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ISSR Markers as a Tool for Assessing Genetic Diversity in Small Cardamom (*Elettaria cardamomum* Maton)

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ABSTRACT: *Fingerprinting with molecular markers allows precise and rapid variety identification. The present investigation was carried out to validate the utility of ISSR markers to characterize small cardamom accessions. There was successful amplification of ISSR markers in small cardamom. The seven polymorphic ISSR primers generated a total of 550 alleles. The number of alleles produced by different primers ranged between five and 12 with an average of 7.86 alleles per primer. Ten small cardamom accessions grouped into two major clusters in UPGMA analysis. Eight of the ten accessions can be unequivocally differentiated, but identical profiles were obtained for two accessions. The results indicated practical utility of ISSR markers in assessing genetic diversity among small cardamom accessions.*

Keywords: *cardamom, ISSR markers, diversity.*

INTRODUCTION

Small cardamom (*Elettaria cardamomum* Maton), is an economically important spice crop universally known as 'Queen of Spices' belongs to the genus *Elettaria* in the family Zingiberaceae (Holttum, 1950). The description 'Queen of Spices' is apt because cardamom has a pleasant aroma and taste and is an export oriented crop in India with a substantial share in foreign exchange earnings. Globally, small cardamom is the second most important spice crop next to black pepper.

Cultivation of cardamom is mostly concentrated in the evergreen forests of the Western Ghats of Southern India, which forms natural habitat of the species. Small cardamom, generally propagated by seeds and suckers, exhibits considerable variation under cultivation. Based on the nature of the panicle, shape, size of the fruit and other general growth characters cardamom is broadly grouped into three main 'cultivated types' – Malabar, Mysore and Vazhuka. The var. *Malabar* is characterized by prostrate panicle and var. *Mysore* possesses erect panicle. The third type var. *Vazhuka* is considered a natural hybrid between the two and its panicle is semi-erect or flexuous. Though cardamom germplasm has abundant variability being a cross pollinated crop, not much work has been done to exploit molecular marker technologies for documenting genetic diversity or for generating informative markers in cardamom.

ISSRs provide great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers. The multiple profiles generated by ISSR primers are highly polymorphic and as such are ideal for the study of genetic variability. The aim of the present study was to investigate the genetic relationships among small cardamom accessions using ISSR markers.

MATERIALS AND METHODS

Plant Materials and Isolation of Genomic DNA

Ten small cardamom accessions representing the three natural varieties- Malabar, Mysore, Vazhukka (Table 1) were collected from the germplasm repository of Indian Cardamom Research Institute, Myladumpara, India. Total genomic DNA of all plants was extracted from young, healthy leaves based on modified CTAB method (Mary et al.,2014). The quantity and quality of DNA was checked with spectrophotometer (Hittachi) and agarose gel (0.8 %)

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electrophoresis. Absorbance ratio between 260 and 280 was computed and the quality of genomic DNA was confirmed.

ISSR Assay

Initially, optimization of PCR was examined including concentration of DNA, primers and number of PCR cycles as well as annealing temperature. Fifteen ISSR (UBC set) primers were tested. The PCR amplifications were performed on an BIORAD-My Cycle Thermocycler (Bio-Rad Laboratories, USA) in 25ìL reaction system containing 25ng genomic DNA, 1×PCR buffer, 2.5mM $MgCl_2$, 15pM of each primer, 200µM of each dNTP (Genei, Bangalore) and 1 unit of Taq DNA polymerase (Genei, Bangalore). The reactions were performed with the following conditions: 95°C for 4 min, followed by 40 cycles of 94°C for 30 s, 45-52°C for 1min and 2min extension at 72°C for 2 min, and a final extension at 72°C for 10min. The PCR products were separated using agarose gel (2.5%) electrophoresis and visualized using EtBr staining. The allele sizes were calculated by comparing with 100bp DNA ladder (Genei, Bangalore).

DATA ANALYSIS

ISSRs were scored as dominant markers: presence of a band was recorded as "1" and absence as "0". All accessions were analysed as a single population. Only reproducible bands were used to calculate the Dice's similarity coefficients (Nei and Li, 1979). An unweighted pair group method using arithmetic average (UPGMA) cluster analysis was performed based on the similarity matrix for 30 small cardamom individuals using NTSYS- pc program version 2.2 (Rohlf, 1998).

RESULTS AND DISCUSSION

The seven polymorphic primers out of 15 generated a sum of 550 unambiguous and reproducible bands that could be scored, with the size ranging from 200 bp to 1750 bp. The highest numbers of polymorphic bands revealed among the 10 populations were obtained from UBC835 (AG microsatellite repeats),UBC 807 (AG repeats) and UBC841 (GA microsatellite repeats), while the lowest number was amplified by UBC880 (GGAGA). This indicates that AG and GA microsatellite repeats were able to reveal high variation among the *E. cardamomum*accessions. The total number of DNA fragments amplified from each primer in each population varied from 5 (UBC866) to 12 (UBC807). This also indicates the ability of AG microsatellite repeats in revealing the variation among the *E. cardamomum*accessions.

Based on Jaccard's similarity coefficient, a dendrogram was generated to represent the genetic relationships among the ten *E. cardamomum*accessions (Fig. 2). The accessions were grouped into two major clusters. A close genetic similarity was observed between two accessions MCC 8 and PV-1, which indicates that they were relatively remote in relationship. The highest divergence was found between the accessions ICRI1 and ICRI3.

The use of molecular markers has become a common practice in studies of population structure, genetic diversity for pre-breeding and breeding germplasm and in distinguishing one individual genotype to preserve the property of breeding rights (Langridge and Chalmers, 2004). In addition, ISSR markers are useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of plant species (Christopoulos *et al.* 2010).

In this study, ISSR markers revealed high genetic variation among 10 accessions studied, allowing an unequivocal identification for each one. The investigation showed that the AG and GA repeats from UBC835 and UBC807 primers produced a great number of polymorphic bands, and thus suggests that the AG and GA microsatellite repeats and their adjacent regions can be utilized for detecting wide range of genetic diversity through the genome of *E. cardamomum*. In addition, these microsatellite repeats can be utilized for designing new SSR markers for further marker-trait associations and QTL

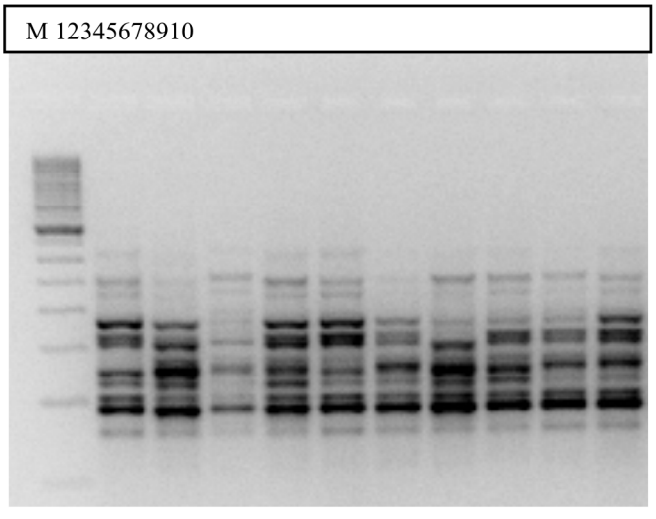

Figure 1: Molecular profiles of 10 cardamom accessions obtained with ISSR primer UBC 807
M- 100bp ladder; 1- ICRI1, 2- ICRI2, 3- ICRI3, 4- MHC 26, 5- MCC4, 6- MCC6, 7- MCC7, 8- MCC8, 9- MCC34, 10- PV1

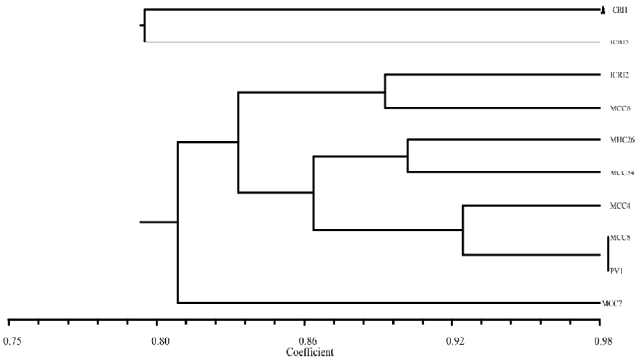

Figure 2: Dendrogram indicating relationships among populations of *E. cardamomum*based on Jaccard's similarity coefficients derived from seven ISSR primer

investigations. The genetic variation obtained could be utilized for improvement of populations with specific characteristics in future breeding programs for the identification of commercial value properties in this plant using these markers.

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