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Analysis of diversity among cumin (*Cuminum cyminum*) cultivars using RAPD markers

SHARDA CHOUDHARY[1], R S MEENA[2], R SINGH[3], M K VISHAL[4], GEETIKA JETHRA[5], MAMTA SAINI[6] and ALKA PANWAR[7]

National Research Centre on Seed Spices, Tabiji, Ajmer, Rajsthan 305 206

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ABSTRACT

Cumin (*Cuminum cyminum*) is a valuable seed spice belonging to the family apiaceae. It is one of the oldest and economically most important seed spice with medicinal properties. In the present study, experiments were conducted to assess the genetic diversity among Indian cumin, accessions by using RAPD molecular markers. After DNA extraction, complement gene locuses were amplified by 20 RAPD primers, out of which only 15 primers showed amplification. These primers produced 1191 bands, of which 218 bands (about 18.30%) were polymorphic. This indicates very low level of genetic diversity among genotypes. Cluster analysis was performed using UPGMA method and Dice's similarity coefficient by NTSYS software. The resulting dendrogram categorized the accessions into three groups showing 53% similarity. Principle Component Analysis was performed and 3 dimensions graphs using 15 primers and 55 genotypes were drawn.

Key words: Cumin, DNA, Genetic diversity, Polymorphism, RAPD, Seed Spice

The genetic diversity among different genotypes can be assessed using morphological, biochemical and molecular markers but morphological and biochemical traits have some confines as they are influenced by environmental factors and the developmental stages of the plants. Inspite of large potential, however, very less reports on genetic diversity using molecular markers is available in cumin (*Cuminum cyminum* L. 2n = 2x = 14). The present study was therefore aimed to focus on understanding the genetic relationship among different accession of cumin, which may further assist in developing and planning breeding strategies for crop improvement programs. Among the various molecular marker techniques, Random Amplified Polymorphic DNA (RAPD) is relatively quick, inexpensive and require no prior information of target genome. Applying DNA molecular markers, for assessment of genetic variation in plants have shown advantages over other markers based on the phenotype; they are neutral, not related to age and tissue type, not influenced by the environmental conditions, feasibility, low cost and are more informative than morphological markers (Da Mata *et al.* 2009). Among all the kinds of DNA markers, RAPD are the most popular markers based on polymerase chain reaction (PCR). Polymorphism detected by RAPD markers has proven to be useful for unraveling of genetic diversity and relationship in several medicinal plant species, viz. *Rhodiola* (Gupta *et al.* 2012.) and fenugreek (Choudhary *et al.* 2013). Presence or absence of DNA bands in the gel are used as RAPD markers to study close genetic relationship (Alam *et al.* 2009), inter and intra-specific genetic variations (Ahmad *et al.* 2010). These PCR based markers reveal variation at DNA level, those variations that are obtained from the huge extent of genetic polymorphism generated by these markers, leading to evaluation of phenotypic variability. It is a dominant class of marker useful for cultivar identification.

The objectives of this study were to identify and characterize fifty five cumin accessions (Table 1) using RAPDs and to estimate the genetic relationships between the selected accessions.

MATERIALS AND METHODS

Since off season germination of cumin is very difficult and as its seeds have high amounts of essence and secondary metabolites (phenolic, polysaccharide and aldehydic ingredients), therefore DNA extraction is very difficult so to avoid this problem, DNA was isolated for the seeds using CTAB method with slight modification.

50 ng genomic DNA was taken to carryout PCR using 0.5 IU Taq DNA polymerase, and 1x reaction buffer supplied with the enzyme (containing 1.5 mM of $MgCl_2$) in a 25 ml reaction volume using MJ PTC 100 thermal cycler (MJ Research Inc., Waltham, MA, USA). The programme

[1]Scientist (email id: shardaajmer@yahoo.com), [2]Scientist (email id: rsm.nrcss@gmail.com), [3]Scientist (email id: mahla_rs@yahoo.com), [4]Scientist (email id: mukeshvishalrns@gmail.com) , [5]Young Professional II (email id: gjethra08@gmail.com), [6]email id: sainimamta318@gmail. com, [7]Senior Research Fellow (email id: simiran.saini@rediffmail.com)

Table 1 Showing details of cumin genotypes

Cumin genotype	Place	Latitude and longitude
RZ 19	Jobner, Rajasthan	26° 582 123 N, 75° 222 483 E
RZ 209	Jobner, Rajasthan	26° 582 123 N, 75° 222 483 E
RZ 223	Jobner, Rajasthan	26° 582 123 N, 75° 222 483 E
RZ3 41	Jobner, Rajasthan	26° 582 123 N, 75° 222 483 E
GC 4	Jagudan, Gujarat	230.40N 720.30E
GC 2	Jagudan, Gujarat	230.40N 720.30E
EC 637344	Jodhpur, Rajasthan	26° 162 483 N, 73° 12 123 E
EC 243375	Jodhpur, Rajasthan	26° 162 483 N, 73° 12 123 E
JC 2002-8	Jagudan, Gujarat	230.40N 720.30E
JC 2002-14	Jagudan, Gujarat	230.40N 720.30E
JC 2002-17	Jagudan, Gujarat	230.40N 720.30E
JC 2002-23	Jagudan, Gujarat	230.40N 720.30E
JC 2002-28	Jagudan, Gujarat	230.40N 720.30E
JC 2002-32	Jagudan, Gujarat	230.40N 720.30E
C.CUMIN 09-4	Barmer, Rajasthan	70° 492 483 N, 72° 522 123 E
C.CUMIN 09-I-2	Jaisalmer, Rajasthan	26° 552 123 N, 70° 542 03 E
C.CUMIN 09-E-3	Jalor, Rajasthan	25° 212 03 N, 72° 372 123 E
C.CUMIN 09-Y-4	Dhorimanna, Barmer	70° 492 483 N, 72° 522 123 E
EC 279081	Jodhpur, Rajasthan	26° 162 483 N, 73° 12 123 E
EC 232684	Jodhpur, Rajasthan	26° 162 483 N, 73° 12 123 E
UC 341	Udaipur, Rajasthan	24° 342 483 N, 73° 402 483 E
UC 346	Udaipur, Rajasthan	24° 342 483 N, 73° 402 483 E
JC 2000-68	Jagudan, Gujarat	230.40N 720.30E
JC 2002-31	Jagudan, Gujarat	230.40N 720.30E
JC 2000-65	Patan, Gujarat	23° 492 483 N, 72° 72 123 E
GKKB 178	Kachchh	23° 542 543 N, 70° 222 1.23 E
JC 2000-20	Jagudan, Gujarat	230.40N 720.30E
EC 243373	Jagudan, Gujarat	230.40N 720.30E
JC 2002-37	Jagudan, Gujarat	230.40N 720.30E
JC 2002-28	Jagudan, Gujarat	230.40N 720.30E
JC 2000-14	Jagudan, Gujarat	230.40N 720.30E

involved initial denaturation at 94 °C for 4 min, followed by 32 cycles of 94 °C for 1 min, 1.5 min at annealing temperature (depending on RAPDs primers used, Table 2) and 72 °C for 2 min. This was followed by a final extension step at 72 °C for 10 min.

Ten micro liters of loading buffer was added into each amplified sample and mixed well. 15 ml of each amplified sample was loaded on 1.5 % agarose gel in 1 × TBE buffer

Table 2 Showing details of 15 primers and their amplification

Primer	Sequence (5'-3)	ÁT in °C	GC%	Alleles amplified	PIC value
OPB 02	TGATCCCTGG	32.2	60%	43	0.37
OPB 03	CATCCCCCT	35.1	70%	47	0.37
OPB 04	GGACTGGAGT	32.2	60%	40	0.37
OPB 05	TGCGCCCTTC	41.1	70%	109	0.76
OPB 06	TGCTCTGCCC	39.8	70%	168	0.86
OPB 07	GGTGACGCAG	38.1	70%	130	0.86
OPB 10	CTGCTGGGAC	36.6	70%	49	0.37
OPB 11	GTAGACCCGT	32.6	60%	101	0.75
OPB 12	CCTTGACGCA	35.7	60%	71	0.57
OPB 13	TTCCCCCGCT	41.8	70%	52	0.37
OPB 14	TCCGCTCTGG	38.8	70%	67	0.58
OPB 15	GGAGGGTGTT	33.2	60%	156	0.83
OPB 16	TTTGCCCGGA	38.0	60%	50	0.59
OPB 17	AAGGAACGAG	33.1	50%	57	0.57
OPB 18	CCACAGCAGT	34.3	60%	51	0.37

to separate the amplified fragments. The electrophoresis was done for 1 and ½ hours at 80 volts. 5ml of molecular weight markers (1 bp, 100 bp DNA ladder) were also loaded to compare the molecular weight of amplified products. After electrophoresis, gel was observed and visualized under UV and photographs of gel were taken with the help of Gel- Documentation system.

DNA amplifications with each RAPD primer were repeated at least twice to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in two separate amplifications for each primer.

DNA fragment sizes were observed by photograph directly. Total number of bands within each line and number of polymorphic bands were noted. RAPDs behave as a dominant marker, it tend to be bistate (present or absent) type of scoring. Each DNA fragment amplified by a given primer was considered as a unit character and the RAPD fragments were scored as a binary variable (one) for presence and (zero) for absence of each of the primer accession combination. Since DNA sample consisted of bulk sample of DNA extracted from individual plants, a low intensity of any particular fragment may be explained by the lesser representation of that specific sequence in the bulk sample of DNA.

Therefore, the intensity of bands was not taken into account and the fragments with the identical mobility were considered but those identical fragments were scored which had only major bands and faint bands were not considered. The presence or absence of polymorphic and non-polymorphic bands was scored in a binary data matrix. The data was further analyzed by deriving the PIC values and by making Dendrogram using NT-sys software.

Polymorphic Information Content (PIC) was calculated using the formula: (Yuan *et al.* 2011)

$$PIC = 1 - \sum_{i=1}^{k} p_i^2$$

RESULTS AND DISCUSSION

Molecular markers are useful complementary tool for morphological and physiological characterization of plants because they have many advantages like they are plentiful, independent of environmental effects and help in early cultivar identification during plant development (Manifesto *et al.* 2001). In the present study, genetic similarity was assessed among fifty five cumin accessions, analysis of the data using 20 RAPD primers showed that 15 primers generated bright and reproducible amplified products which detected polymorphism among the accessions used. Fifteen RAPD primers showed clear bands and polymorphism on profiling (Table 3). Amplification conditions with selected primers, yielding good and clear patterns were standardized. Genetic similarity matrix was calculated on the basis of Jaccard's. Fifteen polymorphic primers generated a total of 1191 bright, reproducible and scorable amplification fragments across all the accessions. Out of the 1 191 bands obtained, 218 were polymorphic, with (18.30%) polymorphism. Some of the primers were able to show a high level of genetic diversity while others produced a little variability. The number of RAPD bands detected by each primer depends on primers, sequence and extent of variation in specific genotype (Shiran *et al.* 2007) therefore the number of bands varied in different accession. The number of bands generated varied from 3 to 8 with an average of 5.2 bands per primer.

Cluster analysis resulted in a classification of fifty five accessions into four clusters (clusters I, clusters II, clusters III and clusters IV) in 13 distance unit (Fig 1). Dendogram based on similarity coefficient showed that the genotype EC 637344 formed separate operational taxonomy unit (OUTs). Remaining accessions were grouped into three clusters showing 15-53% similarity with each other. Cluster I is the biggest cluster containing 28 genotype; RZ 19, JC 2002-17, JC- 95-93, JC 2002-31, RZ 341, C.CUMIN 09-E-3, JC 2000-11, GC 4, JC 2002-37, JC 95-11, GC 2, JC 2000-71, EC 243375, C.CUMIN 09-1-2, JC 2000-9, JC 2002-32, JC 95-77, EC 232684, EC 244375, EC 243373, EC 279069, JC 2000-68, JC 2000-65, JC 2000-3, JC 2000-4, C.CUMIN 07-08-5, JC 2000-44 and UC 243. Genotype RZ 223, JC 2002-23, UC 346, JC 2002-14, C.CUMIN 083, 13JC 2002-28, JC 2000-17, JC 95-104, JC 2000-14, EC-279081, JC 2000-20, C.CUMIN 0850, JC 2000-72 and C.CUMIN 09SI were falling in cluster II, 12 genotypes were falling into cluster III namely RZ 19, UC 341, GKKB 178, JC 2002-8, JC 95-102, C.CUMIN 09-4, JC 95-106, JC 95-112, JC 95-78, JC 2002-28, C.CUMIN 08-44 and C.CUMIN 09-Y-4.

The primers OPB 6 and 7 gave maximum Polymorphic Information Content (PIC) with PIC value 86 followed by PIC value 83 in primer OPB 15. Primers OPB 2, 3, 4, 10, 13 and 18 showed only 37 PIC value. Number of bands obtained per primer indicated the efficiency of the primers to characterize the germplasm. The Polymorphism Information Content values varies from 37 to 86 with an average of 57.26 (Table 2). Genetic similarity matrix was

Table 3 Genetic similarity matrix calculated on the basis of Jaccards algorithm for RAPD data

Cumin acessions	I	L
RZ 19	1	0.486
RZ 209	11	0.422
RZ 223	55	0.365
RZ3 41	24	0.325
GC 4	4	0.529
GC 2	17	0.425
EC 637344	33	0.352
EC 243375	5	0.397
JC 2002-8	29	0.500
JC 2002-14	50	0.290
JC 2002-17	6	0.424
JC 2002-23	39	0.288
13JC 2002-28	8	0.459
JC 2002-32	16	0.384
C.CUMIN 09-4	42	0.374
C.CUMIN 09-I-2	14	0.419
C.CUMIN 09-E-3	44	0.328
C.CUMIN 09-Y-4	20	0.471
EC 279081	54	0.396
EC 232684	28	0.457
UC 341	37	0.266
UC 346	23	0.531
JC 2000-68	25	0.363
JC 2002-31	38	0.327
JC 2000-65	34	0.287
GKKB 178	43	0.249
JC 2000-20	40	0.296
EC 243373	41	0.238
JC 2002-37	3	0.515
JC 2002-28	12	0.350
JC 2000-14	22	0.300
JC 2000-72	10	0.333
JC 2000-11	52	0.262
JC 2000-4	13	0.438
JC 2000-17	35	0.381
C.CUMIN 09S1	48	0.315
EC 279069	31	0.256
JC 2000-3	19	0.289
JC 2000-71	27	0.400
JC 2000-44	51	0.338
UC 243	32	0.484
JC 2000-9	36	0.226
C.CUMIN 07-08-5	2	0.448
JC 95-77	21	0.430
C.CUMIN 08-44	26	0.309
JC 95-106	9	0.444
JC 95-112	49	0.374
JC 95-104	15	0.260
JC 95-102	46	0.321
JC 95-11	47	0.211
C.CUMIN 0850	53	0.192
C.CUMIN 08-3	30	0.231
JC 95-78	45	0.150
EC 244375	18	
JC 95-93	7	

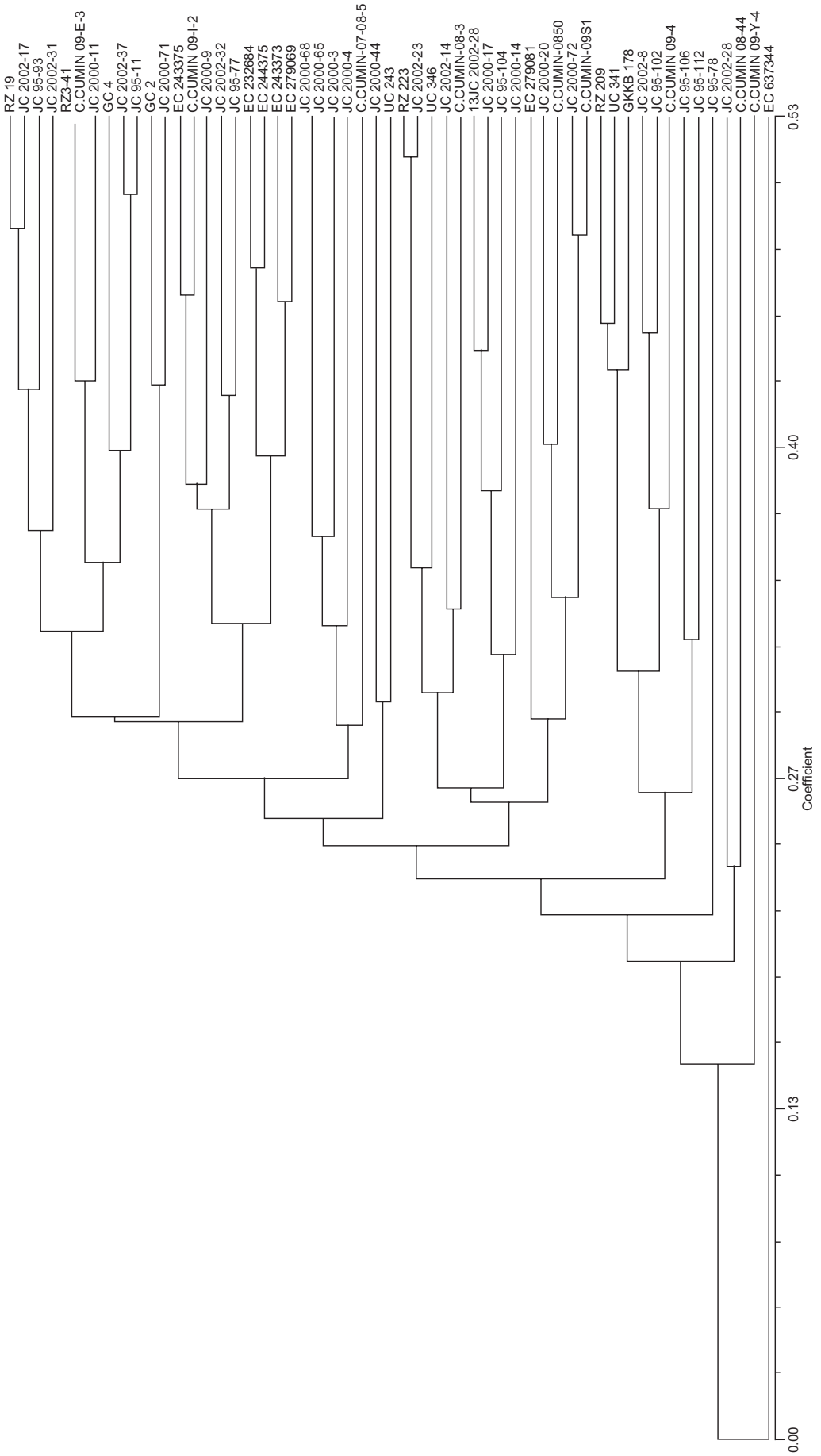

Fig 1 Dendogram showing Cluster analysis

calculated on the basis of Jaccard's algorithm for RAPD data. The pair wise similarity values ranged from 0 to 0.531 (Table 3). Maximum similarity was observed between genotypes JC 2000-68 and JC 2000-65 (53%). The wide range of similarity indicated the presence of high genetic diversity in Indian cumin varieties.

The cluster analysis was conducted by the software NTSYS-pc version 2.00 (Rohlf 1998). Similarity matrices based on Jaccard's similarity coefficient was used to construct a 3D plot and a line graph based on unweighted pair group method with arithmetic average (UPGMA) (Fig 2 a, b). Among 55 genotypes of cumin the similarity value ranges from 0 to 0.53 which exhibits very less genetic diversity among them. The polymorphic pattern observed in the different cumin genotypes and the extent of information generated from RAPD analysis varied with the primers. The observation of molecular data reveals significant diversity among genotypes (Marinello *et al.* 2002). RAPD and ISSR has been extensively used for the identification of either species or cultivars in a wide range of plants (Ahmad *et al.* 2012). The contradiction cases between DNA-based and morphology based classification also has been acknowledged in many plants (Vinod *et al.* 2007). To make sure the meticulousness of evaluation, only variables with strong genetic control should be quantified in morphological trait analysis and other more effective molecular markers such as AFLP, SSR or ISSR should be used (Wang and Bao 2005). The varieties with diverse pattern for RAPD are suggested to be used for advance study and to select parents for inheritance or linkage groups (Prakash *et al.* 2012).

According to RAPD molecular characterization, done in this study the geographical origin of specimens has no influence on the clusters obtained and seems to favor the hypothesis of existence of a common origin. All the accessions were falling into four clusters showing very narrow diversity

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