African Journal of Biotechnology Vol. 10(76), pp. 17477-17483, 30 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.497
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Assessment of genetic diversity among *Capsicum annuum* L. genotypes using RAPD markers

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Accepted 1 June, 2011

Chilli or pepper (*Capsicum annuum* L.) is an important commercial crop cultivated exclusively in tropical and temperate zones of the world. It is a good source of vitamin C, A and E and chilli oleoresin has a considerable use in pharmaceutical industry. India being the largest producer of chilli has tremendous potentiality to increase production in order to promote export besides meeting its domestic requirements. Vast genetic diversity is available in chilli which may facilitate the breeder to develop new varieties, provided the genetic distance between the accessions is properly understood. In this study, 45 accessions of chilli collected from Chilli Research Station, Devihosur, Haveri district of Karnataka State were subjected for RAPD analysis to understand the genetic homology. Among 45 accessions, three RAPD primers (decamer) efficiently amplified genomic DNA. The dendrogram constructed from pooled data revealed 14 clusters. Two clusters are represented by a single genotype exhibiting their genetic divergence. However, one large cluster consists of maximum of 8 genotypes revealing genetic closeness which could be efficiently used in breeding programme.

Key words: *Capsicum annuum*, RAPD, polymorphism, genetic diversity, chilli.

INTRODUCTION

Chilli or pepper (*Capsicum annuum* L.) is an important commercial crop cultivated exclusively in tropical and temperate zones of the world. India contributes one-fourth of the world production of chilli with an average annual production of 20.98 t (Karvy, 2008). The main producers are Hungary, India, Mexico, China and Korea. The genus *Capsicum* L. belongs to family Solanaceae and includes 27 species (Rodriguez et al., 1999; Onus and Pickersgill, 2004; Votava and Bosland, 2002; Ince et al., 2009) consisting of five domesticated species namely: *C. annuum* L., *Capsicum baccatum* L., *Capsicum chinensis* Jacq., *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz and Pavan.

The most important species, *C. annuum* L., bears both pungent (hot) and sweet fruits. The taxa show much variability particularly with regards to fruits. Chilli fruits are the rich source of vitamin C, A and E and also a good source of chilli oleoresin which has varied uses in processed food, beverage industries and in pharmaceuticals. Chilli has diverse uses as spice, condiment, culinary supplement, medicine, vegetable and as ornamental plant (Berke and Shieh, 2001).

India being the largest chilli producers has vast potentiality to increase the production, in order to promote export besides meeting its domestic requirements. However, despite continuous efforts at various levels, the chilli productivity did not gain momentum. This could be attributed to number of constraints such as non-availability of suitable cultivars/ hybrids, biotic and abiotic stresses, genetic drift in cultivars and development of new pathogenic races (Sharma and Singh, 2009). Thus, for enhancing the productivity there is a demand to develop high yielding varieties or hybrids enriched with good quality attributes through genetic reconstructing of the chilli germplasm.

The great genetic diversity available for chilli breeders has facilitated the development of new varieties and hybrids. Several chilli breeders considered the level of heterosis exhibited by chilli hybrids which is directly related with the genetic distance between the parental lines ($VN_{2,}$ Byadgi Dabbi, Punjab lal.).

Therefore, estimation of genetic distance can be useful

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Table 1. *Capsicum annuum* L. accessions.

S\N	Accession number	S/N	Accession number
1.	DCA1	24.	DCA24
2.	DCA2	25.	DCA25
3.	DCA3	26.	DCA26
4.	DCA4	27.	DCA27
5.	DCA5	28.	DCA28
6.	DCA6	29.	DCA29
7.	DCA7	30.	DCA30
8.	DCA8	31.	DCA31
9.	DCA9	32.	DCA32
10.	DCA10	33.	DCA33
11.	DCA11	34.	DCA34
12.	DCA12	35.	DCA35
13.	DCA13	36.	DCA36
14.	DCA14	37.	DCA37
15.	DCA15	38.	DCA38
16.	DCA16	39.	Byadgi Kaddi
17.	DCA17	40.	Byadgi Dabbi
18.	DCA18	41.	VN2
19.	DCA19	42.	Jyoti
20.	DCA20	43.	Nooji
21.	DCA21	44.	Yellow *Capsicum*
22.	DCA22	45.	Red *Capsicum*
23.	DCA23		

for prediction of high performance crossings. Earlier, diversity in *Capsicum* was studied using morphological, cytological and biological markers (Hunziker, 1950, 1956, 1998; Heiser and Smith, 1953; Pickersgill, 1988; Gopinath et al., 2006). Species identification based on morphological characteristics is often difficult, since most of these characteristics are under the influence of environmental factors and might not be distinguish between closely related genotypes (Rodriguez et al., 1999). Characterization of accessions or species is an important step for germplasm conservation, maintenance and breeding studies. Molecular DNA marker analysis has been suggested for the determination of genetic diversity among genotypes (Gilbert et al., 1999).

Amplified polymorphic DNA (RAPD) is one such method (Welsh and McClelland, 1990; Williams et al., 1990) of identifying polymorphism that can be used to elicit information on molecular differences among individuals of a population between lines or accessions or any breeding material. RAPD markers are simple, fast and low cost and it can be done with small amount of DNA. RAPD markers can provide robust classification criteria that could be useful in species separation and systematics. Therefore, the objective of the study was to establish the genetic relationship between the chilli accessions of *C. annuum* L. using RAPD markers which may be useful in improving the productivity and stability of the crop yield.

MATERIALS AND METHODS

Plant material

Forty five (45) accessions of *C. annuum* L. were procured from Chilli Research Centre, Devihosur, University of Horticultural Sciences, Bagalkot, India and were used as source material for this study (Table 1). Seeds were sown and grown in the Botany Department Experimental Garden, Sciences, University of Agricultural Dharwad, India.

DNA extraction

Healthy leaves (4 g) were collected from six plants of each accession, cleaved, surface sterilized and pulverized in liquid nitrogen. Genomic DNA was extracted according to modified c-TAB method described by Doyle and Doyle (1990). Concentration and quality of genomic DNA was determined using spectrophotometer at 260 to 280 nm wavelength and the quality is counter confirmed by a single crisp band on 0.8% agarose gel which is treated as pure and suitable for RAPD analysis.

Table 2. List of random primers used, their sequence and number of PCR amplified bands obtained.

S/N	Primer	Sequence (5' – 3')	Number of band			% Polymorphism
			Total	Monomorphic	Polymorphic	
1.	OPJ-01	CCCGGCATAA	8	2	6	75.00
2.	OPJ-05	CTCCATGGGG	5	1	4	80.00
3.	OPJ-06	TCGTTCCGCA	4	2	2	50.00
4.	OPC-13	AAGCCTCGTC	8	3	5	62.50
5.	OPC-15	GACGGATCAG	6	2	4	66.66
6.	OPC-03	GGGGGTCTTT	6	1	5	83.33
7.	OPA-12	TCGGCGATAG	7	2	5	71.40
8.	OPA-07	CCGATATCCC	9	1	8	88.00
9.	OPA-17	GACCGCTTGT	5	2	3	60.00
10.	OPA-15	TGCCGAGCTA	8	4	4	50.00
11.	OPA-11	CAATCGCCGT	8	2	6	75.00
12.	OPB-11	GTAGACCCGT	15	5	10	66.66
13.	OPI-01	ACCTGGACAC	2	2	0	00.00
14.	OPI-02	GGAGGAGAGG	4	4	0	00.00
15.	OPI-03	CAGAAGCCCA	3	2	1	33.33
16.	OPI-06	AAGGCGGCAG	1	1	0	00.00
Total			99	36	63	
Average band/ primer			6.1	2.25	3.9	
% Polymorphism				63.3%		

PCR amplification

A set of 25 arbitrary decamer primers (Table 2) from Operon technologies, Almedia, USA were used to amplify genomic DNA of *Capsicum* accessions. 20 µl PCR mixture contained 50 ng of genomic DNA, 5 pM primers, 2.5 mM dNTP, 1.5 mM $MgCl_2$, 0.33 µl (1 unit) of Taq DNA polymerase and 1x assay buffer. DNA was amplified on gradient palm cycler, (Corbett research, Australia, Model: CG1-96) and was programmed as, initial denaturation for 5 min at 94°C, 39 cycles each of denaturation for 60 s at 94°C, primer annealing for 60 s at 36°C and 2 min extension at 72°C. The final extension was at 72°C for 7 min followed by cooling at 4°C for 3 min.

Amplified DNA samples were analyzed by electrophoresis on 1.2% agarose gel, λ DNA – double digest (*EcoR*I / *Hind*III) served as a molecular size marker.

Similarity and dendrogram analysis

Polymorphic RAPD amplified product was considered to be a unit character and the populations were manually scored as binary data with presence as "1" and absence as "0". Only clearly distinguishable DNA bands were used in the genetic analysis. The molecular size of the amplification products were calculated from the standard curve based on the known size of the DNA fragments of the ladder. Estimates of genetic similarity were calculated according to Jaccard's similarity index (1908). The matrix obtained was used to evaluate the genetic relationship among *Capsicum* with an Unweighted pair group method with arithmetic averages (UPGMA). All statistical analysis was performed with aid NTSYS-PC computer genotypes with cluster analysis using the SHAN module of NTSY sp. version 2.0. (Rohlf, 1998)

RESULTS

DNA polymorphism among genotypes

A varying level of genetic polymorphism was revealed in the banding pattern across 45 chilli accessions (Figure 1). For a total of 16 primers, 99 marker levels were amplified, of which 63 (63.33%) were polymorphic and 36 (36.36%) were monomorphic across the genotypes. On an average, total number of bands generated per primers was 6 of which 3.9 were polymorphic and 2.25 were monomorphic. Names of primers used to generate PCR products and summary of the total number of mono-morphic and polymorphic DNA fragments amplified is listed in Table 2.

Among all primers used in this study, OPA-07 (88%) and OPC-03 (83.33%) showed higher levels of polymor-phism. OPA-3 exhibits a very low percentage of polymorphism (33.33%). Three primers OPI-01, 02, and 06 did not yield any polymorphism.

Similarity matrix based on RAPD profiles

The similarity coefficient ranged from 0.20 to 0.94 indicated substantial diversity present in the germplasm. Highest diversity was observed between accessions Byadgi Dabbi and DCA-33 at Sij-0.2. Highest degree of similarity at Sij-0.94 was observed between DCA-13 and

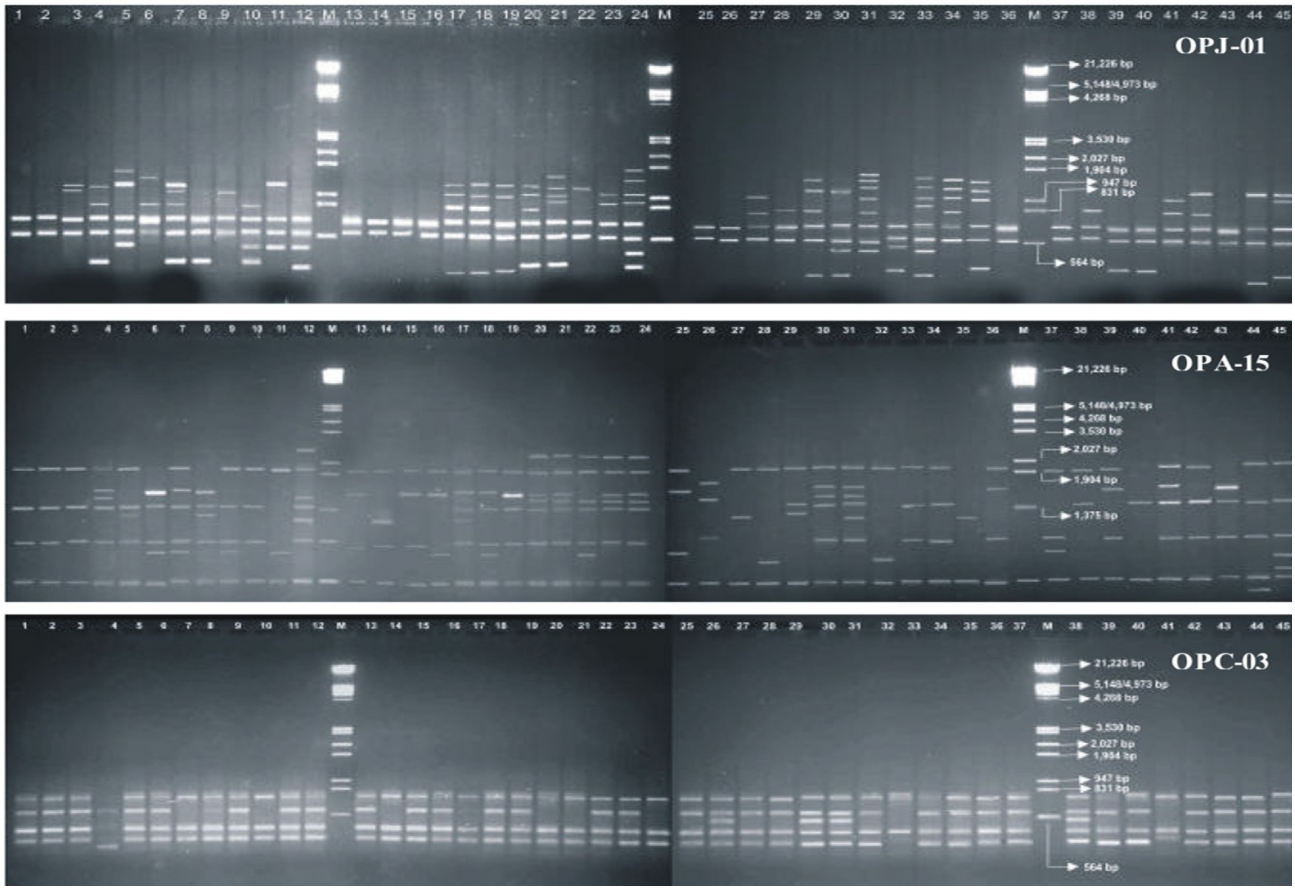

Figure 1. RAPD banding pattern of primers OPJ-01, OPA-15 and OPC-03.

DCA-15.

Dendrogram constructed from pooled data (Figure 2) for 45 genotypes of *Capsicum* and 3 RAPD primers revealed 14 different clusters. Among all these clusters, cluster II and cluster V are observed as solitary clusters (Table 3) containing single genotypes DCA-35 and DCA-26 respectively. Cluster XIV is the largest one which includes eight genotypes in it, among which DCA-1, DCA-2 and DCA-3 are very closely related at Sij 1.0 with no differences between them. Five clusters namely: I, III, IX, X, XIII consist of two accessions each. However, cluster IV consists of three accessions, three clusters VII, VIII and XI consist of four accessions and two clusters VI and XII consist of five accessions each.

DISSCUSSION

RAPD markers represent an efficient inexpensive tool to generate molecular data and thus have been used successfully in various taxonomic and phlyogenetic studies (Nabauer et al., 1999; Ryzhova and Kochieva 2004; Ince et al., 2009; Votava et al., 2005).

OPA-07, OPC-03 primers were successfully used to access genetic diversity by Rodriguez et al. (1999), AV-08 and OPJ primers were used by Pawar (2000) and Yadwad (2005) respectively to assess the genetic diversity. Moderate to high polymorphism in this study is due to wide genetic bases of the accessions and the primers used are potential for polymorphism.

High level of genetic diversity in DCA-35 and DCA-26 genotypes which represent solitary cluster may be due to cross pollination by insects, dispersal of seeds, habitat changes and larger population size in different locations. The significant degree of variation (Sij-0.20) between accession Byadgi Dabbi and DCA-33 reveals maximum genetic diversity as a result of geographical isolation and change in the environmental conditions. Mexican accessions revealed that greater genetic variation was observed among *Capsicum* populations, rather than within populations, presumably due to self pollination and group bottlenecks (Loaiza-Figueroa et al., 1989). Since genetic differentiation was correlated with geographic isolation in Mexico (Loaiza-Figueroa et al., 1989), it may be appropriate to analyze accessions that represent a wide range of geographic origins in order to maximize genetic diversity.

In this study, we determined the genetic relationships

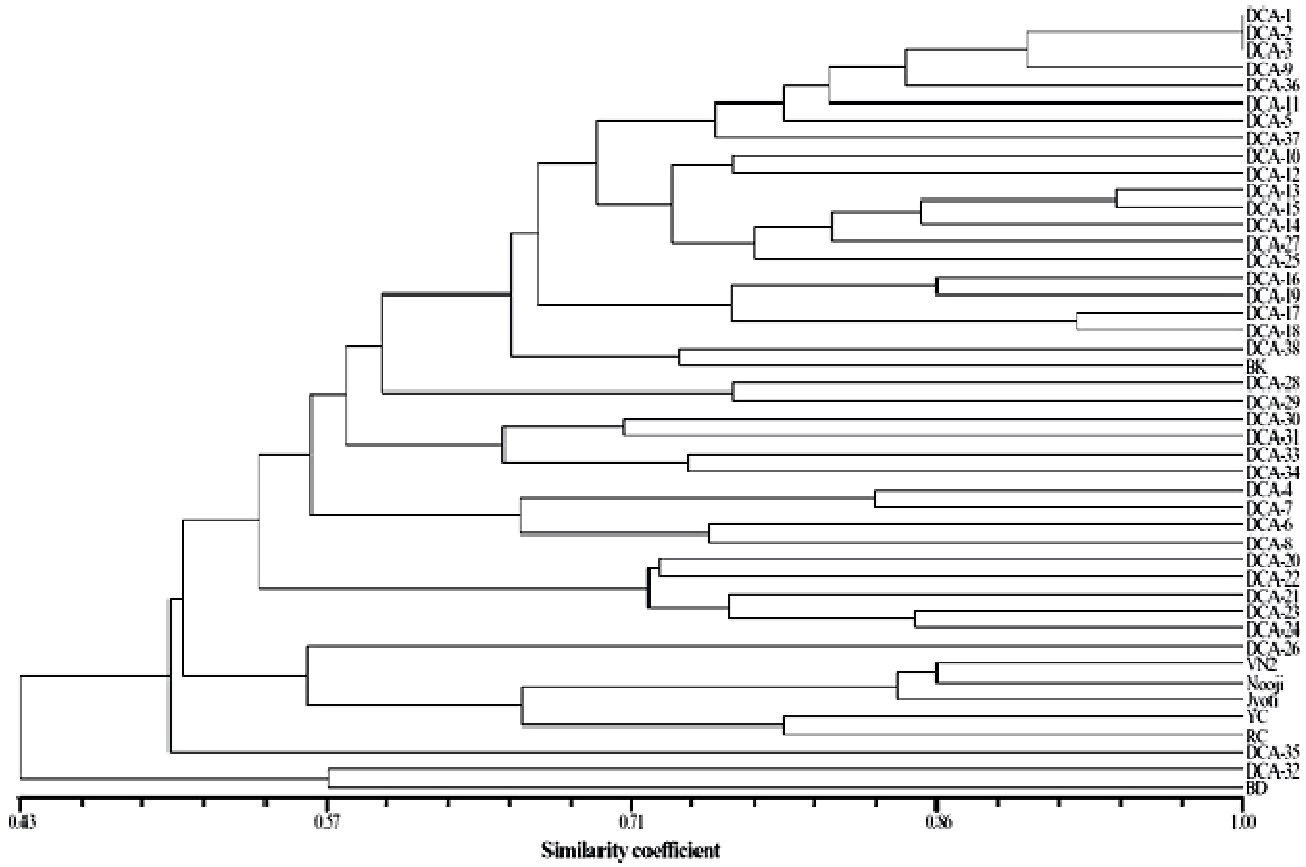

Figure 2. Dendrogram constructed for 45 genotypes of *Capsicum* with 3 RAPD primers (pooled).

Table 3. Cluster of chilli germplasm based on RAPD analysis.

S/N	Cluster	Genotype
1.	I	DCA-32 and BD
2.	II (solitary)	DCA-35
3.	III	YC and RC
4.	IV	VN_2, Nooki and Jyoti
5.	V (solitary)	DCA-26
6.	VI	DCA-21, DCA-23, DCA-24, DCA-20 and DCA-22
7.	VII	DCA-4, DCA-7, DCA-6 and DCA-8
8.	VIII	DCA-30, DCA-31, DCA-33 and DCA-34
9.	IX	DCA-28, DCA-29
10.	X	DCA-38, BK
11.	XI	DCA-16, DCA-17, DCA-18 and DCA-19
12.	XII	DCA-13, DCA-15, DCA-14, DCA-27 and DCA-25
13.	XIII	DCA-10 and DCA-12
14.	XIV	DCA-1, DCA-2, DCA-3, DCA-9, DCA-36, DCA-11, DCA-5 and DCA-37

among *capsicum* accessions of various types. The general pattern of variation was similar to those of the previous studies (Loaiza-Figueroa et al., 1989; Lefebvre et al., 1993) that is, the small-fruited cultivars formed a more divergent group from the large-fruited cultivars, limited variation was observed among the bell-type cultivars. The small-fruited hot cultivars were separated from the cluster of large-fruited cultivars such as yellow

Capsicum and red *Capsicum.*

High level of genetic similarity is expected among Byadgi Dabbi and DCA-32 accessions restricted to Karnataka due to similar geographical conditions. But in contrast, this study revealed broad genetic base indicating earlier introduction of this species and subsequently leading to accumulation of variation. There is a close genetic similarity (Sij 0.94) between the accessions DCA-13 and DCA-15 and it clearly depicts that genetically both accessions are similar. The genotypes DCA-1, DCA-2 and DCA-3 which were developed from same progenitors also showed highest level of similarities with Sij 1.0 and are grouped into same cluster. This study revealed that the aforementioned varieties have the same genetic base.

The local cultivars of Karnataka Byadgi Dabbi and advanced breeding lines of Chilli research centre Devihosur DCA-32 having nearly similar phenotypic characters fall into same cluster indicating genetic similarity where as Byadgi Kaddi and DCA-38 having nearly similar phenotypic characters fall into another cluster indicating diversity from Byadgi Dabbi. Wang et al. (1996) subjected diverse pepper accessions to RAPD analysis and clustered into 4 group indicating that RAPD markers can be effectively and reliably used for classification of *capsicum* species. Similarly, Kang et al. *(*1997) observed sufficient degree of polymorphism to differentiate among *Capsicum* species by RAPD analysis in *Capsicum* germplasm.

These results indicated that the genotypes DCA 1, 2, 3 having similar phenotypes also showed the similarity at genotypic level. The principal co-ordinate analysis supports the major clustering pattern. This may be useful in identifying the duplication of genotypes.

The results of this study are similar to other studies focused on landrace diversity in Mexico and elsewhere (Baral and Bosland, 2002). Migration to geographically remote areas has been shown to bottleneck genetic diversity in *Capsicum* (Pickersgill, 1997) and the pattern of genetic similarity seen in most of the New Mexican long green pod-type landraces is consistent with this observation.

RAPD method used in this study displayed appreciable inter-population variation or molecular polymorphism, which is pre-existed in different collections. In spite of their morphological identity, substantial polymorphism was observed among the accessions under study. The study revealed that though the decamer primers are small in comparison to the large genome of *C. annuum*, they produced appreciable amplicons sufficient to demarcate all accessions used.

Conclusion

Our study reflected the tremendous genetic diversity available among the genotypes. This rich genetic diversity in which breeding efforts depend can be utilized for current and future breeding programs in order to select genetically distinct parents. This study confirms the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions available in the germplasm collection. Accurate estimates of diversity are a pre-requisite for optimizing sampling strategies and for conserving genetic resources.

Currently, it is also proved that the entries that were found to be similar in taxonomical classification based on morphological characters do have divergence at DNA level. The RAPD profiles display vast genetic variation indicative of the evolving nature of the taxa.

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