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DNA profiling of commercial chilli pepper (*Capsicum annuum* L.) varieties using random amplified polymorphic DNA (RAPD) markers

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In the present study, genetic variability in 10 commercial chilli pepper varieties viz. Gemini, G-334, Agnirekha, Pusa jwala, Mangala, Black diamond, Sindura, Pusa jyothi, Badiga-2 and Teja (branch), cultivated in the local area of Andhra Pradesh State, India, using random amplified polymorphic DNA (RAPD) markers was examined. Out of thee RAPD markers used for screening 10 chilli pepper genotypes, DNA amplification was observed only with OPAB02; this primer produced monomorphic band in Gemini,G-334, Black diamond and Sindura and polymorphic bands in Agnirekha, Pusa jwala, Mangala, Pusa jyothi, Badiga-2 and Teja (Branch). Genetic variabilty in terms of DNA pattern produced in the above varieties could be used as a marker to distinguish between them. Dendrogram generated by OPAB02 primer showed that the 10 chilli pepper varieties could be grouped into four clusters. Average genetic similarity index revealed 100% similarity between varieties of first cluster, 50% similarity between varieties of second and fourth clusters and 32% genetic similarity between varieties of third cluster. UPGMA cluster analysis will be useful in chilli pepper breeding programmes and germplasm conservation. Since the commercial value of chilli pepper is based on pungency level, future studies are aimed at molecular marker based pungency phenotyping.

Key words: *Capsicum annuum*, Randomly Amplified Polymorphic DNA, Dendrogram, Polymerase Chain Reaction.

INTRODUCTION

Chilli pepper (*Capsicum* sp.) of Solanaceae family, originated in Central and South Americas, then spread to Europe, Africa and Asia, especially to India, China and Japan (Bosland et al., 1996). Chilli pepper is integral and most important ingredient of different cuisines around the world, as it adds pungency, taste, flavour and colour to the dishes.

Chilli peppers cultivated in India are considered to be world's famous for two important commercial qualities: pungency (hotness) and colour. Some varieties are famous for biting pungency attributed to Capsaicin (Kumar et al., 2001) and fluorescent red colour due to the pigment Capsanthin (Kumar et al., 2006). A natural hybrid of *Capsicum chinense* and *Capsicum rutescens* variety Bhut jolokia, cultivated in Assam was the world's hottest chilli pepper (Mathur et al., 2000; Bosland and Baral, 2007).

The world production of chilli pepper sums up to around7.5 million tonnes and India with a production of 1.27 million tonnes is the world leader, followed by China

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and Pakistan. Around 75,000 tonnes of chilli pepper produced in India (25%), is exported every year and income of around Rs. 450 Crores (100 million USD) in foreign exchange is earned.

Andhra Pradesh (A.P.) is *Numero Uno* in chilli pepper area and production (30%), followed by Karnataka (20%), Orissa (9%), Tamil Nadu (8%) and all other States (18%). The major chilli pepper growing districts in A.P. State are Guntur, Warangal, Khammam, Krishna and Prakasam. Guntur and Warangal are the largest commercial chilli pepper producing districts, contributing more than 30% of the total production of A.P., State, with annual turnover of around Rs. 600 crores. In A.P., State, more than three dozen chilli pepper varieties are cultivated. Guntur and Warangal are the principal chilli pepper growing districts of Andhra Pradesh and they decide the prize at national level.

Conventionally, morphological descriptors like plant height, flower colour, fruit length and orientation and seed characteristics are routinely used to distinguish chilli pepper genotypes (Sitthiwong et al., 2005). In the last century, chromosome morphology (Pickersgill, 1971) and protein/enzyme profiling (McLeod et al., 1983; Panda et al., 1986; Posch et al., 1994; Kumar et al., 2010) has been used to study genetic diversity within the genus *Capsicum.*

However, these methods suffer from many discrepancies like, influence of environment on trait phenotype, epistatic interactions, pleiotropic effects etc.

Genetic markers (molecular markers) are efficient than phenotypic or biochemical markers, since previous knowledge of DNA sequence to design primers is not required, the technique is simple, low cost and does not use radioactive probes (Sitthiwong et al., 2005).

Many research groups have examined genetic diversity in the *Capsicum* spp., using restriction fragment length polymorphism (RFLP) (Prince et al., 1992; Prince et al., 1995), RAPD technique for analysing genetic diversity (Sitthiwong et al., 2005; Thul et al., 2011) genetic distance (Sanatombi et al., 2010), genetic relationship (Ince et al., 2010), phylogeny (Oyama et al., 2006; Adetula et.al., 2006) and amplified fragment length polymorphisms (AFLP) (Paran et al., 1998; Toquica et al., 2003) in region specific accessions and land races (Makari et al., 2009), and national germplasm repositories. There is scanty information on the use of RAPD markers for examining genetic diversity of commercial chilli pepper varieties cultivated in local areas of A.P., State, India.

The objective of the present investigation was to examine the genetic diversity of ten (10) commercial chilli peppers (*Capsicum annuum* L.) varieties, cultivated in the local areas of the A.P., State, India, using RAPD markers.

MATERIALS AND METHODS

Capsicum annuum L. varieties G-334, Pusa jwala, Pusa jyothi and Badiga-2 were procured from Agricultural Research Station (ARS), Lam, Guntur, A.P. India and varieties Gemini, Agnirekha, Mangala, Sindura, Teja (Branch) and Black diamond were procured from ARS, Malyal, Warangal, A.P. India. These varieties were grown in our research field and young leaves without blemish were used for genomic DNA extraction.

DNA extraction

Genomic DNA was extracted from leaf tissue of the above mentioned 10 *C. annuum* L. varieties, using the method of Doyle and Doyle (1990). Briefly, 1 g of leaf tissue was ground with a pestle and mortar in 1.5 ml extraction solution (1.2 M NaCl, 35 mM EDTA (pH 8.0), 150 mM Tris-HCl (pH 8.0) and 2% β-mercaptoethanol). The sample was incubated at 45°C for 1 h with frequent shaking. After incubation, the sample was cooled to room temperature and chloroform and isoamyl alcohol (24:1) was added, centrifuged at 8000 rpm for 10 min. The supernatant was collected and mixed with equal volume of cold isopropyl alcohol and incubated for 5 min at -20°C. After centrifugation, the pellet was dried, washed and dissolved in TE buffer and was used in PCR amplification experiments.

RAPD-PCR amplification

RAPD-PCR amplification was performed by the method of Williams et al. (1990). In this experiment, 03 RAPD primers viz., with base sequence 5' TGCCGAGCTG 3' (OPAB02), 5' GGGCGACTAC 3' (OPAB9) and 5'-GGAGTGCCTC-3'(OPP13) (Annealing temperature, 32°C) was used. The PCR reactions were setup in a 20 µl reaction containing 60 ng genomic DNA (10 varieties each), 1X PCR reaction buffer containing (10 mM Tris hydrogen chloride (pH 8.2), 50 mM Potassium chloride and 0.2% gelatin), 2 µl of 0.2 mM dNTPs (Fermentas, USA), 1.5 mM $MgCl_2$, 0.8 µM primer, 1 µl of 0.3 units of *Taq* DNA polymerase. Amplification was performed on a thermal cycler (Model No. TC-3000 Techne, USA) with a programme of initial denaturation at 94°C for 10 min, followed by 32 cycles of 94°C for 1 min, 32°C for 1 min and 72°C for 2 min, followed by final extension at 72°C for 10 min and finally the amplification produced was stored at -20°C.

Agarose gel electrophoresis (AGE)

The RAPD-PCR products were electrophoresed on 1 % Agarose gel, in 1X TBE buffer at 60 V for 3 h. The size of the amplicons was estimated using 0.1 -10 kb DNA ladder as standard (Fermentos, USA). DNA bands were visualized using Gel Documentation System (Bio-Rad, USA) and photographed. The experiment was repeated thrice to establish reproducibility of DNA banding pattern.

Dendrogram analysis

A dendrogram was constructed on the basis of similarity matrix data by Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis using indigenous software by the method of Sitthiwong et al. (2005). Data were generated by RAPD analysis by the method of Nei and Li (1979) which excludes negative data on this equation. Similarity = $2 N_{ab}/(N_a+N_b)$, where N_a= number of scored amplified fragments in genotype a and N_b= number of scored amplified fragments in genotype b and N_{ab}= number of scored amplified fragments with the same molecular weight shared between genotypes a and b.

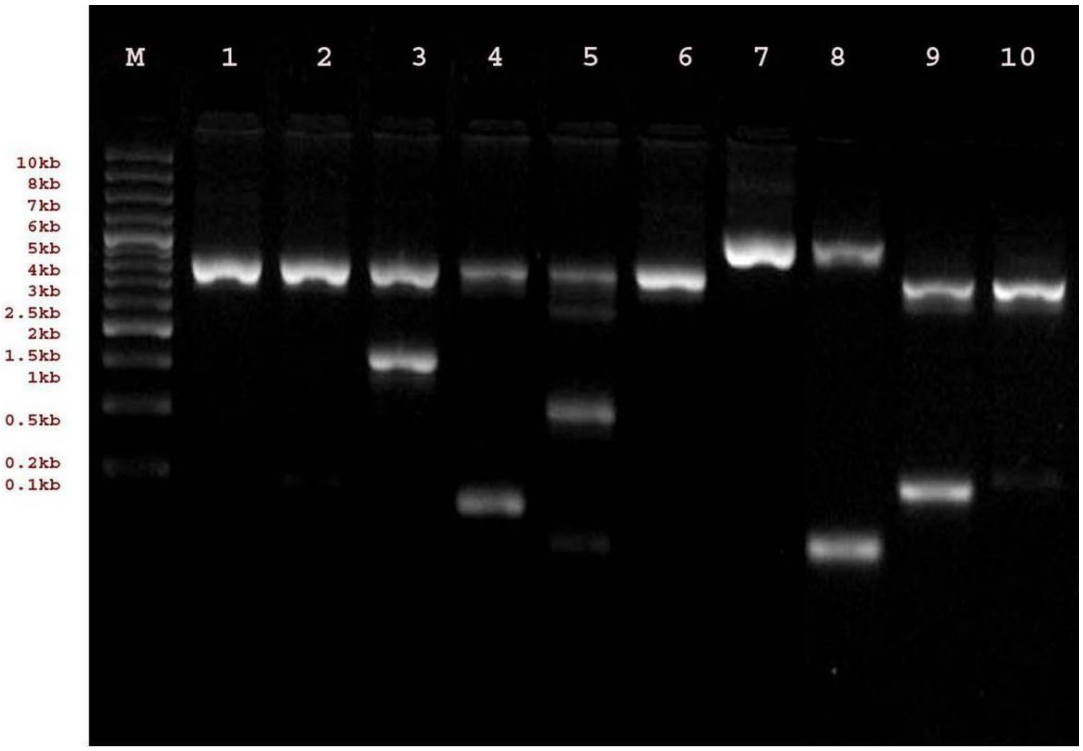

Figure 1. RAPD profiles for 10 *Capsicum annuum* varieties amplified with by primers OPAB02 (bottom): Lane 1; Gemini; Lane 2, G-334; Lane 3, Agnirekha; Lane 4, Pusa jwala; Lane 5, Mangala; Lane 6, Black diamond; Lane 7, Sindura; Lane 8, Pusa jyothi; Lane 9, Badiga-2; Lane 10, Teja (Branch).

Table 1. Number and size of RAPDs in 10 *C.annuum* L.varieties.

S/N	*C.annuum* variety	Number of bands	Size of bands (kb)
1	Gemini	1	4.1
2	G-334	1	4.1
3	Agnirekha	2	4.1, 1.2
4	Pusa jwala	2	4.1, 0.07
5	Mangala	4	4.1, 2.5, 0.5, 0.02
6	Black diamond	1	4.1
7	Sindura	1	5.1
8	Pusa jyothi	2	5.1, 0.02
9	Badiga-2	2	4.1, 0.07
10	Teja (Branch)	2	4.1, 0.08

RESULTS AND DISCUSSION

RAPD-PCR analysis

In the present study, genetic variability among 10 commercial chilli pepper varieties viz. Gemini, G-334, Agnirekha, Pusa jwala, Mangala, Black diamond, Sindura, Pusa jyothi, Badiga-2 and Teja (branch), cultivated in local areas of Andhra Pradesh State, India, using RAPD markers was examined. Out of 03 RAPD markers used for screening 10 chilli pepper genotypes, DNA amplification was observed only with OPAB02 (Figure 1).

OPAB02-PCR product measuring 4.1 kb with reference to DNA ladder was observed in 8 varieties, viz. Gemini, G-334, Agnirekha, Pusa jwala, Mangala, Black diamond, Badiga -2 and Teja (branch) and OPAB02-PCR product measuring 5.1 kb was observed in two varieties, viz. Sindura and Pusa jyothi (Table 1).

OPAB02 primer produced monomorphic band in four

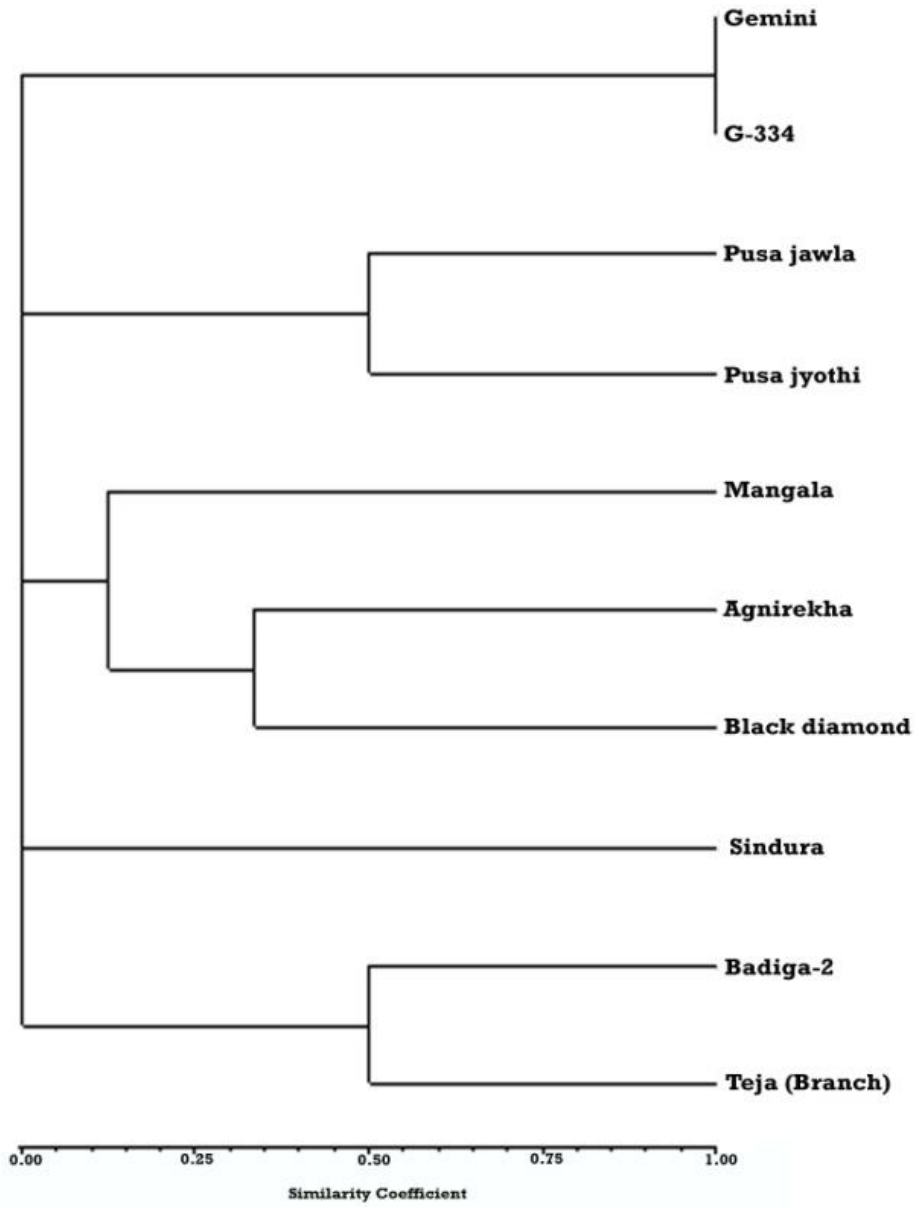

Figure 2. Dendrogram cluster analyses (UPGMA) of *Capsicum annuum L.* varieties

varieties, viz. Gemini,G-334 and Black diamond and Sindura and polymorphic bands in 6 varieties, viz. Agnirekha, Pusa jwala, Mangala, Pusa jyothi, Badiga-2 and Teja (Branch). Some genotypes produced specific bands which could be used as a marker to distinguish between them. Similar results were reported when OPAB02 primer was used to amplify genomic DNA of 10 commercial chilli pepper varieties cultivated in the local area of Karnataka State (Makari et al., 2009).

Dendrogram analysis

Dendrogram generated by OPAB02 primer showed that 10 chili pepper varieties could be grouped into four clusters (Figure 2) .Average genetic similarity index revealed 100% similarity between varieties of first cluster, viz. Gemini and G-334 and which was consistent with in plant height, flower colour, fruit length and orientation (Table 2). The number and molecular weight of OPAB02 generated DNA band in the first cluster was also similar (Table 1). These two varieties might have originated from a common genetic stock (Tiwari, 2009).

Average genetic similarity index generated by RAPD marker OPAB02 revealed 50% similarity between varieties of second cluster, viz. Pusa jwala and Pusa jyothi was consistent in flower colour and fruit orientation (Table 2). The number of OPAB02 generated DNA bands

Table 2. Morphological characters of 10 *C.annuum* varieties.

Variety	Plant height (cm)	Flower colour	Fruit size	Fruit Orientation	Origin and cultivated in
Gemini	78	White	Medium to Long	Pendent	Andhra Pradesh
G-334	89	White	Long	Pendent	Andhra Pradesh
Agnirekha	40	Purple	Short	Pendent	Andhra Pradesh
Pusa jwala	78	White	Medium	Pendent	Andhra Pradesh
Mangala	112	White	Long	Pendent	Andhra Pradesh
Black diamond	80	White	Small	Erected	West Bengal and Andhra Pradesh
Sindura	47	White with yellow spots	Long	Pendent	Maharashtra and Andhra Pradesh
Pusa jyothi	102	White	Long	Pendent	Andhra Pradesh
Badiga-2	103	White	Long	Pendent	Karnataka and Andhra Pradesh
Teja (Branch)	115	White	Long	Pendent	Andhra Pradesh

bands in the second cluster was same, but the molecular weight of the bands was variable (Table 1). Pusa jwala and Pusa jyothi varieties have been bred in IARI, New Delhi (Tewari, 1991).

Average genetic similarity index generated by RAPD marker OPAB02 revealed 50% similarity between varieties of fourth cluster**,** viz. Badiga-2 and Teja (branch) and consistency in flower colour, fruit length and fruit orientation was noticed (Table 2). The number of OPAB02 generated DNA bands was similar, but small deviation in molecular weight was observed (Table 1). Badiga-2 has been bred in Karnataka and Teja (branch) in A.P.

Average genetic similarity index generated by RAPD marker OPAB02 revealed 32% genetic similarity between varieties of third cluster, viz. Mangala, Agnirekha and Black diamond. There was no similarity in morphological characters - purple flower and short and pendent fruit in Agnirekha and white flower and long fruit and pendent fruit in Mangala and white flower and medium and erect fruit in Black diamond (Table 2). Moreover, the number and molecular weight of OPAB02 generated DNA bands was higly variable (Table 1). Agnirekha and Mangala originated in A.P and Black diamond originated in West bengal.

C.annuum L. variety Sindura formed a divergent cluster from others and was of short height (47 cm), possessed white flowers with yellow spots, fruit was long and of pendent orientation. White flowers with yellow spots was found in *Capsicum baccatum* group (Walsh and Hoot, 2001). UPGMA cluster data can be used in chilli pepper breeding programmes and germplasm conservation.

Thus, OPAB02 marker can be useful in identification of genetic variabiltiy in 10 commercial chilli pepper varieties, viz., Gemini, G-334, Agnirekha, Pusa jwala, Mangala, Black diamond, Sindura, Pusa jyothi, Badiga-2 and Teja (branch), cultivated in the local areas of Andhra Pradesh State, India, and the UPGMA cluster data can be used in breeding programmes.

Future studies are aimed at using molecular markers for pungency phenotyping , because the commercial value of chilli pepper is based on pungency level.

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