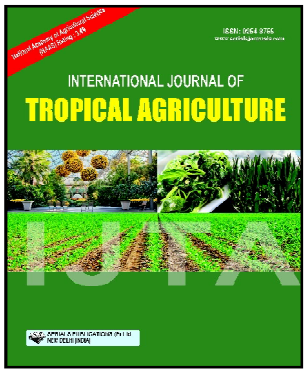

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Genetic Relationship among Coriander Genotypes and Cluster Analysis Based on Simple Sequence Repeats (SSR)

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Abstract: Coriander (*Coriandrum sativum* L.) is grown as seed spice crop all over the world. (*Coriandrum sativum* L.) also called cilantro, or dhania (in Hindi) is an annual herbaceous crop (Ghobadi *et al.*, 2012). Present investigation was carried out to explore the molecular characterization in 24 coriander genotypes using Simple Sequence Repeats. Very limited literature is available for molecular studies in coriander it's a need to develop molecular markers for this crop. Development of SSRs (Simple Sequence Repeats) for new crop like coriander is an expensive and time consuming process, but this can be easily achieved by transferring the microsatellite loci from the same family/genus/species which is a cost-effective approach for developing microsatellite markers for new species. Presently carrot and celery SSRs are available on the public domain for cross-species investigation; both crops are from the same family, *Apiaceae*.

Key words: Coriander, SSR, Molecular marker, Molecular diversity, Polymorphism

INTRODUCTION

Coriander is belongs to the *Apiaceae* family, formerly known as *Umbeliferaeae*. It is also known as the carrot family and has many important members, such as anise, asafetida, caraway, celery, dill, fennel, and parsley. It is a native of Mediterranean and commercially produced in India, Morocco, Russia, East European countries, France, Central America, Mexico, and USA. Coriander is very popular vegetable species, but limited molecular and genomic data hinder the research on it. In addition, a lack of coriander molecular markers limits the process of molecular genetics breeding. Till date very limited literature is available for molecular studies in coriander. A very popular and informative marker SSR is still lacking for coriander crop. Development of a new microsatellite (SSR) marker system for new species is very expensive and requires isolation,

cloning, sequencing and characterization of microsatellite loci. SSR become more widespread if the loci of SSR are transferrable across species even in closely related genera (Rai *et al.* 2013). Cross species transferability is a quick and economic method to enrich SSR databases, particularly for minor crop where little genomic information is available (Satya *et al.* 2016). The impact of evolutionary relationship between source and target species on SSR transfer success have been widely observed in many plant families (Rossetto *et al.*, 2001; Arnold *et al.*, 2002). Here we have used cross species transferable SSRs used by (Choudhary *et al.*, 2017). Therefore, the aim of this study is to identify and characterize coriander genotypes using SSR markers and to estimate the genetic relationship among due genotypes.

MATERIALS AND METHODS

Plant material: Twenty four (24) diverse coriander genotypes developed from nine different geographical regions of the India (Table 1) were used for diversity analysis. The seeds were procured from Gene Bank, ICAR-National Research Center on Seed Spices, Tabiji, Ajmer (Rajasthan), India. Seeds were grown, in pots and kept in seed germinator with controlled conditions, after 20 days of growth; leaves were cut and frozen in liquid nitrogen for DNA extraction. The present study was conducted in Biotechnology Laboratory at ICAR-National Research Center on Seed Spices, Tabiji, Ajmer (Rajasthan), India.

PCR Amplification with Microsatellites and Data Analysis

Hundred microsatellite loci previously developed for carrot by Cavagnaro *et. al.* (2011) were tested for cross amplification in coriander species. PCR reactions (20 µl) were conducted in C1000™ Thermal cycler (BIO-RAD) containing 12.8 µl water, 2 µl 10 × DNA polymerase buffer, 2 µl dNTPs (2 mM each), 0.5 µl of each primer at 100 µM, 0.2 µl Taq Polymerase at 5 u/µl (SRL, biolit™) and 2 µl of genomic DNA. Thermal cycling conditions were as follows: initial denaturation at 94°C (4 min), followed by 40 cycle of appropriate primer annealing temperature (1.0 min), elongation at 72°C (1.0 min), and denaturation at 94°C (20 sec). A final elongation step was allowed at 72°C for 10.0 min.

Electrophoresis was carried out for 3 hours at 150 V on 1.5% high-resolution agarose. TAE gels supplemented with 5 ul (5.0 mg/ml) of ethidium bromide for each 100 ml of TAE.

For each microsatellite locus, sizes of the allele were estimated by comparison with standard DNA marker (100bp, SRL Biolit™) and were scored across all the samples manually. Amplification of products was scored as positive only if sharp and reproducible band was observed.

RESULTS AND DISCUSSION

Simple sequence repeats (SSRs) Markers analysis

All 24 varieties of coriander cultivars were examined for DNA polymorphism using 30 SSR primers showing high (G+C) content. All 30 primers were screened for amplification. Out of 30 primers 20 primers produced amplification whereas 10 primers did not show any amplification. Again out 20, only 2 primers showed variable degree of monomorphism Viz., GSSR-10 and GSSR-139 with 75 and 66.66% respectively. Remaining all other primers shows 100% polymorphism. A total of 49 amplification products were generated, out of which 47 bands were polymorphic across 24 coriander genotypes.

PIC value 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 index (PIC) were found to be 0.75, 0.00 and 0.35, respectively. Since the average value of PIC (0.35) showed a good efficiency of the used primers in discrimination of the individuals. Although the low PIC value obtained

Table 1
Twenty diverse Coriander genotypes and their origin

Code No.	*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	HisarSugandh	CCHAU-Hisar (Haryana)	Mass selection from indigenous germplasm	29° 19′N, 76° 23′E
V10	HisarAnand	CCCHAU-Hisar (Haryana)	Mass selection from Haryana collection	29° 19′N, 76° 23′E
V11	HisarSurabhi	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

Table 2
Details of SSRs Primers used in present study

S. No.	*PrimerName*	*Sequence (5'-3')*	*Mol.wt.*	*GC content*	T_M	*OD/260*	*Yield(µg)*
1.	GSSR-4	5'CAATCTTGCCACTAAAAGAGCA	6696.5	40.9	51.1	17.3	432.1
		5'CAGATACAATAGACAGGAAACATCG					
2.	GSSR-10	5'CTTAGTAGTAGCACACACCAGACG	7330.9	50	57.4	17.2	451.3
		5'GAGCTGAACGAGTCAGAAAGG					
3.	GSSR-16	5'ATGCAAACGACAATATCCACAG	6705.5	40.9	51.1	18	440
		5'GCCCAGCCACTTCCTAGAT					
4.	GSSR-40	5'TAGAAGCTCCAACAAATCACCC	6641.5	45.5	53	16.2	391.5
		5'CAAGGAACCCTAGATCACAAATG					
5.	GSSR-43	5'TTCTTCACCTATGTTGGGGC	6090.1	50	51.8	18	547
6.	GSSR-87	5'CCAACAACCATCCAACAAACTA	6594.5	40.9	51.1	16.5	371.2
		5'AGTCGTCCGATAAGCGAATCTA					
7.	GSSR-92	5'AGAAGGCATCGTGTGTTCATAA	6798.5	40.9	51.1	15.3	427.9
		5'CAACGGTGATTAAGTGGGTTCT					
8.	GSSR-96	5'AGCGTCGTTTTCGCGAGT	5521.7	55.6	50.3	14.5	450.3
		5'CGCGGTTAAAGCAAAGCTAAT					
9.	GSSR-107	5'TTCTGGTCTTTTGACATGAAGG	6771.5	40.9	51.1	16.8	506.7
		5'CGGATTTGAGGTGAGTTGAATA					
10.	GSSR-111	5'GAGGAAGGGTAGATCCAGTCA	6544.4	52.4	54.4	16.6	471.3
		5'ATGGGATGTCTTTCCCCTCAT					
11.	GSSR-112	5'TCTTGTYYAAGAAMACCACA	6057.1	37.5	46.7	14.9	374.7
		5'GTCCACACCAAGTATGCTC					
12.	GSSR-113	5'AGTGGTTGTGAGGTTGATTGTG	6907.5	45.5	53	18.1	612.9
		5'TATGTCGGAAAGGTTCAATGCT					
13.	GSSR-131	5'AAATTACTGGAGATGGAGCGAG	6872.6	45.5	53	16.5	465.9
		5'GTTTGTTGATTCGGACTTTGTG					

contd. table 2

S. No.	*PrimerName*	*Sequence (5'-3')*	*Mol.wt.*	*GC content*	T_M	*OD/260*	*Yield(µg)*
14.	GSSR-138	5'CGCTCGAGTTTCGTAGAGT	5834.9	52.6	51.1	18.1	546.6
		5'CCTCCCAACTCAATCCAAT					
15.	GSSR-139	5'GCAAGTGTTTCGTGACATGC	6148.1	50	51.8	16.4	483.4
		5'AACATGAGTTAATCGAAGGGGA					
16.	GSSR-140	5'GGATACGAAGGAAAGACTCCAC	6786.6	50	54.8	16.6	432.5
		5'AGGAGAGTAAAAGATTGAGGACTTG					
17.	GSSR-143	5'GGGAAGAACTAAAACCAACACA	6763.6	40.9	51.1	16.3	390.3
		5'TCAGTAAATCAGGAGTGCAGAA					
18.	GSSR-149	5'TGAAGCAACTCGTGATACAGAGA	7105.8	43.5	53.5	17.4	462.7
		5'TTCTCTTGTCCTGGTTAGCTC					
19.	GSSR-154	5'CTTATATGTGATGGCGTCGAAA	6789.5	40.9	51.1	18.1	518.7
		5'GACTGCACCGCTCCTAACTC					
20.	BSSR-8	5'TGAAGCTAATATCCAACAAAGGAAA	7978.3	38.5	54.8	17	453.6
		5'AGGAGCATGTTATGCTATTACCAACA					

by some SSR markers may be only due to low number of SSR loci presented. Similar results have been reported by other workers (Pirseyedi *et al.*, 2010)

The effective multiplex ratio depends on the fraction of polymorphic fragments (b). In this study, the highest effective multiplex ratio (EMR) 6.00 was observed with the primer GSSR-111 and lowest EMR is 1 with an average EMR of 2.27 per primer. To determine the general usefulness of the system of markers used, the MI (marker index) for each SSR primer was calculated. Marker index (MI) as a feature of marker diversity was also calculated for all the primers based on the PIC and polymorphic bands. MI value ranged from 0 to 12.01 with an average value 6.00. Highest MI (12.01) was observed with primer GSSR-113 that generated 3 polymorphic fragments across all the 24 genotypes of coriander.

Genetic Relationship among Coriander Genotypes and Cluster Analysis Based on Simple Sequence Repeats (SSR)

The dendrogram generated on the basis of Jaccard's Similarity Coefficient, clearly indicated eight main clusters. The result suggested the level of genetic diversity.

Cluster-I containing 8 genotypes i.e Gcr-1, Gcr-2,Rcr-41, Co-3, Rcr-435, Rcr-446 and Pant Haritima this cluster has 2 subclusters. Sub cluster first have genotypes Gcr-2, Rcr-20, Rcr-20 and Co-3 are closely related. Among these four genotypes Rcr-41 and Rcr-20 originated from SKNRAU, Jobner (Raj.), through recurrent half sib selection from local type.

Cluster-II containing Rcr-435, Rcr-446 and Pant Haritima, from which Rcr-435 and Rcr-446 both are originated from similar place and environment, hence they are closely related and comes in this small subcluster. Cluster second separated at a similarity coefficient at 0.52. Total 5 genotypes comes in this cluster i.e HisarAnand, Swati, Sudha, JD-1 and Rajendra Swathi. Second subgroup also has single genotype *i.e* JD-1 while subgroup third having three genotypes, among these Sudha and Swati are very closely related which are originated from similar place (Andhra Pradesh, Guntur).

Cluster-III containing only a single genotype (Azad Dhaniya) at a similarity coefficient of 0.45. This genotype is belong to diverse origin and geographical distribution. Cluster four also has a single genotype i.eHisarSurabhi at a similarity coefficient 40. This genotype form a separate cluster and it may be probably due to selection of local germplasm from Hisar (Haryana). Cluste-V containing 2 genotypes at a similarity coefficient of 0.30. This cluster includes Acr-1 and HisarSugandh. Both genotypes are highly diverse and originated from different ecological conditions and these genotypes do not shows an ecogenetic relationships with other clusters. Cluster-VI and VII both having single genotypes i.e. Rcr-436 and Sindhu respectively, they are originated from diverse places and heterogenous in nature.

Cluster-VIII includes 5 genotypes at a similarity matrix of 0.25 with 2 subcluster. Subcluster first contain genotype Rcr-684 and subcluster second having Co-1, Co-4 and Sadhana. In this cluster genotype Co-2 is outgrouped from rest all 23 genotypes and shows highly diverse, heterogenous and genetically variable. Hence this can be used for further crop improvement program.

The banding pattern generated and polymorphic patterns was used to calculate the genetic similarity among the 24 coriandergenotypes taken for the present study. Genetic similarity estimates based on SSR banding patterns were calculated using method of Jaccard'scoefficient analysis. The similarity coefficient matrix generated for the primers was subjected to algorithm UPGMA (Unweighted Pair Group Method with Arithmetic averages) and dendrogram (Fig. 1) was generated using NTSYS-pc 2.02 programme (Rohlf, 1998).

The SSR data were used to obtain a similarity matrix. The similarity coefficient for different

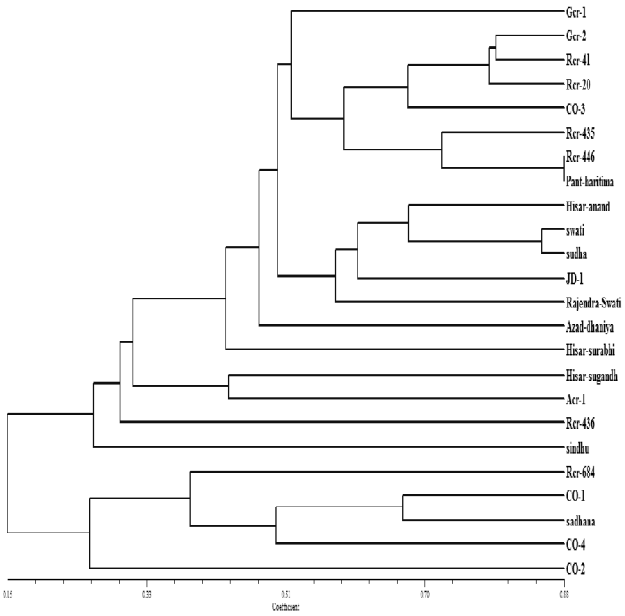

Figure 1: Dendogram generated using SSR primers in 24 coriander genotypes

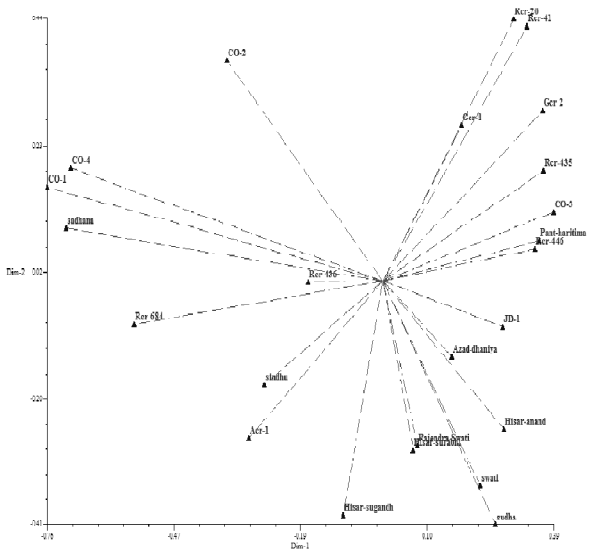

Figure 2: Three dimensional PCA (Principle Component Analysis) scaling of 24 genotypes of coriander using SSR markers

genotypes was in the range of 0.05 to 0.88. The average similarity across all the genotypes was found to be 0.38 indicating an average genetic similarity among the genotypes. This indicated a narrow genetic base of tested cultivars. The maximum similarity coefficient (0.88) was observed between Pant Haritima and Rcr-446. The minimum similarity coefficient (0.05) was observed between Co-1 and Rcr-435 followed by Sadhana and RCr-41.

Similar results were found in the study of genetic diversity in jute by Zhang *et al.* (2014) in which similarity coefficients ranged from 0.520 to 0.910 with an average of 0.749, indicating relatively great genetic diversity among jute genotypes. Singh *et al.* (2015) also found a significant genetic variation ranging between 0.60 and 0.98 among the genotypes of Turmeric.

4.3.1. Principal Component Analysis (PCA)

Three dimensional principal component analysis based on SSR data (Fig. 2) showed similar clustering pattern of 24 genotypes as evident from cluster tree analysis. Most of the genotypes were in eight major cluster. The cluster first having maximum genotypes having two minor clusters included total eight genotypes.

SUMMARY AND CONCLUSION

The Simple Sequence Repeats (SSRs) analysis showed broad genetic diversity. Total 49 fragments were amplified in all the twenty four genotypes; polymorphic fragment were obtained with 20 primers with average 2.45 fragments per primer. Out of 49 bands 47 bands were found to be polymorphic and the level of average polymorphism was 97.08%. Out of 30 primers 20 primers produced amplification whereas 10 primers did not show any amplification. Again out 20, only 2 primers showed variable degree of polymorphism ranging from 66.66 per cent (GSSR-139) to 100 per cent in 18 primers.

Dendrogram generated through SSR marker showed Similarity Coefficient value in range of 0.05 to 0.88. The dendrogram clearly divided the 24 genotypes into 8 main clusters. Cluster I included 8 genotype, cluster II included 5 genotype, cluster III included 1 genotype, cluster IV included 1 genotypes, cluster V included 2 genotypes, cluster VI included

1 genotypes, cluster VII included 1 genotypes and cluster VIII included 5 genotypes respectively.

Two and three dimensional principal component analysis based on SSRs showed similar clustering pattern of 24 genotypes as evident from cluster tree analysis. Most of the genotypes were in eight major cluster. The cluster first having maximum genotypes having two minor clusters included total eight genotypes.

On the basis of molecular studies (SSR), Co-1 appeared superior, genetically diverse and promising; hence could be gainfully utilized.

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