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Screening of SCAR primer for pungency and non-pungency in chilli (*Capsicum annuum* L.)

Vivek Kumar Singh, Kartikeya Srivastva, Rajesh Singh, Ved Prakesh Rai

ABSTRACT

The Chilli strains (*Capsicum annuum* L.) were screened for pungency and non-pungency using a set of nine primers in five different combinations. Two primers combinations of CSF-1 and CSR-2, CSF-1 and CSR-4 were found to be robust primers capable of very low capsaicin detection. The study indicated that the SCAR primers provide simple, fast and economic means of early detection of pungency in capsicum.

Keywords: Germplasm, Pungency, SCAR Markers

Introduction

Pepper, which is commonly known as chilli (*Capsicum* sp.) is one of the most cultivated vegetable and spice crop worldwide, and plays an important role as an ingredients in many of food industry (Bosland and Votata, 2000). *Capsicum annuum* L. also known as hot pepper, chilli, chilli pepper and bell pepper etc, is a dicotyledonous flowering plant and grown on more than 1.5 million hectares worldwide (FAO, 2007). Chilli fruits are rich source of vitamins A, C, E and also a good source of oleoresin. Chilli is diversely used as spice, condiments, culinary supplement, medicine, vegetable and ornamental plants.

The main source of pungency in peppers is the chemical group of alkaloid compounds called capsaicinoids (CAPS), which are produced in fruits. The variety of Chilli differs widely in capsaicin contents. Pungency is measured as Scoville Heat Units (SHU) in given dry weight of fruit tissue. Pungency is unique property and major traits for pepper breeders. One of the goals of pepper breeding program is to control the pungency of the fruits in order to fulfill the various requirements of the industry and of the consumers (Yoon *et al*. 1992). For breeding sweet peppers, accurate selection of non-pungent line is important. The phenotying of pungency depends on the panel tasting of fruit and HPLC analysis. These methods require a great deal of time and labor. Marker Assisted Selection (MAS) may allow pungency phenotyping in early stages of plants development thereby making identification in large population easy, faster and more economical. Sequence characterized amplified regions (SCARs) generated by specific primers can produced reliable PCR data that can be used for MAS (Paran and Michelmore, 1993). In this present investigation, we explored the screening of SCAR marker for pungency and non-pungency in Chilli (*capsicum annuum* L.) using selected seven pungent and non-pungent genotypes.

Vivek Kumar Singh (✉)
The Energy and Resources Institute (TERI), India
e-mail: vivekbhu@gmail.com

Kartikeya Srivastva
Genetics and Plant Breeding, IAS, BHU, Varanasi-221005

Rajesh Singh
Genetics and Plant Breeding, IAS, BHU, Varanasi-221005

Ved Prakesh Rai
Indian Institute of Vegetable Research, Varanasi-221005

Material and Method

Fifteen diverse inbreds of the Chilies were obtained from Indian Institute of Vegetable Research, Varanasi (Table 1). Seeds were grown during *Rabi* 2009-2010 at Vegetable Research Farm, Department of Horticulture, Institute of Agricultural Science, Banaras Hindu University, Varanasi (U.P.), in randomized block design with three replications with

plot size of three rows of 5 meter and spacing of 60 cm x 45 cm. Recommended agronomic practices were followed to raise a good crop.

D^2 Analysis (Clustering of Genotypes)

The 15 lines were grouped into a number of clusters by Tocher's method. The criterion used in the clustering by this method was that any two varieties belonging to the same clusters. Inter and Intra cluster distances were determined and their relationship was diagrammatically represented. Based on pungency and non-pungency performance and genetically diverse genotypes classified through cluster analysis seven (Table 2) representative genotypes were selected. The seven selected genotype LCA 224 (Pungent), BS-35 (Pungent), PBC 367(Pungent), LCA 442(Pungent), Arka Abhir (Non-pungent), SKAUP-7(A) (Non-pungent), Tomato chilli (Non-pungent) were selected from cluster I, II and III given in Table 3.

Table1 The name and source/pedigree of 15 Chilies genotypes.

Genotypes	PEDIGREE/Source of Genotypes
LCA224	APAU Lam Research Station, Guntur
LCA440	APAU Lam Research Station, Guntur
LCA443	APAU Lam Research Station, Guntur
LCA357	APAU Lam Research Station, Guntur
LCA414	APAU Lam Research Station, Guntur
LCA442	APAU Lam Research Station, Guntur
PBC367	AVRDC, Taiwan
KADDI	Local Collection from Dharwad
SKAUP-7(A)	Sher E Kashmir University of Agriculture
ARKA ABHIR	Indian Institute of Horticulture Research, Bangalore
TOMATO CHILLI	Local collection from Warangal
BS-35	INGRO7039 (Local collections) maintained by IIVR, Varanasi
ACS-200104	Gujarat Agriculture University, Ananad , Gujarat
Capsicum-19	Solan, Himachal Pradesh
ACS-200101	Gujarat Agriculture University, Ananad , Gujarat

Table 2 List of nine arbitrary SCAR primers along with the sequence and size.

Primer	OLIGO NUCLEOTIDE	TEMP (°C)	SIZE (nt base pair)	SOURCE OF SEQUENCE
CSF-1	5ATGGCTTTGCATTACCAT 3	54.4	21	
CSR-2	5CCT TCACAA TTA TTCGCC CA-3	55.2	20	
CSR-3	5-CCA ACA AGG GCA GAAGTT GT-3	53.8	20	CS(SB2-66)
CSR-4	5-TCA AAC ACC ACA AAGAC TTG G-3	54.0	22	
BF-5	5-CCA TGA CCA CAT CAT TTT GG-3	53.0	20	
BF-6	5-GAA AGA TCCGAC CTC GTC AA-3	53.7	20	
BR-7	5-GGG GTT GGG TAG AGG TTG TT-3	54.0	20	BAC667116
BR-8	5-TGA CAC CAA TAA GTG GAG TGC T-3	53.4	22	
BR9	5-GAC AAA CAA TAA TGG ACG ATG G-3	53.2	22	

Table 3 Clustering pattern of 15 Chilli strains based on D^2 analysis of 13 traits.

Cluster No.	No inbred lines	Name of inbred lines
Cluster-I	9	***Arka abhir,*** ACS-200101, LCA-442, LCA-224, LCA-357, LCA-443, ***Tomato chilli,*** PBC-367, ***Kaddi***
Cluster-II	1	***SKAUP-7(A)***
Cluster-III	3	LCA-440, CAPSICUM-19, BS-35
Cluster-IV	1	ACS-200104
Cluster-V	1	LCA-414

In above table the lines in italics are non-pungent and rest are pungent genotypes

DNA Isolation

The DNA was extracted out of all the 15 genotypes mentioned above. Genomic DNA was isolated using Chromous Biotech Plant Genomic DNA extraction Kit. Concentration and quality of genomic DNA preparations was determined using spectrophotometer at 260nm/280nm wave length. The quality of DNA of all the genotypes was of high order.

PCR Amplification and Gel Electrophoresis

The DNA of all seven selected genotypes (Choong – Jae Lee *et al*.., 2005) were subjected to PCR amplification. 25µl reaction mixture contained 1 µl template, 1 µl primer (F+R), 1.5 µl d NTPs 0.5µl taq DNA polymerase and 2.5 µl 1 X taq assay buffer were used for PCR reaction. DNA was amplified in eppendorf tube. Amplified DNA samples were analyzed by 1% Agarose gel electrophoresis. DNA fragments amplified by selected presences or absence of particular band.

Results and Discussion

Validation of primer pair BF 6 and BR 8

The primer pair BF 6 and BR 8 amplified clear and distinguishable bands in all pungent and non-pungent genotypes. A band of 1030 bp was amplified in all pungent genotypes while a band of 1060 bp to 264 bp from non-pungent genotype (Figure-4.1).

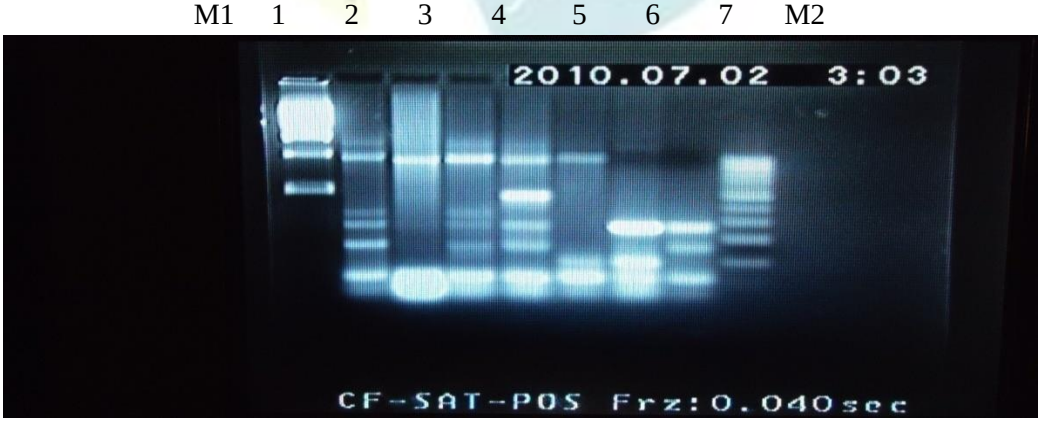

Figure 1 Electrophoretic patterns of the seven pungent and non-pungent genotypes with the primer pair BF 6 and BR 8. Lane M1 and M2 show the 500 bp and 100DNA ladder marker. (Lane Number(LN)1 correspond to-LCA-224 (Pungent), LN-2:BS-35(pungent), LN-3:PBC-367(Pungent),LN-4:LCA-442(Pungent), LN-5:Arka-Abhir (Non-pungent), LN-6:SKAUP-7(A)(Non-pungent)and LN-7: Tomato chilli (Non-pungent).

Validation of primer pair BF 7 and BR-9

The primer pair BF 7 and BR 9 amplified clear and distinguishable bands in all pungent and non-pungent genotypes. A bands of 900 bp was amplified in all pungent genotypes and one non-pungent (Arka-Abhir), while no band obtained from non-pungent genotype (Fig 4.2).

Validation of primer pair CSF-1 and CSR-4

The primer pair CSF-1 and CSR-4 amplified clear and distinguishable bands in all pungent and non-pungent genotypes. A band of 1334 bp was amplified in all pungent genotypes and while no band obtained from non-pungent genotype(Fig 4.3).

Validation of primer pair CSF-1 and CSR-2

The primer pair CSF-1 and CSR-2 amplified clear and distinguishable bands in all pungent and non-pungent genotypes. A band of 410 bp was amplified in all pungent genotypes and while no band obtained from non-pungent genotype.(Fig 4.4).

Validation of primer pair BF-5 and BR-8

The primer pair BF-5 and BR-8 amplified clear and distinguishable bands in all pungent and non-pungent genotypes. A band of 1120 bp was amplified in all pungent genotypes and one non-pungent (Arka Abhir) genotype and while no band obtained from non-pungent genotype.

A set of nine SCAR markers were used in five different combinations to screen the pungency and non-pungency among the seven genotypes mentioned above.. Varieties LCA-224, BS-35, PBC-367, LCA-442 were found to be pungent and Arka Abhir, SKAUP-7(A), Tomato chilli were found to be non-pungent. Kim and Kim (2005) developed SCAR markers of 607 and 708 bp length and used it to distinguish between N and S cytoplasm of chillies. Tanaka *et al*. (2009) used SCAR to screen the F_2 population created by crossing Himo and No.3446, a pungent accession. SCAR markers are characterized by many advantages including their specificity, low cost, ease and fast use. SCAR markers have been employed with success in plant and animal species identification (Parent and Page 1998, Mariniello *et al.* 2002, Yau *et al.* 2002, Bautista *et al*. 2003). Usually SCAR markers have been developed from RAPD fingerprints (e.g. Parasnis *et al.* 2000, Koveza *et al*. 2001, Arnedo-Andrés *et al.* 2002, Bautista *et al.* 2003) or from AFLP fingerprints (e.g. Negi *et al.*2000, Xu and Korban 2002, Schmidt *et al.* 2003).

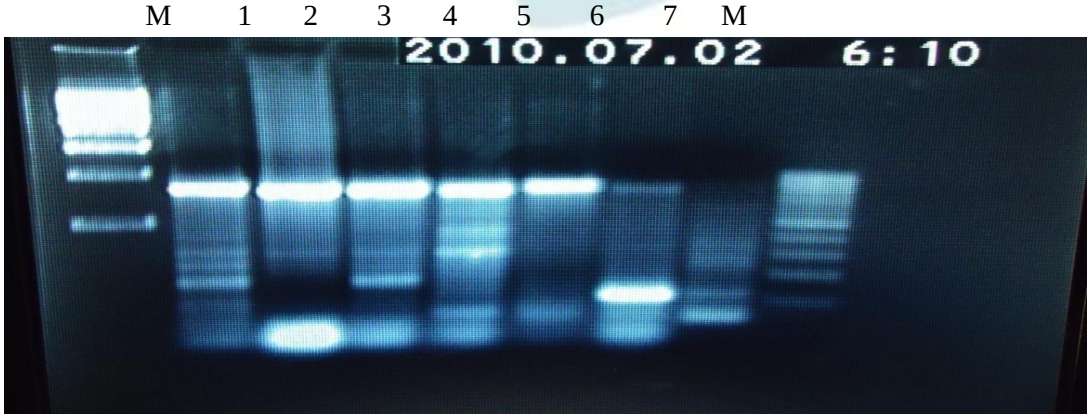

Figure 2 Electrophoretic patterns of the seven pungent and non-pungent genotypes with the primer pair BF 7 and BR 9. Lane M1 and M2 shows the 500 bp and 100DNA ladder marker, respectively. (Lane Number(LN)1 correspond to-LCA-224 (Pungent), LN-2:BS-35(pungent), LN-3:PBC-367(Pungent),LN-4:LCA-442(Pungent), LN-5:Arka-Abhir (Non-pungent), LN-6:SKAUP-7(A)(Non-pungent)and LN-7:Tomato-chilli(Non-pungent).

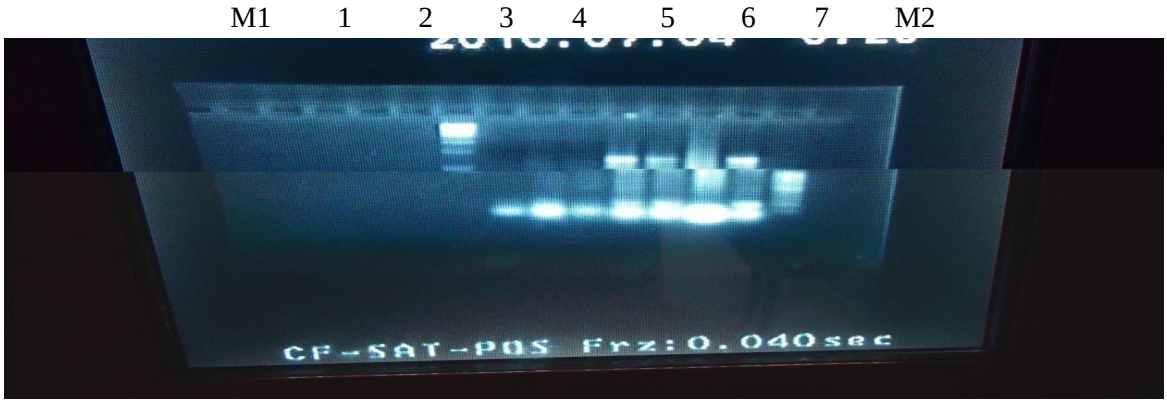

Figure 3 Electrophoretic patterns of the seven pungent and non-pungent genotypes with the primer pair CSF 1 and CSR 4. Lane M1 and M2 show the 1000 bp and 100DNA ladder marker respectively. (Lane Number(LN)1 correspond to-LCA-224 (Pungent), LN-2:BS-35(pungent), LN-3:PBC-367(Pungent),LN-4:LCA-442(Pungent), LN-5:Arka-Abhir (Non-pungent), LN-6:SKAUP-7(A)(Non-pungent)and LN-7: Tomato chilli (Non-pungent)

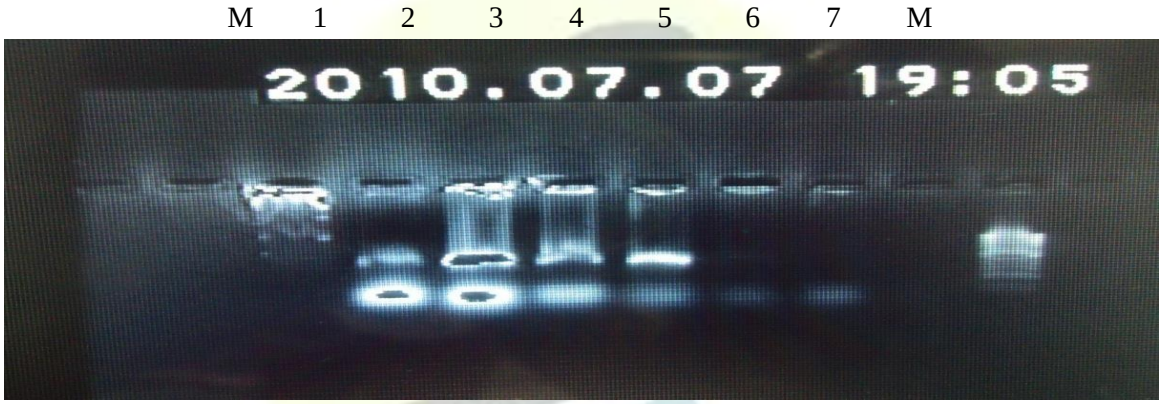

Figure 4 Electrophoretic patterns of the seven pungent and non-pungent genotypes with the primer pair CSF-1 and CSR-2. . Lane M1 and M2 show the 1000 bp and 100 DNA ladder marker respectively. (Lane Number(LN)1 correspond to-LCA-224 (Pungent), LN-2:BS-35(pungent), LN-3:PBC-367(Pungent),LN-4:LCA-442(Pungent), LN-5:Arka-Abhir (Non-pungent), LN-6:SKAUP-7(A)(Non-pungent)and LN-7: Tomato chilli (Non-pungent).

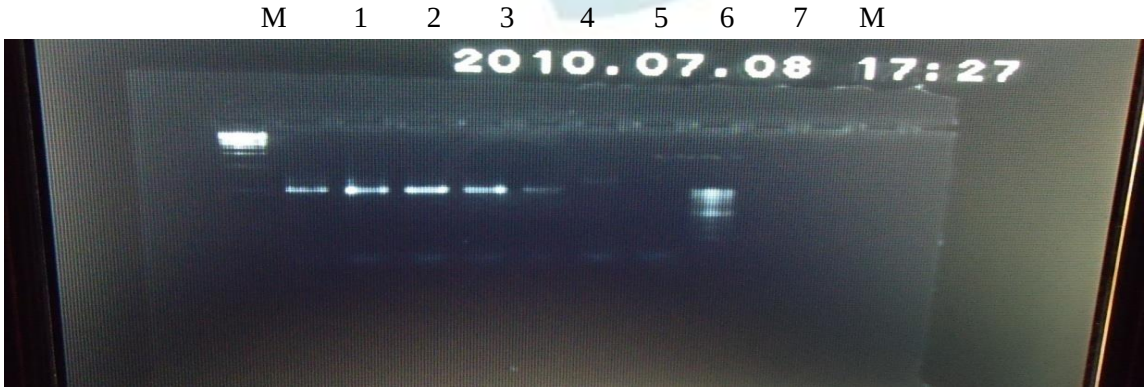

Figure 5 Electrophoretic patterns of the seven pungent and non-pungent genotypes with the primer pair BF 5 and BR 8. . Lane M1 and M2 show the 1000 bp and 100DNA ladder marker respectively. . (Lane Number(LN)1 correspond to-LCA-224 (Pungent), LN-2:BS-35(pungent), LN-3:PBC-367(Pungent),LN-4:LCA-442(Pungent), LN-5:Arka-Abhir (Non-pungent), LN-6:SKAUP-7(A)(Non-pungent)and LN-7: Tomato chilli (Non-pungent).

In the present study the SCAR markers has been successfully used to differentiate the pungent and non pungent genotypes. Molecular markers based on DNA amplification using the polymerase chain reaction (PCR) are relatively easy to use (Saiki *et al*., 1987) and various types of PCR-based markers have been developed. RAPD markers are typically obtained using short (10−14 nucleotides) arbitrary primers (Williams *et al.,* 1993). RAPDs have the obvious advantage that they require no prior knowledge of DNA sequences. However, they are typically inherited in a dominant manner that precludes unambiguous classification of genotype. Moreover, they suffer from a lack of reproducibility because they are sensitive to subtle differences in reaction conditions (Neale and Harry, 1994). Although many RAPD markers can be developed at relatively modest initial cost, no marker system is suitable for detecting the multiple alleles that are common in many out breeding species. It is also advised to use SSR and AFLP markers for better understanding of Chilli genome.

The primer combinations CSF1 and CSR4, CSF1 and CSR2, and BF7 and BR9 were dominant markers, while the primer combinations BF5 and BR8, BF6 and BR8 were co-dominant markers. Co-dominant SCAR markers are much more informative as non-pungent peppers generate a visible, deleted fragment. The lack of a product band with dominant SCAR markers does not positively identify a non-pungent pepper. These markers have been very effective in defining the pungency of various cultivars. In some cases, chilies classified as non-pungent by panel tasting turned out to be very mildly pungent by tests using the SCAR markers and HPLC analysis. Extremely low capsaicinoid contents could not be detected by the taste panel method, but could be detected with more sensitive methods such as HPLC of concentrated extracts. HPLC determination has to wait until mature fruits are available, whereas SCAR markers can determine the pungency trait earlier and with small amounts of leaf material. A sample of 50 ng of genomic DNA from less than a leaf is sufficient for a single PCR amplification. The SCAR markers developed provide an accurate, simple, fast and economic means of early detection of pungency in *Capsicum*. These SCAR markers should also greatly facilitate phylogenetic studies of *Capsicum* and the Solanaceae.

In present study, the five primer pairs CSF-1 + CSR-2 and CSF-1+ CSR-4 validated pungency and non-pungency in *Capsicum annuum*, genotypes. These primers are robust primers and sensitive to very low capsaicin contents. Hence these primers can be used to detect the pungency in the early stages of the developments of the plants.

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