Evaluation of intra species genetic variability of Capsicum annuum L. through RAPD marker

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Abstract

In the present investigation, a total eight varieties of Capsicum annuum L. were used for evaluation of intra species genetic variability through RAPD molecular marker. Total 15 primers were tested for vatietal identification, out of which 8 gave maximum scorable bands, in which 6 primers showed polymorphism. A total of 89 polymorphic amplified bands were obtained from 10 decamer RAPD primers, which discriminated all the varieties. The PIC value ranged from 0.4281 to 0.9843. Based on the jaccard's similarity coefficient, the similarity index was observed in the range of 0.210to 0.894. By using RAPD marker, tested varieties of capsicum annuum L. could provides key platform for further crop improvement and cross breed.

Key words: *Genetic diversity, RAPD, Capsicum annuum L., UPGMA*

Introduction

Chilli (Capsicum annuum L.) popularly known as hot, bell and sweet pepper belongs to genus Capsicum of Solanaceae family. It is diploid (2n = 2 x = 24), annually worldwide cultivated and short-lived perennial plant. The genus Capsicum consists of approximately 22 wild and 5 cultivated species. The cultivated species include C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens. 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.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. Recent medical research has also documented antimicrobial and antifungal activity of capsaicin obtained from several Capsicum species, and on-going studies are exploring its use in cancer treatment.

Since form time of evolution, the role of genetic diversity in different ecosystem has its own importance for sustaining the existence of living system. It deals with the total number of Genetic genetic characteristics in the genetic makeup of a species.In present time, genetic diversity in vegetable crops has its own importance to maintain the variability in important traits and characteristic. Same time genetic diversity is usually mentioned with reference to agriculture and maintaining food security.Molecular markers are also widely utilized in the plant production sector of the developing world even if the present uptake of molecular marker technologies does not reflect their actual potential. Molecular markers have proven useful for assessment of genetic variation in germplasm collections (Hausmann et al., 2004 and Maccaferri et al., 2006). During the one and half decades, molecular markers have been entered the scene of genetic improvement in a wide range of vegetable crops.

Randomly amplified polymorphic DNA (RAPD) has been widely used for the identification of genetic relationship between cultivars [(Faruque et al., (2011); Makari et al., (2009) and Biswas et al., (2009)], for species identification (Welsh and McClelland 1990). it is a PCR based technique for identifying genetic variation.The molecular markers, such as RAPD (Randomly Amplified Polymorphic DNA), do not depend on the environmental conditions and are present in all plant parts (Rom et al. 1995). They can identify a great number of polymorphisms that allow the

distinction among accessions and the identification of possible duplicates (Bastianel et al. 1998). Simplicity, cheapness and quick results are the main advantages of RAPD and make it efficient to analyze the genetic diversity in germplasm collections. The technique has been successfully used to distinguish accessions, to evaluate genetic diversity among them and to recognize duplications in germplasm collections (Waycott and Fort 1994, Virk et al. 1995, Daher et al. 2002, Teixeira-Cabral et al. 2002, Picoli et al. 2004, Palomino et al. 2005).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

Plants materials

Eight varieties of Capsicum annuum L. (chilli) viz., GVC111(GC111), GCA283(GC283),AVNPC131(AVN131), JWALA(JAW) procured from Vegetable Research Department, Anand Agriculture University (VRD,AAU) and Gayatri(GAYT), Nisha(NISHA), Picador(PICA), Omega(OMEG) procured from Anand seeds market. Seeds materials were properly sowing in pots, once they acclimatized; young leaves were used for detecting genetic variability within and among the tested varieties of chilli by RAPD method.

Genomic DNA Isolation

Genomic DNA was extracted from young leaf tissue following the procedure given by Doyle with some modification. DNA quality and quantity were assessed on a 0.8 % (w/v) agarose gel stained with ethidium bromide and also by using a NanoDrop® ND-1000 spectrophotometer.

RAPD analysis

A total of 10 decamer primers were used for RAPD analysis. Polymerase chain reaction were performed in 25 µl system containing 2.5 µl 10 X assay buffer (10mM Tris -Cl , pH-9.0 , 1.5 mM Mgcl2, 50 mM Kcl and 0.01 % gelatin), 0.5 µl Tag DNA polymerase (Bangalore Genei pvt. Ltd.), 0.5 µl of each dNTPs (dATP , dTTP , dCTP , dGTP), 2 µl of primer & 2 µl of template DNA. 17.7 µl PCR water. DNA amplification was performed in a Thermal Cycler (Eppendorf).The PCR program was started with an initial cycle of 94°C for 4 min followed by 40 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C. Finally, extension was performed at 72°C for 7 min. PCR products were examined by electrophoresis on a 1.5 % agarose gel containing ethidium bromide (4µl per 100 ml) at 100V for 1-2 h in 0.5x Tris-borate-EDTA buffer. The amplified DNA fragments were observed under the UV Transilluminator (Lab net India).

Data Analysis

The presence/absence of bands in RAPD was recorded in binary form in 1 and 0 respectively. All the bands (polymorphic and monomorphic) were taken into account for calculation of similarity with a view to avoid over-/underestimation of the distance (gherardi *et al*., 1998). Jaccard's coefficients of similarity (jaccard, 1908) was measured and a dendrogram based on similarity coefficients generated by the unweighed pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and SAHN clustering. The statistical analysis was done using the computer package NTSYS-PC (Rohlf, 1997).

Result and Discussion

In the present finding, genomic DNA extracted from eight tested varieties of Capsicum annuum L. were subjected to eight RAPD primers to study the genetic diversity among the varieties, various parameters were taken under consideration to establish the phylogenetic relationship viz., polymorphic bands, monomorphic bands, PIC (polymorphic Index content), and percentage polymorphism, 2D & 3D plots schematically and diagrammatically presented the scenario in a definitive form.

For individual tested varieties of chilli the number of polymorphic bands generated by distinct primers ranged between 6 and 24, forming a total of 89 polymorphic bands and 03 monomorphic bands (Table no 1).

Table no. 1 List of primers, their sequences, total number of amplified fragments and number of polymorphic bands generated by PCR using 08 RAPD primer for selected varieties of Capsicum annuum L.

sr.no	primer	sequence	Tm	GC %	Total Bands	Monomorphic Bands	Polymorphic Bands	PIC Value	perecentage
1	OPA-1	5'-CAGGCCCTTC-3'	38.2	70	12	00	12	0.4375	100
2	OPA-2	5'-TGCCGAGCTG-3'	42.4	70	08	00	08	0.000	100
3	OPA-4	5'-AATCGGGCTG-3'	39.3	60	12	00	12	0.4281	100
4	OPA-7	5'-GAAACGGGTG-3'	34.5	60	06	00	06	0.4375	100
5	OPA-8	5'-GTGACGTAGG-3'	22.9	60	24	02	22	0.6510	91.6
6	OPA-9	5'-GGGTAACGCC-3'	38.7	70	11	00	11	0.4921	100
7	OPA-10	5'-GTGATCGCAG-3'	29.8	60	08	01	07	0.9843	88.5
8	OPA-13	5'-CAGAACCCAC-3'	27.7	60	11	00	11	0.4296	100
total					92	03	89		

A total of 92 clear and reproducible bands were amplified with different lengths from tested varieties of chilli using the 08 selected RAPD primers, of which 89 (91.98%) were polymorphic and remaining 03 (8.1%) were monomorphic which is in agreement with the findings of Lanteri et al., (2003) who reported 41.6 % polymorphism and with the findings of Adetula, (2006) who reported 67.67 % polymorphism. Vazquez et al. (1996) also studied the RAPD fingerprinting in different chilli varieties and showed 45% polymorphism.. In our result all the chosen primers amplified with the number of amplified fragments ranging from six to (OPA-7) to 24 (OPA-5) and which varied in size from 200 bp to 2,500 bp and yielded 92 fragments. Average numbers of polymorphic bands per primer as 11.50 and 11.12 respectively. Percentage polymorphism ranged from 87.5% (OPA-10 primer) to a maximum of 100% (OPA-1, 2, 4, 7, 9 and 13 primers). For the identification of varieties, GVC111 and Picador could be identified by using primers OPA-8. However, GCA283 could be identified by using primer OPA-10.

The PIC values, a reflection of allele diversity and frequency among the varieties, were uniformly higher for all the RAPD loci tested. The PIC value ranged from 0.4281 (OPA-04) to 0.9843 (OPA-10) (Table no.1). The Jaccard's similarity analysis depicted a good degree of genotypic diversity existing in the chilli genotypes studied. The minimum and maximum similarity values were 0.210 and 0.842. (Table no. 2).

Table no. 2. Jaccard's coefficient table calculated on the basis of banding traits of all primers in Capsicum annuum L.

rows/COLs	GVC111	GCA283	AVNPC131	Jawala	Gayatri	Nisha	Picador	Omega
GVC111	**1.0000000**							
GCA283	**0.7894737**	**1.0000000**						
AVNPC131	**0.6842105**	**0.6842105**	**1.0000000**					
Jawala	**0.5789474**	**0.5789474**	**0.8947368**	**1.0000000**				
Gayatri	**0.7894737**	**0.8947368**	**0.7894737**	**0.6842105**	**1.0000000**			
Nisha	**0.7368421**	**0.7368421**	**0.7368421**	**0.6315789**	**0.7368421**	**1.0000000**		
Picador	**0.3157895**	**0.2105263**	**0.4210526**	**0.5263158**	**0.2105263**	**0.4736842**	**1.0000000**	
Omega	**0.6842105**	**0.5789474**	**0.7894737**	**0.6842105**	**0.6842105**	**0.8421053**	**0.5263158**	**1.0000000**

UPGMA cluster analysis of all primers:

The results from dendrogram based on similarity coefficients generated by the unweighed pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and SAHN clustering showed presence of high genetic diversity in the chilli cultivars and discriminated all the varieties from each other (Figure no. 4.17). The use of cultivars from various clusters and sub clusters offers the possibility of obtaining an appropriate genetic variability in hybrid populations. The UPGMA dendrogram obtained from the cluster analysis of RAPD divided the cultivars into two main clusters with one stand-alone variety Picador in cluster I, while on the other hand second major cluster again further divided in two sub cluster II A and II B. In case of sub cluster II A comprised of four varieties in two groups, first group holds omega and nisha while in second group jwala and AVNPC131 present. For sub cluster II B, total three varieties present viz. gayatri, GCA283 and GVC111. The second clusters showed to form the largest clusters containing 80.0 % of the population studied. Similar results can be seen in 2-D and 3-D plots as shown in Figure no. 4.18 and Figure no. 4.19 respectively. The dendogram reflect a good genetic analysis, which is based on amplification signals from RAPDs proving that it is a good marker to evaluate the genetic relationships among chilli accessions as previously reported (Prince et al., 1995; Wang et al., 1996; Makari et al., 2009 and Akbar et al., 2010). In last, all these results indicate that the present study using RAPD molecular markers can not only help in identify authenticated varieties of chilli from the false

varieties but also Germplasm characterization provides an important link for conservation through use of the genetic endowment of a species. The systematic description of each accession should lead to classification in small and

well-organized groups of genebank accessions that will facilitate their enhanced utilization. Most of the descriptors, which are identifiable and measurable traits, for germplasm characterization and evaluation are species-specific in vegetable sector.

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