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DETECTION OF PUNGENT AND NON-PUNGENT CHILLI PEPPER (CAPSICUM SP.) VARIETIES AT SEED LEVEL, USING PUN ALLELE SPECIFIC PRIMER MAP1

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ABSTRACT

Genomic DNA isolated from zygotic embryos (seeds) of 7 chilli pepper varieties, namely *Capsicum annuum L.,* California wonder, Yolo wonder, G-5, Pusa sadabahar, Pusa jyothi and LCA-335 and *Capsicum chinense Jacq*. variety Kotpar, was PCR-amplified using *Pun1* allele specific primer *MAP1*. A DNA band admeasuring 479 bp was observed in *C annuum* L., varieties California wonder and Yolo wonder, while a DNA band admeasuring 494 bp was observed in the remaining 5 varieties. This polymorphism was used to discriminate non-pungent varieties namely California wonder and Yolo wonder from pungent varieties namely G-5, Pusa sadabahar, Pusa jyothi, LCA-335 and Kotpar. This method is useful to distinguish non-pungent varieties from pungent varieties, at seed level itself.

KEYWORDS: Capsicum annuum L., Capsicum chinense, Jacq, MAP1 primer, zygotic embryo, genomic DNA, PCR.

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INTRODUCTION

Chilli pepper (*Capsicum* sp.), belonging to Solanaceae family, probably originated in South and Central America[1]. In 1510, the Portuguese introduced chilli pepper into India, and India with a production of 1.27 million tonnes is the world leader, followed by China and Pakistan. Andhra Pradesh ranks *Numero Uno* in chilli pepper production; Maharashtra, Karnataka and Tamil Nadu are the other major chilli pepper growing States in India[2]. Most of 250 varieties of chilli peppers cultivated in India belong to *Capsicum annuum* L. complex and the varieties within this species complex show resemblance with their morphometrics, including seed phenotype .Conventionally, morphological descriptors are routinely used for establishing the identity of the varieties. However, these morphological descriptors suffer from many drawbacks, such as influence of environment on trait expression, epistatic interactions, pleiotropic effects etc[3]. For example, seeds of non-pungent *C. annuum* L. variety California wonder and seeds of pungent *C. annuum* L. variety Pusa jwala are phenotypically indistinguishable. Furthermore, the evaluation of plant material is often laborious and time consuming especially, when a large number of accessions are to be analyzed. Farmers cultivate pungent varieties and hybrids of *C. annuum* L., expecting high yields and lucrative price. Interestingly, atleast one of the parents in most of these hybrids is (less pungent). In other words, most of these popular brands are duplicates being sold under different names; consequently spurious seeds have flooded the seed market. A major problem arising from mid-nineties was crop failure in chilli pepper because of spurious nature of the seed[4]. The commercial chilli peppers, particularly the hybrids have narrow genetic base and consequently distinguishing pungent from non-pungent using morphometrics becomes impossible. Marker-assisted detection provides an alternative to the pungency phenotyping. There are a couple of papers on the use of molecular markers for use in pungency phenotyping [5, 6]. Using molecular markers is a convenient strategy to pungency detection, since it allows to discriminate this trait at early developmental stage, even before fruit setting; it is not affected by environmental conditions and is faster and more economical[6]. The objective of the present study is to distinguish non-pungent chilli pepper varieties from pungent varieties, at seed level, using *MAP1* primer.

MATERIALS AND METHODS

Capsicum annuum L. varieties G5, LCA-335 were procured from Agricultural Research Station (ARS), Lam, Guntur, A.P. India. *C. annuum* L. varieties Pusa sadabahar & Pusa jyothi were procured from Indian Agriculture Research Institution (IARI), Pusa complex, New Delhi, India.*C. annuum* L. varieties California wonder & Yolo wonder were procured from ARS, Malyal, Warangal, A.P. India. Seeds of *C.Chinense,* jacq.variety Kotpar were procured from Tezpur, Assam, India. These varieties were grown in our research field for one year and seeds collected from the fruit were used in this experiment.

DNA extraction

Zygotic embryos were excised from the soaked seeds of the above said 7 chilli pepper varieties[7]. Genomic DNA was extracted from zygotic embryo tissue[8]. Briefly, 250mg of zygotic embryos were macerated with a pestle and mortar in 0.5ml extraction solution (1.0 M NaCl, 30mM EDTA (pH 8.0), 150mM Tris-HCl (pH 8.0) and 2% β-mercaptoethanol).The sample was incubated at 55°C for 30 mins.After incubation the sample was cooled to room temperature and chloroform and isoamylalcohol (24:1) was added, centrifuged at 6000 rpm for 6 mins. The supernatant was collected and mixed with equal volume of cold isopropyl alcohol and incubated for 5 mins then washed the pellet with 70% ethanol and incubated after centrifugation. The pellet was dried, washed and dissolved in

TE buffer and was used in PCR amplification experiments.

PCR amplification

In this experiment *Pun1* allele specific primer *MAP1F* (5' CCAGTCGTTCATTTTTGTTTG 3') and*MAP1R*(5' TCTGCCCTTGTTGGATTTTC3') were used[6]. The PCR reactions were setup in a 32µl reaction containing 80ng genomic DNA of zygotic embryo 5µl, 4 µl of each primer (20 pmol/µl) and 1X PCR reaction buffer containing (10mM Tris hydrogen chloride, pH 8.2, 50mM Potassium chloride and 0.2% gelatin), 4µl of 0.2mM dNTPs (Fermentos, USA) 1.5mM $MgCl_2$, 0.8 µM primer, 1µl of 0.2 Units of Taq DNA polymerase was used. Amplification was performed on a thermal cycler (Model No MG25+, USA) with a program of initial denaturation at 94°C for 10 min, followed by 32 cycles of 94°C for 1 min, 51°C for 50sec and 72°C for 1 min followed by final extension at 72°C for 10 min and finally stored at -20°C.

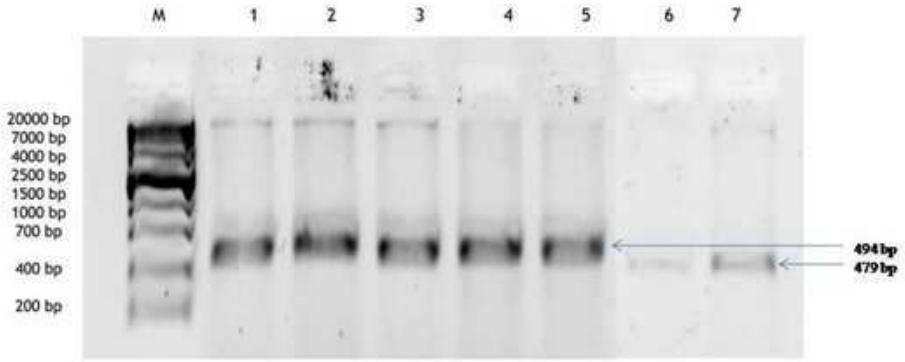
Agarose gel electrophoresis (AGE)

The PCR products were electrophoresed on 1.2 % Agarose gel, in 1X TBE buffer at 60 V for 1 hrs. The size of the amplicons was estimated using 2Kb 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.

RESULTS AND DISCUSSION

Genomic DNA was isolated from zygotic embryo (seeds) of six chilli pepper *C. annuum* varieties California wonder, Yolo wonder, G-5, Pusa sadabahar, Pusa jyothi & LCA-335 and one *C.chinense* Kotpar and PCR amplification was carried out using *Pun1* allele specific primer *MAP1*. DNA polymorphism was observed among the chilli pepper varieties analysed (Fig 1). DNA band admeasuring 479 bp was observed in California wonder and Yolo wonder, while the DNA band admeasuring of 494 bp was observed in remaining 5 varieties G-5, Kotpar, Pusa sadabahar, Pusa jyothi and LCA-335. It is well known that *C. annuum* varieties California wonder and Yolo wonder are non-pungent and are grouped under sweet pepper/bell pepper category[6], while the remaining four *C annuum* varieties G-5, Pusa sadabahar, Pusa jyothi, LCA-335 and one *C chinense,* Jacq. variety Kotpar are pungent. This differential amplification of the *MAP1* marker distinguished non pungent varieties from pungent varieties. In this study within 24 hours we could discriminate non pungent varieties from pungent varieties using zygotic embryos (seeds). This method is rapid and inexpensive and will be useful for seed companies and chilli pepper export business houses.

Figure 1
PCR amplification of genomic DNA isolated from zygotic embryos of six C.annuum L., varieties and one C.Chinese, Jacq. variety using MAP1 primer



Lane M: Marker, Lane 1: G-5, 2: Kotpar, 3: Pusa sadabahar, 4: Pusa jyothi, 5: LCA-335, 6: California wonder, 7: Yolo wonder

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