 27 polymorphic primers (18 random and 9 ISSR) were used. Amplification of genomic DNA of 24 genotypes, using RAPD analysis, yielded 83 fragments, in which 57 (70.46%) were polymorphic. The 9 ISSR primers produced 37 bands across 24 genotypes, of which 17 (49.81%) were polymorphic. Based on the similarity matrix data dendrogram were prepared using UPGMA method. Genotypes were also classified into groups and several subgroups. Principal coordinate analysis (PCA) confirmed the separation of coriander genotypes into groups comparable to those from UPGMA analysis. The high rate of polymorphic lines generated by RAPD and ISSR markers indicated that the method is efficient to analyze molecular diversity in coriander genotypes and that the molecular divergence can be used to establish consistent heterotic groups between coriander genotypes.

Key words: Coriander, ISSR, Molecular diversity, Molecular marker, Polymorphism, RAPD

Coriander (*Coriandrum sativum* L.) is an annual aromatic herb that belongs to Apiaceae family with a wide variety of uses. Coriander has long been cultivated in the Mediterranean region, Southern Europe, Asia and the Caucasus. The fresh green herb and a dry spice are the two main products obtained from coriander plants besides steam distilled oil and solvent extracted oleo-resin for the aroma and flavour industry (Islam *et al.* 2009, Msaada *et al.* 2009) and a good source of phenolics and flavinoids content (Choudhary *et al.*, 2017). The data from RAPD analysis have indicated greater genetic diversity than allozymes in plant species (Esselman *et al.* 2000, Esselman *et al.* 1999). RAPD uses 10-base pair primer to amplify the random portion of genome.

Being an open cross-pollinated crop and high rate of out crossing with the abundant molecular variability, tremendous scope for development of improved varieties and characterization of germplasm. The methods based on morphological features are commonly used but they not always allow the most accurate information due to genotypes-environment interaction; it is well reported that molecular methods overcome these problems. Since not much molecular information is available in literature for coriander crop using molecular markers, thus RAPD and ISSR marker have been used with success to identify and determine relationships at the species, population and cultivar levels in many plant species, including several aromatic and medicinal plants (Haouari and Ferchichi, 2008). These methods are widely applicable because they are rapid, inexpensive, require small amounts of template DNA and, unlike SSR markers, do not require prior designing of primer sequences (Godwin *et al.* 1997). RAPD and ISSR markers have been efficiently used for the study of molecular diversity among different medicinal plant species and various agricultural crops such as *Achillea millefolium* (Farajpour *et al.* 2012), Fenugreek (Choudhary *et al.* 2013), cumin (Choudhary *et al.* 2015) etc.

MATERIALS AND METHODS

Twenty four diverse coriander genotypes from nine different geographical regions of the India (Table 1) were used for diversity analysis. The seeds were procured from Gene Bank, ICAR-NRCSS, Tabiji, Ajmer (Rajasthan), India. The present study was conducted at Biotechnology Laboratory at ICAR-National Research Center on Seed Spices, Tabiji, Ajmer (Rajasthan), India.

PCR amplification was carried out by using master cycler gradient thermal cycler (Bio Rad C1000^{TM}). Amplification was carried out in 20 µl reaction volume containing 1x Taq polymerase buffer, 2.5 mM magnesium chloride 2.0 µl, 200 µM each dNTP, 200 µM primer, 1 unit of the Taq DNA polymerase enzyme and 50 ng of template

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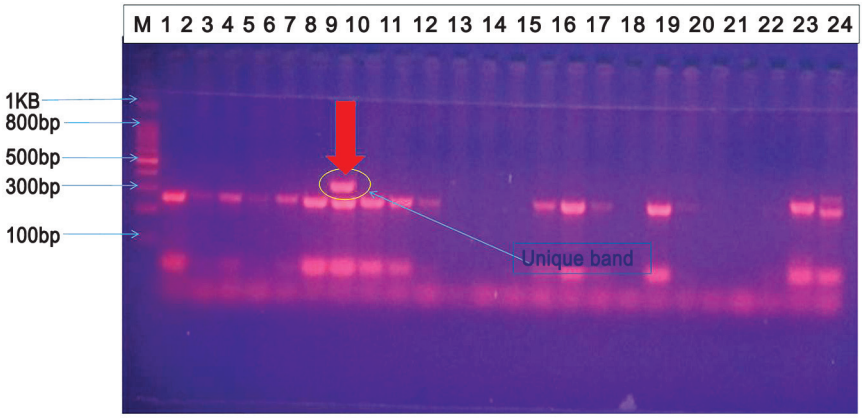
Table 1 Twenty Four diverse coriander genotypes and their origin

Code	Genotypes	Origin/Sources	Pedigree/Parentage	Latitude and Longitude
V1	GCR-1	SDAU, Jagudan (Gujarat)	Selection from germplasm	23° 51' N, 72° 41'E
V2	GCr-2	SDAU, Jagudan (Gujarat)	Reselection from Co.2	23° 51' N, 72° 41'E
V3	RCr-41	SKRAU, Jobner (Rajasthan)	Recurrent half sib selection from local type from "Kota"	26° 97' N, 75° 38'E
V4	RCr-20	SKRAU, Jobner (Rajasthan)	Recurrent half sib election from Jaipur local	26° 97' N, 75° 38'E
V5	RCr-435	SKRAU, Jobner (Rajasthan)	Recurrent selection from local germplasm from Jalore	26° 97' N, 75° 38'E
V6	RCr-436	SKRAU, Jobner (Rajasthan)	Recurrent half sib selection from local germplasm from Kota	26° 97' N, 75° 38'E
V7	RCr-446	SKRAU, Jobner (Rajasthan)	Half sib selection from local type from Jaipur local	26° 97' N, 75° 38'E
V8	RCr-684	SKRAU, Jobner (Rajasthan)	Mutation breeding of gamma rays. Induced mutant of Rcr-20	26° 97' N, 75° 38'E
V9	Hisar Sugandh	CCHAU, Hisar (Haryana)	Mass selection from indigenous germplasm	29° 19' N, 76° 23'E
V10	Hisar Anand	CCCHAU, Hisar (Haryana)	Mass selection from Haryana collection	29° 19' N, 76° 23'E
V11	Hisar Surabhi	CCHAU, Hisar (Haryana)	Mass selection from local germplasm	29° 19' N, 76° 23'E
V12	Rajendra Swati	RAU, Dholi(Bihar)	Pureline selection from Muzaffarpur collection	25° 85' N, 85° 78'E
V13	Azad Dhaniya-1	CSAUAT, Kanpur (UP)	Mass selection from Kalyanpur germplasm collection	26° 50' N, 80° 30'E
V14	CO-1	TAU, Coimbatore (TN)	Selection from Koilpatti local	11° 01' N, 76° 97'E
V15	CO-2	TAU, Coimbatore (TN)	Reselection from culture P2 of Gujarat	11° 01' N, 76° 97'E
V16	CO-3	TAU, Coimbatore (TN)	Reselection from Acc.695 of IARI, New Delhi type	11° 01' N, 76° 97'E
V17	CO-4	TAU, Coimbatore (TN)	Reselection from germplasm ATP77 guntur collection	11° 01' N, 76° 97'E
V18	Pant Haritima	GBPUAT, Pantnagar (UK)	Selection from local type Pant Dhania	28° 97' N, 79° 41'E
V19	JD-1	JNKVV, Jabalpur (MP)	Local collection from Gwalior	23° 10' N, 79° 59' E
V20	ACr-1	NRCSS, Ajmer (Rajasthan)	Reselection from EC-467683 from Russia	26° 45' N, 74° 64'E
V21	Swati	APAU, Guntur (AP)	Mass selection from Nandyal germplasm	16° 18' N, 80° 29' E
V22	Sadhana	APAU, Guntur (AP)	Mass selection from local Alur collection	16° 18' N, 80° 29' E
V23	Sindhu	APAU, Guntur (AP)	Mass selection germplasm,Warangal local	16° 18' N, 80° 29' E
V24	Sudha	APAU, Guntur (AP)	Reselection from Guntur collection	16° 18' N, 80° 29' E

DNA. Thermal cycler with an initial denaturation at 94°C for 5 min. followed by 32 cycles was done. Each cycle consisted of denaturation at 94°C for one minutes, primer annealing at 37°C for 1 min, extension at 72°C for 2 min, with final extension at 72°C for 10 min. PCR products were separated on 1% agrose gel in 1x TAE buffer using ethidium bromide stain. The size of amplified fragments was determined by using size standard (100bp DNA ladder). DNA fragments were visualized under UV light and photographed using gel documentation system.

DNA fingerprints were scored for the presence (1) or absence (0) of bands for various molecular weight and sizes in the form of binary matrix. Initially, the potential of both the markers for estimating molecular variability of coriander genotypes was examined by measuring the marker information counting the bands. Primer banding patterns such as number of total bands (TB), number of polymorphic bands (PB) and percentage of polymorphic bands (PPB) were obtained. To analyze the suitability of both the markers for evaluation of molecular profiles of coriander genotypes, the performance of the markers was measured using two basic parameters: polymorphic information content (PIC) and marker index (MI). The PIC value for each locus was calculated using formula (Roldan-Ruiz *et al.* 2000); PIC$i = 2fi\ (1 - fi)$, 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. Effective multiplex ratio was calculated using formula; EMR (effective multiplex ratio) = n × b, where n is the average number of fragments



1: Gcr-1, 2: Gcr-2, 3: Rcr-41, 4: Rcr-20, 5 5: Rcr-435,: 6: Rcr-436, 7: Rcr-446, 8: Rcr-684, 9: Hissar Sugandh, 10: Hisar Anand, 11: Hisar Surbhi, 12: Rajendra-Swati,13: Azad Dhaniya, 14: CO-1, 15: CO-2, 16:Co-3, 17:CO-4, 18: Pant Haritima, 19: JD-1, 20: Acr-1, 21: Swati, 22: Sadhana, 23: Sindhu, 24: Sudha

Fig 1 RAPD banding pattern generated through OPB series of primers. *Arrow indicate putative genotype specific band

amplified by accession to a specific system marker (multiplex ratio) and b is estimated from the number of polymorphic loci (PB) and the number of nonpolymorphic loci (MB); b = PB/(PB+MB). Marker index for both the markers was calculated to characterize the capacity of each primer to detect polymorphic loci among the genotypes. Marker index for each primer was calculated as a product of polymorphic information content and effective multiplex ratio (Varshney *et al.* 2007); MI = EMR × PIC. Data were analyzed to obtain Jaccard's coefficients (Jaccard, 1908) among the isolates by using NTSYS-pc version 2.02e (Rohlf, 1998). The data matrix of both markers was then converted into molecular similarity matrix using Jaccard coefficient (Jaccard 1908) in SPSS 17.0 (SPSS Inc.) and NTSYS-PC 2.02j (Rohlf 1998). The data matrix was used to determine the molecular diversity, molecular differentiation and gene flow. Further, principal component analysis (PCA) was performed to highlight the resolving power of the ordination based on similarity coefficient of data realized from RAPD and ISSR average similarity indices using SPSS statistics 17.0 software (SPSS Inc.).

RESULTS AND DISCUSSION

RAPD band pattern

Information on molecular diversity and relationship among individuals, population, plant varieties and species are important to plant breeders for the improvement of crop plants. Molecular diversity studies can identify alleles

Table 2 Performance of 18 RAPD primers in the molecular diversity analysis of coriander genotypes

Primer*	Sequence 5' to 3'	G:C (%)	A : T (%)	Size (bp)	TGA	TB	PB	MB	PP	PIC	ß	EMR	MI
OPB-01	GTTTCGCTCC	60	33	200-1400	24	5	4	1	80	0.48	0.80	3.2	1.53
OPB-02	TGATCCCTGG	60	32.2	300-700	24	3	2	1	66.66	0.63	0.66	1.32	0.83
OPB-03	CATCCCCCT	70	35.1	80-1850	24	7	1	6	14.28	0.25	0.14	0.14	0.03
OPB-04	GGACTGGAGT	60	32.2	170-1200	24	5	4	1	80	0.38	0.8	3.2	1.21
OPB-05	TGCGCCCTTC	70	41.1	250-1000	24	4	3	1	75	0.75	0.75	2.25	1.68
OPB-06	TGCTCTGCCC	70	39.8	300-800	24	6	5	1	83.33	0.54	0.83	4.15	2.24
OPB-07	GGTGACGCAG	70	38.1	150-1200	24	3	3	0	100	0.80	1	3	2.4
OPB-08	GGACCCTTAC	60	29.7	200-1600	24	4	3	1	75	0.37	0.75	2.25	0.83
OPB-09	TGGGGGACTC	70	37	250-1600	24	3	3	0	100	0.38	1	3	1.14
OPB-10	CTGCTGGGAC	70	36.6	400-800	24	7	6	1	85.71	0.36	0.85	5.1	1.83
OPB-12	CCTTGACGCA	60	35.7	300-600	24	4	1	3	25	0.42	0.25	0.25	0.10
OPB-13	TTCCCCCGCT	70	41.8	300-1100	24	6	4	2	66.66	0.56	0.66	2.64	1.47
OPB-14	TCCGCTCTGG	70	38.8	400-1700	24	3	2	1	66.66	0.56	0.66	1.32	0.73
OPB-15	GGAGGGTGTT	60	33.2	200-1800	24	5	4	1	80	0.76	0.80	3.2	2.43
OPB-16	TTTGCCCGGA	60	38	200-1600	24	4	4	0	100	0.63	1	4	2.52
OPB-17	AAGGAACGAG	50	33.1	80-1050	24	5	3	2	60	0.68	0.60	31.8	21.6
OPB-18	CCACAGCAGT	60	34.3	200-900	24	4	2	2	50	0.35	0.50	1	0.35
OPB-20	GGACCCTTAC	60	29.7	400-1000	24	5	3	2	60	0.37	0.60	1.8	0.66
Average						83	57	26	70.46	0.51	0.70	4.09	2.42

*Operon series code, TGA=Total Number of Genotype Amplified, TB=Total Number of bands, PB=Polymorphic bands, MB=Monomorpic bands, PP=Percent polymorphism, PIC=Polymorphic information content, EMR=Effective multiplex ratio, MI=Marker Index

that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification 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). Thirty RAPD primers having more than 60% GC content were used for the present investigation. Out of them only eighteen primers (Table 2) were polymorphic and reproducible. All these 18 primers resulted in the amplification of 83 amplified bands from which 57 were polymorphic and showed 70.46% polymorphism indicating the presence of high degree of molecular variation in the studied coriander varieties. The DNA amplicon size and polymorphism generated among various genotypes of coriander using RAPD primers are presented in Table 2. The total number of bands observed for every primer was recorded separately and polymorphic bands were checked subsequently. The total number of amplified bands varied between 3 (primer OPB-02, OPC-09 and OPB-14) and 7 (primer OPB-3 and OPB-10) with an average of 4.6 bands per primer. The polymorphism of all 24 coriander genotype were 70.46% and the overall size of PCR amplified products ranged from 80 bp to 1850 bp. Similar to the present finding Choudhary *et al.* (2013) obtained high level of polymorphism of 57.66% among Indian fenugreek varieties. Based on RAPD similarity matrix data, the value of similarity coefficient ranged from 0.48 to 0.97. The average similarity across all the genotypes was found be 0.72 showing that genotype were polymorphic genetically. PIC is a feature of a primer and, therefore, PIC values were calculated for all the primers. Maximum, minimum and average values of Polymorphism information content (PIC) index were found to be 0.80, 0.35 and 0.51, respectively (Table 2). Since the average value of PIC (0.51) showed a good efficiency of the used primers in discrimination of the individuals. Although the low PIC value obtained by some RAPD markers may be only due to low number of RAPD loci studied. Similar results have been reported by other workers (Pirseyedi *et al.* 2010, Soriano *et al.* 2011). Marker index (MI) as a feature of marker diversity was also calculated for all the primers based on the PIC and polymorphic bands are showed in Table 2. Average MI values were 2.42 in 24 genotypes of coriander.

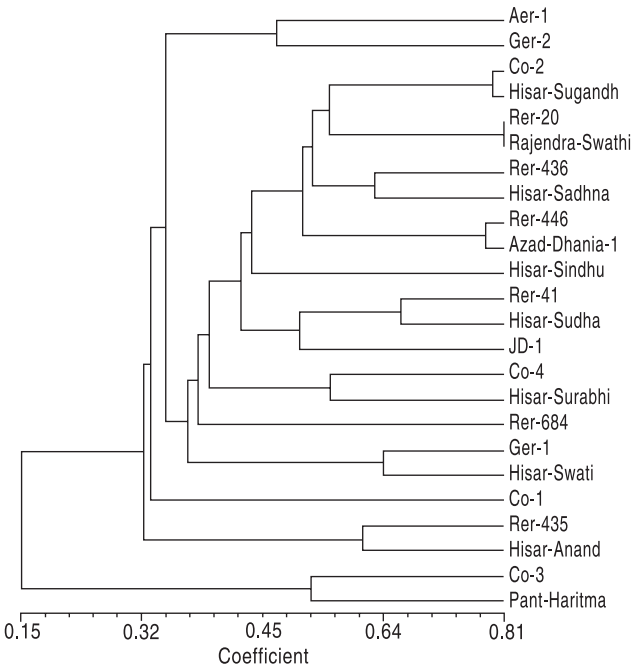


Fig 2 Dendrogram constructed with UPGMA clustering method of 24 genotypes of Coriander using RAPD primers.

The RAPD cluster tree analysis of 24 coriander genotypes showed that they were mainly divided into main four clusters (Fig 2). Cluster I contains two genotypes, in which first main group contain Co-3 and Pant haritima. Cluster II also having two genotypes with diverse origin and different geographical distribution, includes, Rcr-435 and Hisar anand. These genotypes are out grouped from

Table 3 Performance of 9 ISSR primers in the molecular diversity analysis of coriander genotypes

Primer*	Sequence 5' to 3'	GC (%)	AT (%)	Size (bp)	TGA	TB	PB	MB	PP	PIC	ß	EMR	MI
UBC807	AGAGAGAGAGAGAGAGT	47	47.0	200-1400	24	5	3	2	60	0.50	0.6	1.8	0.90
UBC808	AGAGAGAGAGAGAGAGC	53	48.8	300-700	24	2	1	1	50	0.67	0.5	0.5	0.33
UBC810	GAGAGAGAGAGAGAGAC	53	45.5	80-2150	24	4	1	3	25	0.67	0.25	0.25	0.16
UBC814	CTCTCTCTCTCTCTCTA	47	44.7	170-1200	24	4	1	3	25	0.29	0.25	0.25	0.07
UBC816	CACACACACACACACAT	47	50.1	250-1000	24	5	4	1	80	0.15	0.8	3.2	0.48
UBC864	ATGATGATGATGATGATG	33	46.6	300-800	24	2	2	0	100	0.64	1	2	1.28
UBC873	GACAGACAGACAGACA	50	43.4	150-1200	24	6	2	4	33.33	0.37	3	6	2.22
UBC880	GGAGAGGAGAGGAGA	60	44.7	200-1600	24	5	0	5	0	0.56	0	0	0
UBC851	GTGTGTGTGTGTGTGTYG	50	48	250-1600	24	4	3	1	75	0.27	0.75	2.25	0.60
Average						37	17	20	49.81	0.45	0.79	16.25	0.67

*Operon series code, TGA=Total Number of Genotype Amplified, TB=Total Number of bands, PB=Polymorphic bands, MB=Monomorpic bands, PP=Percent polymorphism, PIC, EMR=Effective multiplex ratio, MI=Marker Index

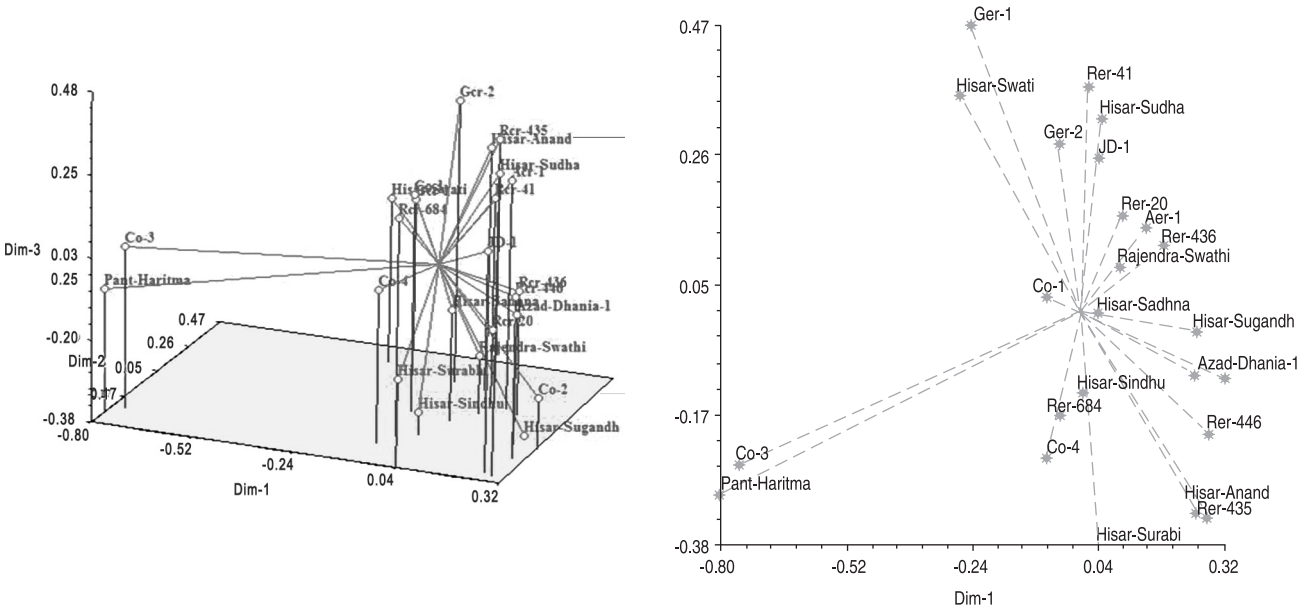


Fig 3 Two and three dimensional PCA analysis using RAPD markers.

rest of all genotypes at a similarity coefficient of 0.32. In cluster III only one genotype was present, viz. Co-1 and out grouped at a similarity coefficient of 0.35.

Cluster IV is biggest cluster having 19 genotypes with many sub group. Rcr-20 and Rajendra swati are the most similar varieties and Acr-1 and Gc-2 are creating a subgroup with in cluster IV although these both genotypes are very much diverse from each other. The clusters presented by the coriander varieties are not following the pattern of geographical distribution.

ISSR band pattern

9 ISSR primers amplified 37 clear and scorable bands across 24 coriander genotypes (Table 3). The total number of amplified bands varied between 2 (primer-UBC-808 and UBC-864) and 6 (UBC-873) with an average of 4.1 per primer. The polymorphism percentage ranged from as low as 0% (UBC-880) to as high as 100% in primers (UBC-864). Average polymorphism across all the genotypes was found to be 49.81% showing abundant molecular diversity at the population level. Overall size of PCR amplified products ranged from 100bp to 2150bp. Maximum, minimum and average values of Polymorphism information content index (PIC) were found to be 0.67, 0.15 and 0.45, respectively (Table 3). Since the average value of PIC (0.45) showed a good efficiency of the used primers in discrimination of the individuals. Although the low PIC value obtained by some ISSR markers maybe only due to low number of ISSR loci studied. Similar results have been reported by other workers (Pirseyedi *et al.* 2010, Soriano *et al.* 2011). Marker index (MI) as a feature of marker diversity was also calculated ranged from 0 to 2.22 with an average value 0.67. Highest MI (2.22) was observed with primer UBC-873 that generated 2 polymorphic fragments across all the genotypes (Table 3).

The ISSR cluster analysis of 24 genotype showed that they were mainly divided into four major clusters at similarity coefficient of 0.56 (Fig 4). The cluster I contain one genotype Co-1 and out grouped from other at a similarity coefficient 0.56. Cluster II contain four genotypes viz.

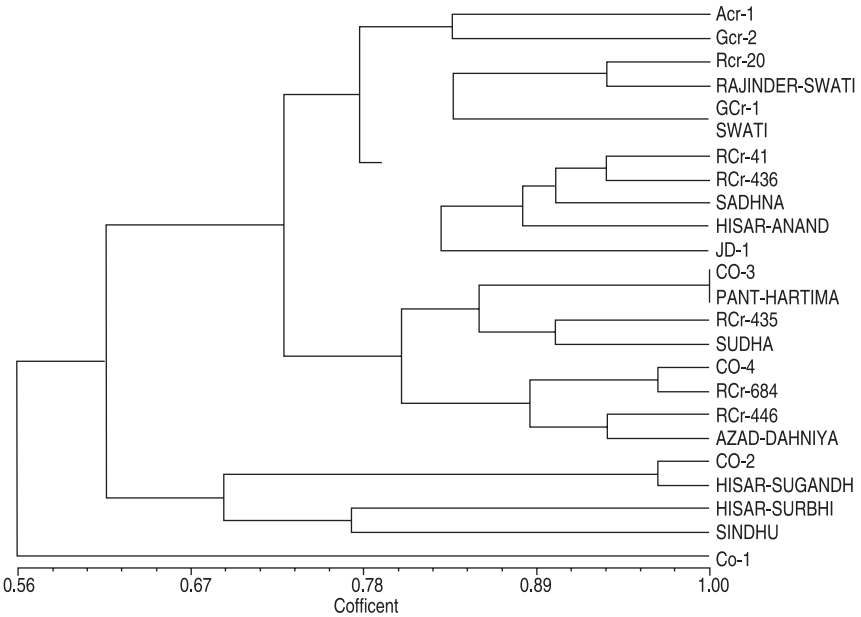


Fig 4 Dendrogram constructed with UPGMA clustering method of 24 genotypes of Coriander using ISSR primers.

Sindhu, Hisar surbhi, Hisar sughandh and Co-2 at similarity coefficient 0.61. Cluster III containing eight genotypes at similarity coefficient of 0.72. These genotypes belongs to diverse origin and geographical distribution, includes Azad dhaniya, Rcr-446, Rcr-684, Co-4, Sudha, Rcr-453, Pant Haritima and Co-3. Cluster IV having eleven genotypes *i.e*, JD-1, Hisar anad, Sadhna, Rcr-436, Rcr-41, Swati, Gcr-1, Rajendra swati, Rcr-20, Gcr-2, Acr-1. In principal component analysis, 24 genotypes were also grouped into 4 groups as shown in two and three dimensional graph (Fig 3).

Unique bands in 24 coriander genotypes

One primer OPB-16 generated a unique band in a specific coriander genotype Rcr-446 with 550 bp size. This primer produced a specific DNA band which distinguished this genotype from the rest (Fig 1).

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