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GENETIC DIVERSITY ANALYSIS IN CHILLI (*CAPSICUM ANNUUM* L.) USING RAPD MARKERS

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ABSTRACT
Genetic diversity in 23 chilli genotypes was analyzed by RAPD markers of the 13 random primers used 9 were polymorphic. The amplification profile of these 9 primers consisted of 63 fragments used with size ranging from 391 bp to 2754 bp of which 35 were monomorphic and 28 were polymorphic with 44.4 % polymorphism. The number of bands generated by each primer varied from 5 (OPB-10) to 9 (OPB-01 and OPB-15) with an average of 7 fragments per primer. The percentage of polymorphic bands with different primers ranged from 14.29 to 66.67 %. The similarity coefficients detected by RAPD marker ranged from 0.42 to 0.97 which revealed existence of genetic variation among chilli genotypes. The consensus tree constructed showed three major clusters. First cluster comprised of 13 genotypes, second cluster of 7 genotypes and third cluster of 3 genotypes. The RAPD technology proved useful in describing genetic diversity among chilli genotypes.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a member of the *Solanaceae* family, originated from South and Central America. It is one of the most important spice crop worldwide, with a global production 30.71 lakh tonnes and 20.378 lakh ha area harvested, in 2010-11. In India chilli ranked first in spice crops in terms of production (12.23 lakh tonnes) and area harvested (7.92 lakh ha), in the year 2010-11 (FAOSTAT, 2012). Chilli is grown for its pungent fruits, which are used both green and ripe/dry form. Chilli is becoming an important crop worldwide due to its wide diversity and high quality in flavor, concentration of vitamins and other antioxidants (Ashrafi *et al.,* 2012).

Information regarding genetic diversity and genetic relationships among different genotypes is very valuable in crop improvement. Morphological characteristics, is often difficult, since most of these characteristics are under the influence of environmental factors. Molecular markers are useful for the genetic identification, estimating the genetic diversity among plant genotypes and allow more reliable differentiation of genotypes. Genetic diversity analysis and varietal identification in chilli was carried out using isozymes (Litoriya *et al.,* 2010); RAPD (Bhadragoudar and Patil, 2011, Thul *et al.,* 2012); AFLP (Lafebvre *et al.,* 2001; Ibiza *et al.,* 2012); SSR (Ibiza *et al.,* 2012) and ISSR markers (Thul *et al.,* 2012)

Analysis of genetic diversity is useful in selecting diverse parental combinations, reliable classification of accessions, and for exact identification of variety. Germplasm characterization is important for conservation and utilization of plant genetic resources (Thul *et al.,* 2012). RAPD markers have been extensively used for the diversity analysis in crop plants due to its simplicity and efficiency even without the prior knowledge of sequence information. Therefore, objective of the present study was to assess the genetic diversity amongst 23 chilli genotypes and utilize in the breeding programme for genotypes improvement.

MATERIAL AND METHOD

Plant materials

The seeds of 23 Chilli (*Capsicum annuum* L.) genotypes were obtained from Chilli and Vegetable Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Table 1). Seeds were surface sterilized with 1 % (w/v) mercuric chloride for 5 min followed by washing with 70 % (v/v) ethanol. The seeds were further rinsed five times with sterile distilled water and then germinated on germination paper in germinator.

Genomic DNA extraction

Genomic DNA has been extracted from young leaves of 14 days old chilli plants by the following CTAB-based protocol. About 2 g of each leaf sample was crushed in a mortar and pestle in the presence of liquid nitrogen to obtain fine powder. The powder was transferred to 50 ml centrifuge tube containing prewarmed 15 ml of extraction buffer (100 mM Tris–HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2 % w/v CTAB and 140 mM β-ME) and mixed by inversion. Then the tubes were kept in thermostatic water bath at 65°C for 60 min. After centrifugation (10 min, 5,000 rpm), the supernatant was transferred to a clean tube and the genomic DNA was purified by two successive extraction with phenol: chloroform: isoamyl

alcohol (25:24:1; v: v: v). The genomic DNA was then precipitated with ice-cold isopropanol by centrifugation (10,000 rpm, 10 min) followed by two washing steps with chilled 70 % ethanol (v/v). DNA pellet was air dried and re-dissolved in 100 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0).

RAPD analysis

RAPD analysis was performed using 13 random decamer primers [OPB series (01-04,06-08, 10-12, 14, 15, 17) purchased from Operon Technologies (Alameda, CA). Amplification reactions contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 100 μM of each dNTPs, 0.400 μM of each RAPD primer, 30 ng template DNA, and 1 unit *Taq* DNA polymerase in a reaction volume of 25 μl. After a pre-denaturation step of 5 min at 94°C, amplification reactions were cycled 45 times at 94°C for 30 sec, 40°C for 1 min and 72°C for 2 min. A final amplification was allowed for 10 min at 72°C. Upon completion of the amplification, aliquots of 20 μl of amplification products were then resolved in 1.2 % agarose gel. Gel was stained with ethidium-bromide and visualized under UV-transilluminator. Size of the amplicons was estimated with 1 kb DNA ladder which was resolved along with amplified product. Reproducibility of the results was confirmed by repeating the amplification twice.

The clearly resolved PCR amplified RAPD bands with 9 polymorphic primers were scored manually for their presence (1) and absence (0) in the binary data sheet. Data was analyzed and similarity matrix was constructed from binary data with dice similarity coefficients which was calculated as per model suggested by Nei and Li (1979). Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) was employed for cluster analysis using the computer package NTSYSpc 2.02i (Rohlf, 1998).

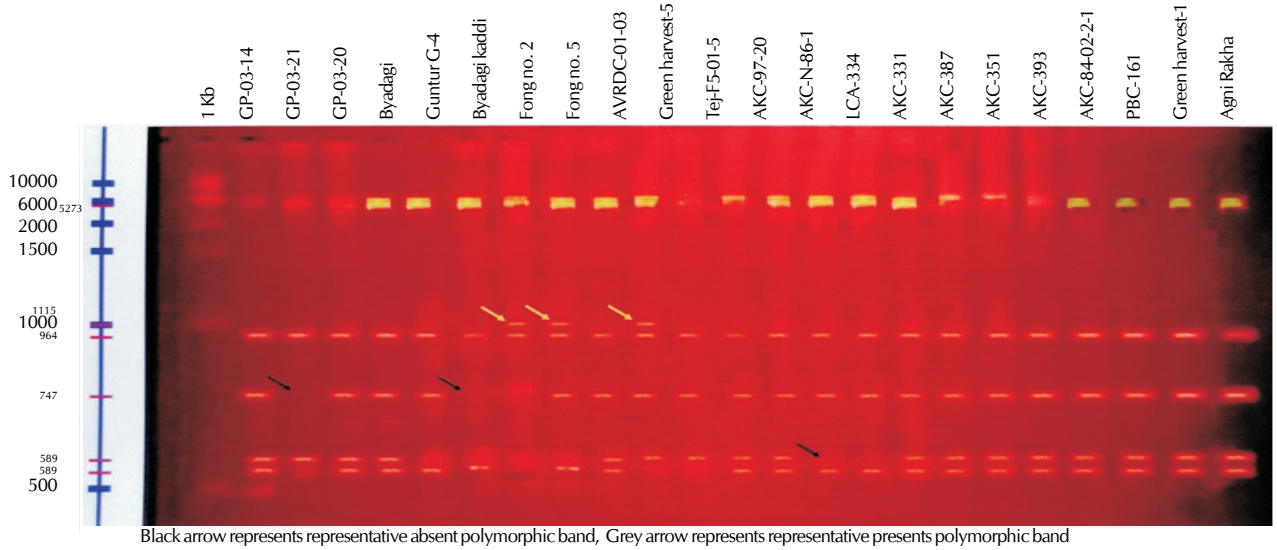
RESULTS AND DISCUSSION

Phenotypic descriptors do not always allow the quantification of the genotypic difference or similarities between cultivars as do genetic distances based on DNA polymorphism (Lafebvre *et al.*, 2001). RAPD markers have been widely used to quantify genetic variation and mapping studies in chilli (Ince *et al.*, 2010, Bhadragoudar and Patil, 2011 and Thul *et al.*, 2012). Multi-band amplification profile obtained in RAPD analysis is useful in solving ''pattern recognition'' problems, like the clustering of different varieties at intraspecies level. Then, the defined clusters can be assigned and correlated to specific groups and characteristics. RAPD markers are technically simple and have good throughput with relatively low cost.

Table 1: List of random decamer primers, their sequences, and details of amplified fragment

Sr. No.	Random primer	Sequence of Primers (5′ to 3′)	T	M	P	%P	Fragmentsize (bp)
1	OPB-01	GTTTCGCTCC	09	04	05	55.56	391-2754
2	OPB-04	GGACTGGAGT	07	03	04	57.14	430-1279
3	OPB-05	TGCGCCCTTC	07	03	04	57.14	750-2611
4	OPB-07	GGTGACGCAG	07	06	01	14.29	574-1121
5	OPB-08	GTCCACACGG	06	04	02	33.33	459-1321
6	OPB-10	CTGCTGGGAC	05	03	02	40.00	500-1629
7	OPB-12	CCTTGACGCA	06	02	04	66.67	569-1115
8	OPB-14	TCCGCTCTGG	07	04	03	42.85	534-1595
9	OPB-15	GGAGGGTGTT	09	06	03	33.33	624-1593
Total			63	35	28	44.44	391-2754
Average			7	3.89	3.11		

T = Total number of bands; M = Monomorphic bands; P = Polymorphic bands; %P = Percent polymorphic bands



Black arrow represents representative absent polymorphic band, Grey arrow represents representative presents polymorphic band

Fig 1. RAPD amplification (OPB-12) profile of 23 chilli genotypes. M = marker (1 kb), Lanes 1-23 are chilli genotypes

Table 2: Jaccard's similarity coefficient of 23 chilli genotypes based on RAPD data analysis

	GP-03-14	GP-03-21	GP-03-20	Byadagi	Guntur G-4	Byadagi kaddi	Fong No.2	Fong No.5	AVRDC-01-03	Green harvest-5	Tej-F5-01-5	AKC-97-20	AKC-98-27	AKC-N-86-1	LCA-334	AKC-331	AKC-387	AKC-351	AKC-393	AKC-84-02-2-1	PBC-161	Green harvest 1	Agni Rekha
	1.00																						
GP-03-21	0.65	1.00																					
GP-03-20	0.77	0.68	1.00																				
Byadagi	0.68	0.90	0.65	1.00																			
Guntur G-4	0.87	0.58	0.71	0.61	1.00																		
Byadagi kaddi	0.81	0.65	0.71	0.61	0.81	1.00																	
Fong No.2	0.68	0.45	0.58	0.48	0.81	0.74	1.00																
Fong No.5	0.90	0.55	0.74	0.58	0.84	0.84	0.77	1.00															
AVRDC-01-03	0.87	0.52	0.71	0.55	0.81	0.81	0.81	0.90	1.00														
Green harvest-5	0.77	0.55	0.68	0.52	0.71	0.77	0.71	0.87	0.84	1.00													
Tej-F5-01-5	0.81	0.58	0.65	0.55	0.74	0.68	0.68	0.77	0.81	0.77	1.00												
AKC-97-20	0.87	0.65	0.84	0.68	0.81	0.81	0.68	0.90	0.87	0.77	0.74	1.00											
AKC-98-27	0.77	0.55	0.68	0.58	0.71	0.71	0.65	0.81	0.77	0.74	0.84	0.84	1.00										
AKC-N-86-1	0.71	0.42	0.55	0.45	0.65	0.71	0.65	0.74	0.84	0.81	0.77	0.71	0.74	1.00									
LCA-334	0.77	0.48	0.55	0.52	0.77	0.65	0.58	0.74	0.71	0.68	0.71	0.71	0.68	0.68	1.00								
AKC-331	0.81	0.52	0.65	0.55	0.87	0.74	0.81	0.77	0.87	0.77	0.81	0.74	0.71	0.77	0.71	1.00							
AKC387	0.77	0.55	0.61	0.58	0.77	0.71	0.71	0.74	0.77	0.68	0.90	0.71	0.81	0.74	0.68	0.84	1.00						
AKC-351	0.81	0.52	0.65	0.55	0.81	0.74	0.74	0.77	0.87	0.77	0.87	0.74	0.77	0.84	0.71	0.94	0.90	1.00					
AKC-393	0.84	0.55	0.68	0.58	0.84	0.77	0.74	0.81	0.84	0.74	0.90	0.77	0.81	0.81	0.74	0.90	0.94	0.97	1.00				
AKC-84-02-2-1	0.87	0.52	0.71	0.55	0.81	0.81	0.74	0.90	0.94	0.77	0.81	0.87	0.77	0.77	0.71	0.81	0.77	0.81	0.84	1.00			
PBC-161	0.71	0.55	0.74	0.58	0.71	0.71	0.65	0.74	0.77	0.68	0.71	0.84	0.74	0.74	0.61	0.77	0.74	0.77	0.81	0.84	1.00		
Green harvest 1	0.71	0.87	0.68	0.97	0.65	0.65	0.52	0.61	0.58	0.55	0.58	0.71	0.61	0.48	0.48	0.58	0.61	0.58	0.61	0.58	0.61	1.00	
Agni Rekha	0.97	0.61	0.74	0.65	0.90	0.84	0.71	0.94	0.90	0.81	0.84	0.90	0.81	0.74	0.81	0.84	0.81	0.84	0.87	0.90	0.74	0.68	1.00

Hence, these marker systems could be effectively used for diversity analysis.

In the current study molecular characterization of 23 chilli genotypes was carried out using 13 RAPD primers in order to assess genetic diversity. Out of which 9 were polymorphic, 2 monomorphic and 2 primers did not amplify. Data of those nine primers were used in analysis. A representative amplification profile obtained by using random primer OPB 12 is depicted in Fig 1. The number of bands amplified with each random decamer primer along with their details is given in Table 1.

The size of the amplified DNA fragments was ranged from 391 bp to 2754 bp. A total of 28 polymorphic amplicons were observed out of a 63 amplicons generated by the 9 random decamer with 44.4 % polymorphism. Primers vary in their polymorphism content with the percent polymorphism varied between 14.29 % (OPB-07) to 66.67 % (OPB-12). The average number of polymorphic bands per primer was 3.1. Earlier different levels of polymorphic bands were reported by Litoriya *et al.,* (2009) 50.8 %; Paran *et al.,* (1998) 22 %, Lafebvre *et al.,* (2001) 45 %

In the present investigation the number of bands generated by each primer varied from 5 (OPB-10) to 9 (OPB-15, OPB-01) with an average of 7 fragments per primer. Previously Bhadragoudar and Patil (2011), and Paran *et al.* (1998) reported an average of 6 and 7.5 bands, respectively.

In the present investigation the similarity coefficients range from 0.42-0.97 with an average of 0.73 which revealed existence of genetic variation among 23 chilli genotypes. Lowest similarly coefficient of 0.42 was present between genotypes AKC-N-86-1 and GP-03-21 while maximum similarly coefficient 0.97 was observed between genotypes Green harvest 1 and Byadagi. Bhadragoudar and Patil (2011) and Ince *et al.* (2010) and found similarity coefficients ranging between 0.20-0.94; 032-0.88 and respectively indicating diversity in chilli genotype studied.

The consensus tree constructed showed three major clusters. First two major clusters joined at 80 % level of similarity (fig 2). Largest cluster one was composed of thirteen chilli genotypes *viz.,* GP-03-14, Agni Rekha, Fong. No. 5, AVRDC-01-3, AKC-84-02-2-1, AKC-97-20, Guntur G-4, Byadagi kaddi, Tej-F5-01-5, AKC-387, AKC-351 and AKC-393 which joined at 0.80 similarity index, while the cluster two consisted of seven chilli genotypes *viz.,* 6 AKC-98-27, Green harvest 5, AKC-N-86-1, PBC-161, Fong No-2, LCA-334, and GP-03-20 which also joined at 0.80 similarity index to form one distinct cluster. On the other hand, cluster 3 with three chilli genotypes *viz.,* GP-03-21, Byadagi and Green harvest 1 appears to be the most diverse of the total accessions, which has lowest of 58 % similarity value with rest of genotypes.

RAPD patterns separated all the varieties of chilli from each other and confirm the variability at molecular level. Our study has established that the RAPD markers are useful for studying genetic diversity among chilli and thereby helps in selecting superior crosses and for distribution of genotypes into different groups.

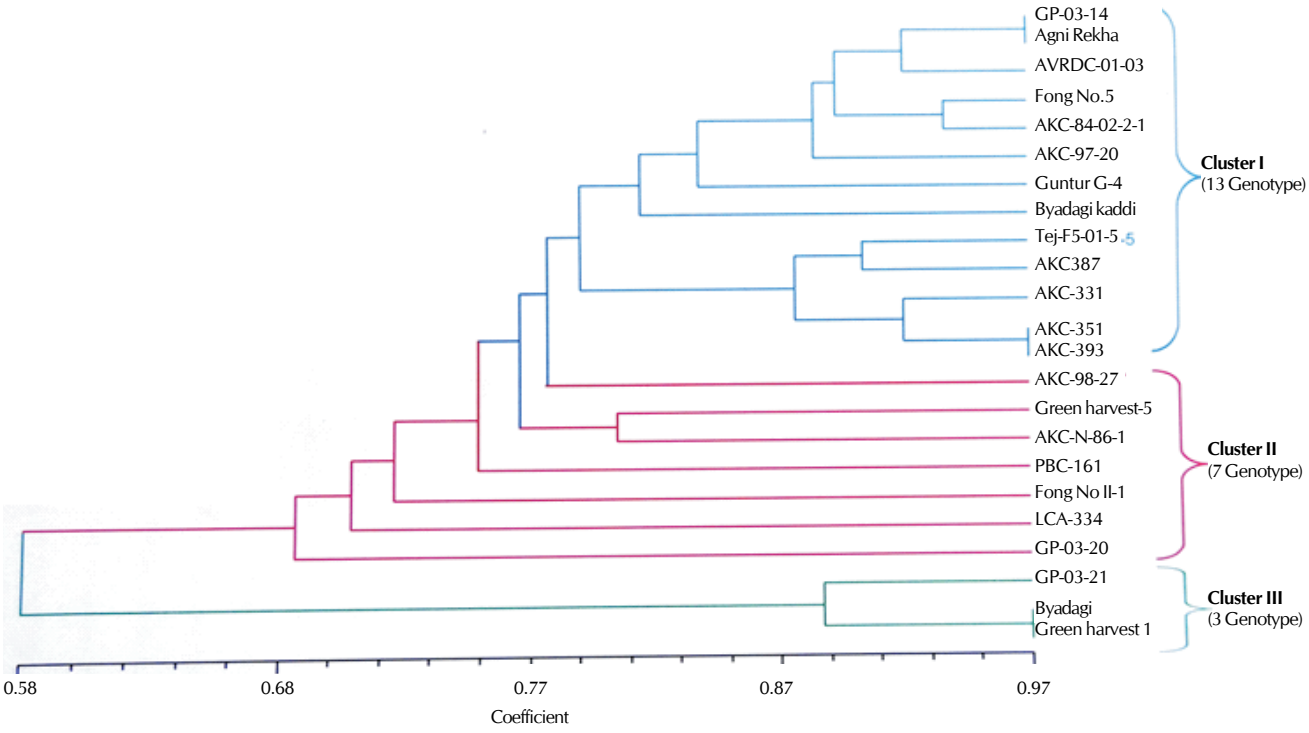


Fig 2. Consensus tree showing clustering of 23 chilli genotypes using NTSYS pc2.02i software

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