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MOLECULAR ANALYSIS OF CULTIVAR DIVERSITY AMONG CHILLI IN NORTHERN KARNATAKA, INDIA USING RAPD MARKERS

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Abstract

Ten chilli cultivars were used for the molecular characterization through RAPD technique, viz., Capsicum Elephant Trunk, Vijay, CG4, Byadgi Kaddi, Triloka, Capsicum Sarpan Nag-10, Byadgi Dabbi, Sitara, Ajay and Guntur. Among the twenty primers, OPA06 produced maximum number of polymorphic bands that indicated a high level of polymorphism as against the primer OPA04 which generated the least number of polymorphic bands. Band size ranging from 110 to 400 bp of PCR amplified products were considered and scored for primers. The reproducibility of the banding pattern in chilli cultivars was confirmed by three replicated reactions with the same primer. Intra genotype similarity indices were higher as they ranged from 86.67 to 100.00%. The highest intra genotype similarity indices were observed in Capsicum Elephant Trunk, CG4, Capsicum Sarpan Nag-10, Guntur, Ajay, Triloka and Byadgi Kaddi (100.00%), whereas the lowest intra genotype similarity indices were found in Byadgi Dabbi (92.43%). The highest similarity value disclosed lower genetic variability within the individuals which was more homogenous than those of Byadgi Dabbi in which similarity indices value was found to be the lowest. The values of the pairwise comparison of Nei's (1972) genetic distance among ten chilli varieties and genotypes computed from combined data from the twenty primers ranged from 0.0488-0.7490. The genetic distance value between variety Guntur and Vijay F1 hybrid was highest (0.749) with lowest genetic identity (0.472) among the other pairwise variety and genotype. The genetic distance between variety Byadgi, Kaddi and CG4 chilli was the lowest (0.048) with the highest genetic identity (0.952). From the difference between the highest and the lowest genetic distance value, there were wide variations among ten chilli varieties and genotypes. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that the variety Guntur and Vijay F1 Hybrid showed the highest genetic variation, while the lowest genetic variation was observed between variety Byadgi kaddi and CG4, the two latter cultivars can be used as parental source for breeding line to improve chilli varieties.

Key Words*: Capsicum annum, cultivars, dendrogram, genetic diversity, RAPD.*

INTRODUCTION

Genus *Capsicum* represents 30 species of which only five have been extensively cultivated, viz., *C. annuum* L., *C. frutescens* L., *C. chinese* Jacq., *C. baccatum* L., and *C. pubescens* R. & P. (Eshbaugh, 1980). *Capsicum annuum* L. belonging to family Solanaceae has its unique taste and smell. Chilli contains capsaicin which is well known for its medicinal properties and above all chilli has a good fruity flavor. The fruits of the chilli give high levels of vitamin C and both ripe and dried chilli are rich source of vitamin A and beta carotene (Mateos et al., 2013). Hence, it is one of the most widely consumed vegetable crops globally. During the last three decades, intensive hybrid breeding has resulted in narrowed genetic base (Van de Wouw et al., 2010). India is the fourth largest producer and second largest exporter of *Capsicum* wherein Karnataka ranks second to a country with an average production of 15% after Andhra Pradesh 49%. Within Karnataka, northern region is the major producer of chilli (Rajur et al., 2008; Veeranagouda et al., 2011; Sreedhara et al., 2013).

Globally, agricultural intensification has resulted in drastic change in genetic material of vegetable crops. Hence, for the proper utilization and conservation of genetic resources, knowledge on genetic variation is essential wherein this may further find application in selection of parental line, classification of varieties and genotypes and exact variety identification (Rao & Hodgin, 2002; Govindaraj et al., 2015). Identification and characterization of species with the aid of

morphological markers are quite difficult (Ganiea et al., 2015). Hence molecular markers are extensively used for the studies on genetic diversity, systematic and phylogenetic relationships. A single or multiple markers are used to construct genetic maps and genetic linkage studies. Molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP) and microsatellites have been successfully applied to characterize closely related genotypes among a wide range of crop plants (Arif et al., 2010). RAPD markers are widely used for the diversity analysis of crop plants due to the ease of polymorphic content, reproducibility and co-dominant in nature (Awasthi et al., 2003; Bansala et al., 2012). Since RAPD primers are arbitrary sequences, prior knowledge of DNA sequence is not essential.

The genetic diversity analysis of chilli cultivars, genotypes and varieties using several molecular markers has been reported in recent past (Paran et al., 1998; Rodriguez et al., 1999; Ilbi et al., 2003; Lanteri et al., 2003; Costa et al., 2006; Ince et al., 2009; Patel et al., 2009; Litoriya et al., 2009; Makari et al., 2009; Bhadragoudar & Patil, 2011; Thul et al., 2012; Peeraullee et al., 2013; Samuel et al., 2013; Orenthung & Changkija, 2013; Sema et al., 2013; Savadatti et al., 2015). Hence, studies on genetic diversity provide prospects to enhance agricultural yield by way of improved cultivars. Furthermore, understanding genetic relatedness and variation among germplasm facilitate in the identification of superior parents in breeding programs. Therefore, the aim of the present study was to evaluate genetic diversity amongst 10 chilli varieties using RAPD marker, and thus, information generated may find useful in the breeding programmes.

MATERIALS AND METHODS

Plant Material

The present experiment was carried out at the Department of Botany at Karnatak University, Dharwad, Karnataka, India. Ten chilli cultivars, *viz*., (1) Capsicum Elephant Trunk, (2) Vijay, (3) Capsicum G4, (4) Byadgi Kaddi, (5) Triloka, (6) Capsicum Sarpan Nag-10, (7) Byadgi Dabbi, (8) Sitara, (9) Ajay and (10) Guntur were selected for the present study. Seeds were collected from different locations of Northern Karnataka region in India. These seeds were raised in nursery bed for the period of one month, tender leaves were collected and stored in -80°C freezer to carry out further study.

DNA Extraction

CTAB protocol was followed for DNA extraction (Doyle & Doyle, 1987) by taking approximately 40 mg of leaf sample from young leaves of chilli cultivars. The quality and integrity of DNA was confirmed by running the DNA sample in 0.8% agarose gel electrophoresis whereas DNA quantification was performed by using Nanodrop spectrophotometer with a wavelength of 260/280 nm.

PCR Amplification and Scoring of Amplified Products

A total of 20 RAPD decamer primers (Operon Technologies Inc, USA) used in the current study are listed in Table 1. These primers were screened by amplifying DNA sample with a 20µl reaction mix containing master mix. Conditions for RAPD amplification reactions were maintained following the protocol reported by Williams et al. (1990) with some variations in reaction mixture. The PCR reactions were performed in a thermal cycler (Veriti, Applied Biosystems, USA). Amplification of DNA samples was performed using the cycle profile reported by Belaj et al. (2004): initial denaturation at 95°C for 5 min followed by 45 cycles at 95°C for 1 min, annealing at 36°C for 1 min and a elongation at 72°C for 2 min and final extension at 72°C for 10 min. All the reactions were performed in triplicate using DNA of different extractions and different lots of the AmpliTaq DNA polymerase (Bangalore Genei, India). The PCR products were run on 1.5% agarose gel electrophoresis and RAPD bands were scored as 1 for present, whereas 0 for absent. Triplicate analysis was performed to confirm the reproducibility of bands and only well-defined bands were selected for scoring. Further Nei's (1972) genetic distance was calculated and the varieties were grouped by

cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA).

Table 1. List of Primers with their Annealing Temperature Used for RAPD Analysis

Primers	Sequence (5′→3′)	Annealing temperature (°C)
OPA-01	CAGGCCCTTC	70
OPA-02	TGCCGAGCTG	70
OPA-03	AGTCAGCCAC	60
OPA-04	AATCGGGCTG	60
OPA-05	AGGGGTCTTG	60
OPA-06	GGTCCCTGAC	70
OPA-07	GAAACGGGTG	60
OPA-08	GTGACGTAGG	60
OPA-09	GGGTAACGCC	70
OPA-10	GTGATCGCAG	60
OPA-11	CAATCGCCGT	60
OPA-12	TCGGCGATAG	60
OPA-13	CAGCACCCAC	70
OPA-14	TCTGTGCTGG	60
OPA-15	TTCCGAACCC	60
OPA-16	AGCCAGCGAA	60
OPA-17	GACCAGCGAA	60
OPA-18	AGGTGACCGT	60
OPA-19	CAAACGTCGG	60
OPA-20	GTTGCGATCC	60

RESULTS AND DISCUSSION

RAPD assay was performed to estimate genetic polymorphism in ten chilli cultivars. Out of 20 primers, four primers (OPA04, OPA06, OPA09, OPA11) showed amplification of genomic DNA. The four primers generated 21 distinct bands of which 17 were considered as polymorphic. The percentage of polymorphic loci was (80.95%) indicating a higher level of polymorphism (Table 2). The four primers generated 5.25 scorable bands per primer and 4.25 polymorphic RAPD markers per primer. Biswas et al. (2009) reported a similar study on eggplant cultivars wherein four primers successfully amplified after screening 21 RAPD primers which generated a total of 76 scorable bands out of which 44 fragments exhibited polymorphism, with an average of 19 amplicons per primer. In the present study, out of four primers, OPA06 could produce higher number of polymorphic bands in comparison to that of OPA04 which generated the lowest number of polymorphic bands (Table 2). OPA6 indicated a high level of polymorphism as against the primer OPA4. The banding patterns of different chilli varieties using OPA04 and OPA09 primers are shown in Figure 1 and 2. Band size ranging from 110 to 400bp of PCR amplification product scored for primers. The reproducibility of the banding pattern of ten chilli cultivars was confirmed by three replicated reactions with the same primer. Strong and weak bands were produced in the RAPD reactions. Weak bands indicated low homology between the primer and the pairing site (Thormann et al., 1994). A diverse level of polymorphism in different crops has been reported in eggplant (57.89%) by Biswas et al. (2009), in tomato (90.19%) by Moonmoon (2006) and in chilli (90%) by Paran et al. (1998).

Table 2. Amplified RAPD Primers with Bands and Their Size Range with polymorphic bands Detected in Ten Chilli Varieties

Primer code	Sequence (5'→3')	Size range (bp)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPA-04	AATCGGGCTG	180-120	3	2	66.67
OPA-06	GGTCCCTGAC	200-120	7	6	85.71
OPA-09	GGGTAACGCC	400-110	5	4	80.00
OPA-11	CAATCGCCGT	400-140	6	5	83.33
Total	-	-	21	17	315.71
Average	-	-	5.25	4.25	80.95

Intra-genotype similarity indices were higher, as they ranged from 86.67 to 100.00% (Table 3). The highest intra-genotype similarity indices of 100 % were observed in Capsicum Elephant Trunk, Capsicum G4, Capsicum Sarpan Nag-10, Guntur, Ajay, Triloka and Byadgi kaddi, on the other hand the least intra-genotype similarity indices were observed in Byadgi dabbi with a similarity index of 92.43% (Table 3). The highest similarity value disclosed lower genetic variability within the individuals which were more homogenous than those of Byadgi dabbi in which similarity indices value was found the lowest.

Table 3. Summary of Similarity Indices Within and Between Individuals of Ten Different Chilli Varieties

Varieties	Band sharing values (%)				
	OPA04	OPA06	OPA09	OPA11	Average
C. Elephant Trunk	100.00	100.00	100.00	100.00	100.00
Vijay F1 hybrid	86.67	93.94	100.00	92.59	93.30
Capsicum G4 chilli	100.00	100.00	100.00	100.00	100.00
C. Sarpan Nag-10	100.00	100.00	100.00	100.00	100.00
Guntur	100.00	100.00	100.00	100.00	100.00
Ajay	100.00	100.00	100.00	100.00	100.00
Triloka	100.00	100.00	100.00	100.00	100.00
Byadgi kaddi	100.00	100.00	100.00	100.00	100.00
Byadgi dabbi	86.67	90.47	92.59	100.00	92.43
Sitara	86.67	100.00	100.00	90.47	94.28
Average	96.00	98.44	99.26	98.30	98.00

Table 4. Summary of Nei's Genetic Identity (Above Diagonal) and Genetic Distance (Below Diagonal) values between Ten Chilli Varieties

Variety	Capsicum Elephant Trunk	Vijay	Capsicum G4	C. Sarpan Nag-10	Guntur	Ajay	Trilok	Byadgi kaddi	Byadgi dabbi	Sitara
Capsicum Elephant trunk	****	0.810	0.619	0.714	0.619	0.571	0.619	0.666	0.662	0.731
Vijay	0.209	****	0.613	0.514	0.472	0.719	0.556	0.605	0.573	0.603
Capsicum G4	0.479	0.488	****	0.809	0.714	0.761	0.809	0.952	0.803	0.731
C Sarpan Nag-10	0.336	0.664	0.211	****	0.809	0.666	0.714	0.857	0.860	0.787
Guntur	0.479	0.749	0.336	0.211	****	0.666	0.619	0.666	0.719	0.592
Ajay	0.559	0.328	0.271	0.405	0.405	***	0.571	0.714	0.753	0.682
Trilok	0.479	0.586	0.211	0.336	0.479	0.559	****	0.857	0.761	0.634
Byadgi kaddi	0.405	0.501	0.048	0.154	0.405	0.336	0.154	****	0.852	0.780
Byadgi dabbi	0.411	0.556	0.219	0.150	0.328	0.282	0.272	0.159	****	0.755
Sitara	0.312	0.505	0.312	0.238	0.523	0.381	0.455	0.248	0.280	****

Nei's (1972) genetic distance values between ten chilli varieties were computed using the collective values of all four primers. The values ranged from 0.0488-0.7490 (Table 4). The genetic distance value between variety Guntur and Vijay F1 hybrid was highest (0.749) with lowest genetic identity (0.472) among the other pairwise variety and genotype. The genetic distance between Byadgi kaddi and Capsicum G4 chilli was the lowest (0.048) with the highest genetic identity (0.952). Wide variation among 10 chilli varieties was observed between the highest and lowest genetic distance. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that the variety Guntur and Vijay F1 hybrid showed the highest genetic variation, while the lowest genetic variation was observed between variety Byadgi kaddi and Capsicum G4 chilli, the two latter cultivars can be used as parental source for breeding line to improve chilli varieties. Moonmoon (2006) indicated that molecular markers may act as better tools when compared to conventional markers such as morphological and biochemical markers.

Dendrogram based on Nei's (1972) genetic distance using UPGMA indicated segregation of ten chilli varieties and genotypes into two main clusters. Variety C Elephant trunk and Vijay F1 hybrid formed cluster-1 and the remaining eight varieties grouped in cluster-2 (Figure 3). In cluster-1 Capsicum Elephant trunk, formed sub cluster-1 and Vijay F1 hybrid formed sub cluster-2. Again in cluster-2 Guntur formed sub cluster-1, and seven entries formed sub cluster-2. Again in sub cluster-1, Ajay alone formed sub sub cluster-1 and remaining six entries formed sub sub cluster-2.

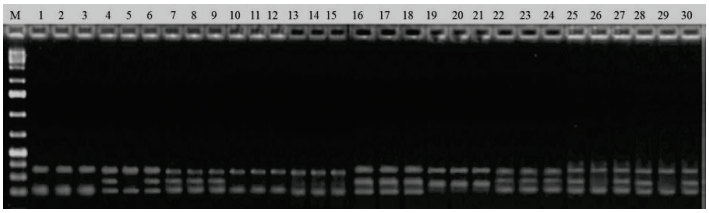

Figure 1. RAPD Profiles of Ten Chilli Varieties using Primer OPA04. Lane 1-3: C. Elephant Trunk, 4-6: Vijay, 7-9: Capsicum G4, 10-12: C. Sarpan Nag-10, 13-15: Guntur, 16-18: Ajay, 19-21: Triloka, 22-24: Byadgi kaddi, 25-27: Byadgi dabbi, 28-30: Sitara

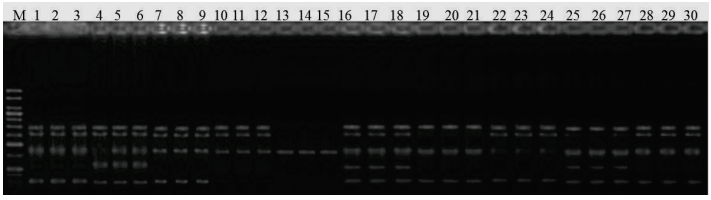

Figure 2. RAPD Profiles of Ten Chilli Varieties using Primer OPA09. Lane 1-3: C. Elephant Trunk, 4-6: Vijay, 7-9: Capsicum G4, 10-12: C. Sarpan Nag-10, 13-15: Guntur, 16-18: Ajay, 19-21: Triloka, 22-24: Byadgi kaddi, 25-27: Byadgi dabbi, 28-30: Sitara

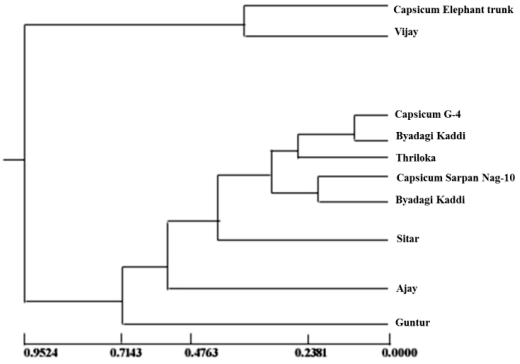

Figure 3. Dendrogram Based on Nei's (1972) Genetic Distance in Ten Chilli Cultivars

In sub cluster-2, Sitara formed group 1 and Capsicum G4, Capsicum Sarpan Nag-10, Triloka, Byadgi kaddi, Byadgi dabbi formed group-2, respectively. In group-2, Capsicum Sarpan Nag-10 and Byadgi dabbi formed sub group-1 and Capsicum G4, Triloka, Byadgi kaddi, formed sub group-2. In sub group-2, Triloka formed sub sub group-1 and Capsicum G4, Byadgi kaddi, formed sub sub group-2. Variety Byadgi kaddi was closer to the Capsicum G4 with the least genetic distance (0.048), hence, they fall under sub sub-group-2 and the highest genetic distance (0.749) was found among Guntur and rest of the varieties and genotypes. Thus, Guntur and other varieties fall under cluster-2 and these varieties and genotypes probably are identical based on morphological characters.

CONCLUSIONS

The present study reflected the genetic diversity amongst 10 different chilli cultivars. The information could thus be resourceful for the future breeding programs in selection of genetically distinct parents. Our study offers evidence against the suitability of RAPD markers that are simple, fast and elegant tool for evaluation of genetic diversity amongst different accessions. DNA based data can readily be used for studying the phylogenetic

relationships among various accessions of a species based on geographic origin. The polymorphism exhibited by different accessions can be exploited in breeding programme to capitalize on genetic resources which may add to improved chilli varieties. The present study revealed that highest genetic identity remains between Capsicum G4 and Byadgi kaddi (0.9524). On the other hand, the lowest genetic identity was observed between the Capsicum G4 *vs* Byadgi kaddi and Vijay *vs* Capsicum G4 (0.4728). This could be used in plant breeding programme for development of new chilli varieties. RAPD markers act as a fast, efficient and reliable tool for assessing genetic relationship and variability, therefore these markers are currently used in plant genetic resource management. It is also evident from the dendrogram that Guntur and Vijay varieties were most distantly related to each other and hence it is recommended that these two genotypes should be used in a hybridization program to create maximum genetic diversity for the improvement of *Capsicum* in Karnataka.

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