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RESEARCH PAPER

Identification of chilli genotypes through simple sequence repeats (SSR) makers

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Abstract : Investigations was carried out to study the varietal characterization of chilli genotypes through SSR markers. Twelve SSR primer pairs (CM0002, CM0004, CM0005, CM0008, CM0009, CM00010, CM00011, CM00012, HpmsE015, HpmsE016, HpmsE035 and Tom 196) were used in the study. Among them, Tom 196 and HpmsE016 reported highly polymorphic primer. TOM 196 primer was useful for the identification of chilli genotype PLR1 from rest of the genotypes. HpmsE016 was used for identification of chilli genotypes *viz*., CO1, K2, PKM1, KKM1, CA97, Sln1 and CCH1.

Key Words : Simple sequence repeats, Chilli genotypes, Varietal identification polymorphic, Monomorphic

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INTRODUCTION

Chilli (*Capsicum annuum*) is an important vegetable crop grown in the tropical, subtropical as well as temperate regions. India is the largest producer and consumer of chillies in the world with an annual production of around 1.1 million tonnes. Consequently India also has the maximum area to the production of this crop with major states being AP, Karnataka, Maharashtra, UP, Punjab, TN, Rajasthan, Orissa, West Bengal and M.P. There is little genetic diversity among and within the cultivated chilli. However, general knowledge is that commercial varieties, particularly hybrids of vegetable crops are based on increasingly narrower genetic variation.

Varietal identification is gaining importance worldwide in the view of introduction of plant variety protection (PVP). The uniqueness of a variety is established by tests for novelty, distinctness, uniformity and stability (DUS) for which the International Union for the Protection of New Varieties of Plants (UPOV) has provided guidelines in the case of most economically useful plant species which is absent for chillies in India. The tests are mainly based on morphological characters called descriptors obtained by growing the varieties side by side. Since, the traditional method of DUS testing is time consuming and expensive, requiring large hectares of land and skilled personnel (Cooke, 1995), modern approach of DNA fingerprinting using molecular markers like simple sequence repeats (SSR) that enlarge differences among nucleotide sequences are used as they remain unaffected by growth stage and environment. SSR markers represent highly polymorphic, reproducible and co-dominant (Becher *et al*., 2000). They are used in variety identification, genetic purity test and genome mapping (Mc Couch *et al*., 1997). Therefore, the present study was conducted to identify a specific SSR marker to differentiate the selected chilli genotypes.

MATERIAL AND METHODS

Chilli varieties (K 1, K 2, CO 1, CO 2, PMK1, PKM 1, PLR 1, KKM 1, hybrids (CCH 1) and its parental lines (Sln 1 x CA 97) were used for characterization of genotypes. The genomic DNA was isolated from chilli seedling using

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Table A : Primer used in the study and their

Marker	Forward primer	Reverse primer
CM0002 (AT)6	GTACCTATGGGAATAAGCAAA	CCAATTTGTCTGAAGTTGAGT
CM0004 (CT)6	ACAAACATATCTATAGTGCAAATT	ATTGTGCTCTGTCAAAACAA
CM0005 (CCA)8	CATGACCACCATGAGGATA	GATAGCCACGAGCATAGTATT
CM0008 (ATATA)5	F ATAGCTCACATGCCCTATAAA	AATCTTGAGCAATAATTGGAC
CM0009(AT)5	TGAGGTCTGAAAAAGGTAAAG	TTAGTTGAACTTGCAGAATCC
CM0010 (AT)6	TTGGTTTTTGCTACTGGTAAT	AAACTGTCATATATTTGTGTGACT
CM0011 (AC)5(TA)8	TCTGCTTTAAAAACACATACAT	CATTCTAACTGAAATTGCATG
CM0012 (AAT)11	GGGATTTAATAAGGAACAATG	TCAAATATCGACATTAGCATG
HpmsE015 (GCA)8	TTGTGAGGGTTTGACACTGGGA	CCGAGCTCGATGAGGATGAACT
HpmsE016(TACA)3)	CCAAGTTCAGGCCCAGGAGTAA	TGCAGAGAAGACTCACCAGTCC
HpmsE035 (AAG)7	GCTCAATCCATGATCGAGGACA	CTGCAGCTTGGAGTTCCTCTCC
Tom 196	CCTCCAAATCCCAAAACTCT	TGTTTCATCCACTATCACGA

modified Cetyl Tri- methyl ammonium bromide (CTAB) method. Young leaf tissue of germinated twelve day old seedlings was homogenized in liquid nitrogen and added with 500 ìl of CTAB buffer (1.0 M pH 8.0 Tris-HCl, 3 ml NaCl, 0.5 EDTA, 1% PVP-360) incubated at 65°C for 30-45 min followed by addition and blending of 500 ìl of chloroform: isoamyl alcohol mixture (24:1). After centrifugation (20 min, 12,000 rpm), supernatant layer was pipetted out into a new eppendorf tube and added 100 µl sodium acetate (3.0 M), ice cold iso propanol (approximately equal volume) and stored at -20°C overnight. Again eppendorf tube was centrifuged (20 min, 12,000 rpm). Settled DNA pellet was separated upon discarding the supernatant. Later ethanol was added and centrifuged (5min, 12,000 rpm), to remove contaminant and stored after vacuum drying in TE buffer. The DNA quality was checked by agarose gel electrophoresis. Based on the quality data, DNA dilutions were made using 1X TE buffer.

Three SSR primer pairs (Table A, Medox Biotech India Pvt. Ltd, Chennai) were used in the study. In general, primer pairs have been derived from sequence information obtained from published DNA libraries (*www. gramene.org*). The 15 ìl reaction mixture volume consists of 40 ng of template DNA, 1 × PCR buffer with 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.25 ìM each of forward and reverse primers and 1U of Taq DNA polymerase. Thermal cycler was programmed for 30cycles at 94°C (5 min), 50-56°C (40 sec.), 72°C (45 sec.), followed by final extension at 72°C for 7 min. PCR products (7.0-7.5 ìl) were used for electrophoresis on 1.5 per cent agarose gels and stained with ethidium bromide and photographed using gel documentation unit under UV light. The size of the amplified fragments were estimated with the help of Alphaease software utility using 100 bp DNA ladders (Medox Biotech India Pvt. Ltd, Chennai) as standard. A dendrogram showing the distinct clusters among the 11 chilli genotypes was constructed using numerical taxonomy and multivariate analysis system (NTSYS) and similarity coefficients were calculated by simple matching produced by UPGMA (Rohlf, 2005).

RESULTS AND DISCUSSION

The present study utilized 12 SSR markers *viz.*, CM0002, CM0004, CM0005, CM0008, CM0009, CM00010, CM00011, CM00012, HpmsE015, HpmsE016, HpmsE035 and Tom 196 SSR markers for identification of eleven chilli genotypes. Among the 12 SSR markers, Tom 196 and HpmsE016 showed polymorphic primers and others showed monomorphic. Based on above mentioned SSR marker, the genotypes were differentiated. With help of monomorphic markers (CM0002, CM004, CM005, CM008, CM009, CM0010, HpmsE015 and HpmsE035), the genotypes K1, PMK1, Sln1, PLR1, CCH1, CO2 and PKM1 were distinguished. Tom 196 had differentiated only one genotype (PLR1) gave polymorphic banding patterns of two alleles amplified at 200bp and 300bp that were amplified with rest of the genotypes (>700 bp). HpmsE016 produced only one allele in genotypes CO1 (180bp), K2 (180bp), PKM1 (180bp), Sln 1 (200bp) and CCH1 (200bp) and more than one allele (200bp and 220bp) in genotypes (KKM 1 and CA 97) and other genotypes showed null alleles.

The monomorphic SSR markers based dendrogram depicted 100% similarity between CO1, KKM1, K2 and CA 97. The dendrogram was separated into two clusters at 44% similarity level (Fig. 1). Jaccard similarity coefficient of chilli genotypes is given (Table 1). CO2, K2, CA97 and PKM1 formed a separate cluster, whereas remaining seven genotypes were formed the second cluster. The pair wise similarity matrix revealed that 4%, 25%,18% and 7% showed 100%, 80-88%, 75%, 60-67% and 50% similarity level, respectively. Some of the primers showed null alleles.

The polymorphic Tom 196 primer showed 100% similarity between all the genotypes except PLR1. The similarity coefficient ranged from 0% to 100% with the

average similarity coefficient of 50%. The dendrogram was separated into two clusters at 0% similarity. PLR1 formed a separate cluster and remaining genotypes (ten) formed another cluster (Fig. 2). The pair wise similarity matrix (Table 2) revealed that, among 55 comparisons 24%, 21% and 10% showed 100%, 50% and 0% similarity level, respectively. The polymorphic HpmsE016 primer showed 100% similarity between nine genotypes (CO1, CO2, K1, PMK1, PLR1, K2, PKM1, Sln1 and CCH1). The similarity coefficient ranged from 38% to 100% with average similarity coefficient of 69%. The dendrogram was separated into two clusters at 38% similarity level. CA97 and KKM1 formed separate cluster from others (Fig. 3). The pair wise similarity matrix (Table 3) revealed that, among 55 comparisons 20%, 31%, 33%, 12.7% and 3.6% showed 100%, 75%, 50%, 25% and 0% similarity level, respectively. Similar findings related to SSR

Table 1 : Pairwise similarity indices using monomorphic SSR markers in chilli genotypes

	CO1	CO2	K1	K2	PLR1	PKM1	PMK1	KKM1	CA97	Sln1	CCH1
CO1	1.000										
CO2	0.750	1.000									
K1	0.833	0.625	1.000								
K2	0.625	0.875	0.500	1.000							
PLR1	0.500	0.750	0.375	0.625	1.000						
PKM1	0.500	0.375	0.600	0.250	0.286	1.000					
PMK1	0.667	0.500	0.800	0.375	0.250	0.400	1.000				
KKM1	1.000	0.750	0.833	0.625	0.500	0.500	0.667	1.000			
CA97	0.625	0.875	0.500	1.000	0.625	0.250	0.375	0.625	1.000		
Sln1	0.667	0.500	0.800	0.375	0.250	0.750	0.600	0.667	0.375	1.000	
CCH1	0.500	0.375	0.600	0.250	0.286	0.200	0.750	0.500	0.250	0.400	1.000

Table 2 : Pairwise similarity indices using polymorphic SSR marker (TOM 196) in chilli genotypes

	CO1	CO2	K1	K2	PLR1	PKM1	PMK1	KKM1	CA97	Sln1	CCH1
CO1	1.000										
CO2	1.000	1.000									
K1	1.000	1.000	1.000								
K2	1.000	1.000	1.000	1.000							
PLR1	1.000	1.000	1.000	1.000	1.000						
PKM1	0.000	0.000	0.000	0.000	0.000	1.000					
PMK1	1.000	1.000	1.000	1.000	1.000	0.000	1.000				
KKM1	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000			
CA97	0.500	0.500	0.500	0.500	0.500	0.000	0.500	0.500	1.000		
Sln1	0.500	0.500	0.500	0.500	0.500	0.000	0.500	0.500	1.000	1.000	
CCH1	0.500	0.500	0.500	0.500	0.500	0.000	0.500	0.500	1.000	0.100	1.000

Table 3: Pairwise similarity indices using polymorphic SSR marker (HpmsE016) in chilli genotypes

	CO1	CO2	K1	K2	PLR1	PKM1	PMK1	KKM1	CA97	Sln1	CCH1
CO1	1.000										
CO2	1.000	1.000									
K1	1.000	1.000	1.000								
K2	0.750	0.750	0.750	1.000							
PLR1	0.750	0.750	0.750	1.000	1.000						
PKM1	1.000	1.000	1.000	0.750	0.750	1.000					
PMK1	1.000	1.000	1.000	0.750	0.750	1.000	1.000				
KKM1	0.500	0.500	0.500	0.250	0.250	0.500	0.500	1.000			
CA97	0.250	0.250	0.250	0.000	0.000	0.250	0.250	0.750	1.000		
Sln1	0.750	0.750	0.750	0.500	0.500	0.750	0.750	0.750	0.500	1.000	
CCH1	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	1.000

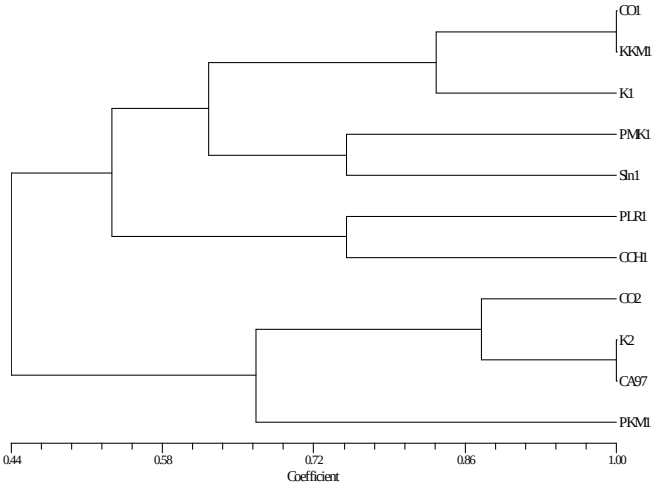

Fig. 1 : Dendrogram representing the grouping of eleven chilli genotypes formed through UPGMA based on monomorphic SSR markers

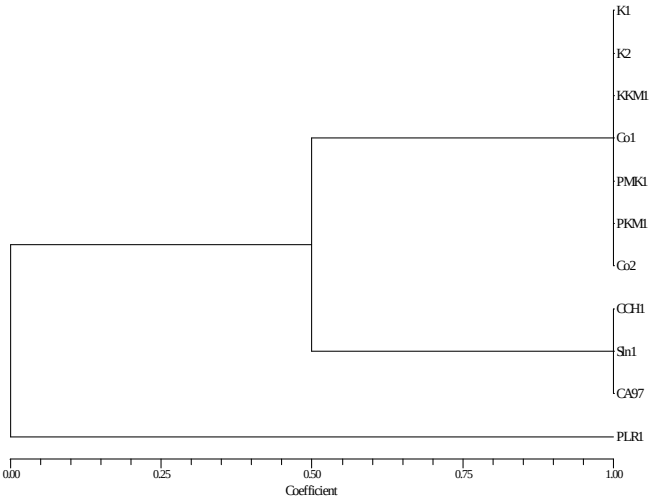

Fig. 2 : Dendrogram representing the grouping of eleven chilli genotypes formed through UPGMA based on polymorphic SSR marker (Tom 196)

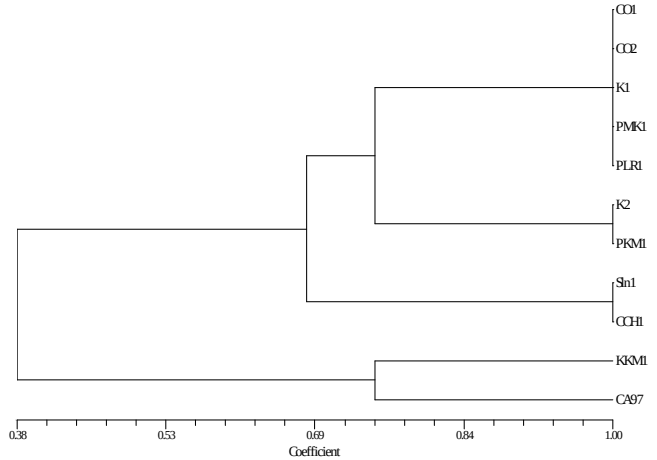

Fig. 3 : Dendrogram representing the grouping of eleven chilli genotypes formed through UPGMA based on polymorphic SSR marker (HpmsE016)

marker used for genotypes characterization have been reported by McCouch *et al*. (1997); Yashitola *et al*. (2002); Nandakumar *et al*. (2004); Sundaram *et al*. (2008) in rice and also SSR marker have been developed for pepper by Jang *et al*. (2004); Kang *et al*. (2001); Lee *et al*. (2004); Lefebvre *et al*. (2001); Moon *et al*. (2003); Paran *et al* (1998) and Prince *et al*. (1992). Aranzana *et al*. (2003) characterized the two hundred cultivars of peaches and nectarines using a selected set of 16 microsatellite markers. Simple sequence repeats (SSR) primer were widely used in cultivar identification of tomato (Martina *et al*., 2009), chilli (Lekha *et al*., 2001); egg plant (Stagel *et al*., 2008) and grapevine (Galbacs *et al*., 2009).

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