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ISSR markers for genetic diversity analysis in spices – An appraisal

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ABSTRACT

Inter-simple sequence repeat (ISSR) is a multilocus DNA marker increasingly being used for DNA typing of plants. Three non-anchored primers were tested for assessing the suitability of its use in genetic diversity analysis of spices germplasm. Optimum annealing temperature for different primers varied from 42 to 63 °C depending on the base sequences of the primers. All the primers were successfully used in amplifying inter-microsatellite regions of small cardamom, large cardamom, different species of *Vanilla* and *Piper*. Band profiles obtained with ISSR primers were found to be highly polymorphic and reproducible. The ISSR primer $(GACA)_4$ yielded reproducible and consistent DNA banding pattern in *Piper* and was found to be useful in distinguishing the seven species selected for the study. It is suggested that ISSR markers, in addition to being simple and time efficient, allow rapid identification of polymorphisms within spices germplasm.

Key words: Spices, inter-simple sequence repeat, genetic diversity analysis.

INTRODUCTION

Identification and characterisation of the germplasm collections of different spices are essential for their maintenance as well as for furthering breeding prospects. Molecular markers are proved to be highly heritable and exhibit enough polymorphism to discriminate genotypes of different crops (Kumar, 6). They can be very useful in identifying accessions and varieties at early stages of growth and characterise the genotype comprehensively. Application of molecular markers as complementary approach for genetic characterisation has been reported in many crops (Karp *et al.*, 5). Characterisation in spices have been done mostly based on the morphological characters. Efforts are on at different laboratories in India to characterise them on molecular basis, especially using RAPD (Pradeepkumar *et al.*, 9). Among the various molecular marker techniques developed over the past two decades, ISSR-PCR has been one of the most reliable DNA technique used owing to its simplicity, efficiency and reproducibility. The objective of the study was to investigate into the potential for exploitation of ISSR-PCR in genetic diversity analysis of some selected, economically important spices.

MATERIALS AND METHODS

The plant materials selected for the present study include small cardamom (*Amomum subulatum*), large cardamom (*Elettaria cardamomum*), three species of *Vanilla viz., V. andamanica*, *V. planifolia* and *V. vatsalana*, different *Piper* species viz., *P. nigrum* (var. Sreekara), *P. longum, P. chaba, P. brachystachyum, P. colubrinum, P. bababudani* and *P. trichostachyon*. The leaf samples were collected from the IISR Experimental Farm at Peruvannamuzhi. Total DNA from the leaves of *Piper* species and other spices were prepared by using an extraction technique modified from the original CTAB method (Johnson *et al.*, 4). The quality of DNA was checked by agarose gel electrophoresis (1%), the approximate DNA yields were calculated by a spectrophotometer and the DNA samples were stored at -20°C. Three non-anchored ISSR primers $(GATA)_4$, $(GACA)_4$ and $(CAG)_4$ (Bangalore Genei, India) were screened by polymerase chain reaction (PCR). PCR reaction were carried out based on protocols of Zietkiewicz *et al.* (10) for ISSR–PCR. The PCR reaction mixtures were prepared in a total volume of 20 µl each containing 1 x PCR buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% Triton X-100), 2 mM of $MgCl_2$, 200 µM each of dNTPs, 0.25 µM of primer and 1.0 unit of *Taq* DNA polymerase and 30 ng of template DNA. Time and thermal profile of the PCR were as follows; 3 min. of initial denaturatrion at 94°C, followed by 30 cycles each consisting of a denaturation segment of 1min. at 94°C, annealing temperature of 42 to 63°C for 1 min. depending on the melting temperature of the primers and an extension segment of 2 min. at 72°C. PCR was terminated with a final extension of 10 min. at 72°C. The amplification products were separated by electrophoresis in 2% agarose gels, with 1x TAE buffer, stained with 0.5 µg/ml ethidium bromide (EtBr) and photographed under exposure to UV light.

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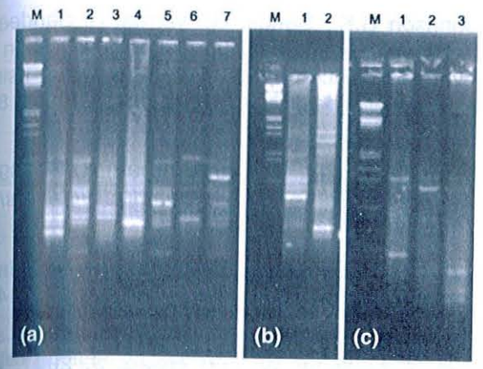

Fig. 1. ISSR-PCR products resolved in 2% agarose gels: (a) ISSR profile of seven selected *Piper* sp. using $(GACA)_4$ primer: 1- *P. chaba*, 2- *P. nigrum var. Sreekara*, 3- *P. longum*, 4- *P. colubrinum*, 5- *P. brachystachyum*, 6- *P. trichostachyon*, 7- *P. bababudani;* (b) ISSR profile of small and large cardamom using $(GATA)_4$ primer: 1- *Amomum subulatum*, 2- *Elettaria cardamomum*; and (c) ISSR profile of three selected *Vanilla* species using $(GACA)_4$ primer: 1- *V. planifolia,* 2- *V. andamanica,* 3- *V. vatsalana*. M- λDNA/EcoRI-HindIII double digest.

RESULTS AND DISCUSSION

Molecular technique like ISSR has been widely used in the analysis of genetic diversity in plant species. The present study was on characterisation of different *Piper* species and cultivars, species of *Vanilla*, small cardamom and large cardamom using ISSR-PCR. DNA from the selected accessions of spices were amplified using three non-anchored primers for ISSR-PCR. All primers used were successful in amplifying and discriminating the accessions tested. No significant differences were observed in different PCR experiments using the same primer, although occasionally variation in the intensities of individual bands was detected. The size of the amplification products ranged from 150 bp to 2 kb. Out of the three ISSR primers tested, $(GACA)_4$ could yield better amplicons in all the seven species of *Piper* compared to the other two primers. A significant variation in banding pattern could be observed among the selected species, useful in identification of all the seven species of *Piper* tested (Fig. 1a). The best primers determined to produce interpretable and variable banding patterns in different plant samples are given in Table 1. The presence or absence of bands amplified with different primers were sufficient enough to distinguish all the accessions tested. Five unique band were scored in *Piper* when amplified with $(GACA)_4$. Large cardamom and small cardamom was easily distinguishable with all the three primers tested and amplification using $(GATA)_4$ primer was more informative which amplified unique band for each of the sample (Fig. 1b). Similarly the 3 species of *Vanilla* could also be differentiated, the most informative primer being $(GACA)_4$ to amplify two unique amplification products corresponding to *V. planifolia* and *V. vatsalana* (Fig. 1c).

The present study demonstrated the merits of ISSR in discriminating different species of spices. Bornet and Branchard (2) reported the use of non-anchored ISSR primers in DNA profiling of seven different dicot species. One of the advantages of the technique is that no prior knowledge on the micro-satellites in the plants to be studied are required for the investigation. ISSR markers had already proved useful in the assessment of genetic relationship in cultivated tea clones and native wild tea (Lai *et al.*, 7), genetic diversity studies of gooseberry (Lanham and Brennan, 8) and DNA fingerprinting of Indian cashew (Archak *et al.*, 1). Studies conducted in cultivated chestnut by Goulao *et al.* (3) revealed the importance of ISSR due to its high effective multiplex

Table 1. Primers used in inter-simple sequence repeat (ISSR) analyses and size of the unique amplification products obtained.

Plant sample	ISSR primer sequence	Unique polymorphic ISSR bands (in base pairs- approximate)	Remarks
Piper species	$(GACA)_4$	950 bp	Band unique to *P. trichostachyon.*
		560 bp	Band unique to *P. longum*
		500 bp	Band unique to *P. colubrinum*
		300 bp	Band unique to *P. chaba*
Small cardamom and large cardamom			$(GATA)_4$ 400 bp band unique to large cardamom (*A. subulatum*)
		200 bp	Band unique to small cardamom (*E. cardamomum*)
Vanilla species	$(GACA)_4$	500 bp	Band unique to *V. planifolia*
		400 bp	Band unique to *V. vatsalana*

ratio and reproducibility. One of the advantages of the ISSR-PCR is the requirement of a low quantity of DNA as described by Lanham and Brennan (8). The preliminary assessment of the utility of ISSR analysis for detecting polymorphism in spices was done in the present study. The advantages of non-anchored primers were exploited for the genetic analysis of selected spices. The study also indicates the potential use of ISSR markers in assessing the wealth of genetic variations in germplasm collections and its utilization in the improvement of spices.

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