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Short Communication

Molecular characterization of some elite genotypes of cardamom (*Elettaria cardamomum* Maton)

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Cardamom (*Elettaria cardamomum* Maton) is one of the important spice crops cultivated for its valued dried ripe fruit (capsule), the cardamom of commerce. It is a native of the moist evergreen forests of the Western Ghats of South India. Cardamom is also grown in Sri Lanka, Papua New Guinea, Tanzania and Guatemala. In India, cardamom is mainly cultivated in Kerala, Karnataka and Tamil Nadu at elevations ranging from 600 M to 1300 M as an undercrop in the evergreen rain forests of the Western Ghats. Characterization of fourteen elite genotypes of cardamom using molecular tools has been attempted presently so as to find out the genetic relationship between them since such tools are more precise and dependable.

Fourteen elite genotypes of cardamom maintained at Indian Cardamom Research Institute, Myladumpara, Kerala, India including two released clones ICRI-1 and ICRI-2; eight unreleased selections MCC-12, MCC-16, MCC-21, MCC-40, MCC-73, MCC-85, MCC-260 and MCC-346 and four promising hybrids MHC-18, MHC-24, MHC-26 and MHC-27 have been used for the present study.

Genomic DNA was successfully isolated from young and fresh leaves of the plants using CTAB method [1]. The genomic DNA was purified, vacuum dried and completely dissolved in TE buffer. Quantification of genomic DNA was done by standard ethidium bromide fluorescent quantification. Different combinations and concentrations of dNTPs, Taq polymerase and $MgCl_2$ were tested for good and consistent amplification of genomic DNA. Among the different combinations tested, 1X assay buffer (1.5 mM of $MgCl_2$, 150 µM of dNTPs, 1 U of Taq) gave good amplification with clear bands without any non-specific banding. Hence, this combination was used in PCRs for primer screening and RAPD analysis.

100 operon primers were screened for amplification and polymorphism and eight primers were found to be polymorphic and were used for the study (Table 1).

Table 1. Operon primers successfully used for developing RAPD profiles in cardamom

Sl. No.	Primers	Sequence	% of GC
1	OPA 04	5'AATCGGGCTG 3'	60
2	OPA 16	5'AGCCAGCGAA3'	60
3	OPB 01	5'GTTTCGCTCC3'	60
4	OPB 17	5'AGGGAACGAG3'	60
5	OPC 10	5'TGTCTGGGTG3'	60
6	OPE 15	5'CACACTCCAG3'	60
7	OPC 16	5'CCGATATCCC3	60
8	OPF 02	5'ACGCACAACC3	60

The RAPD products were resolved in 2% agarose gel (Fig. 1). The data obtained from the gels were scored for the presence and absence of bands. Cluster analysis of the genomic fingerprints was carried out using NTSYS pc 2.0 software to analyze the genetic relationship among the genotypes. Paired Affinity Indices were calculated as per the following formula:

$$PAI = \frac{\text{Number of similar bands} \times 100}{\text{total number of bands}}$$

Dendrogram was also constructed (Fig. 2) based on the relationships.

Out of the eight primers found to be successful in DNA amplification in the case of cardamom, the most informative were OPC 10, OPC 16 and OPA 04 (Fig. 1). Among the fourteen genotypes studied, the highest similarity was observed between ICRI-1 and ICRI-2 (95%) even though they are two morphologically distinct genotypes as well as released varieties. MCC-16 and MCC-73 formed another such cluster with 95% similarity. MCC-16, MCC-73 and MHC-24 formed a cluster at 90% of similarity. MCC-85 and MCC-260 also formed a cluster at 90% similarity. At 71% of similarity, the fourteen genotypes could be grouped in to two groups, ICRI-1 and ICRI-2 forming the first group and all the other 12 genotypes forming the second group.

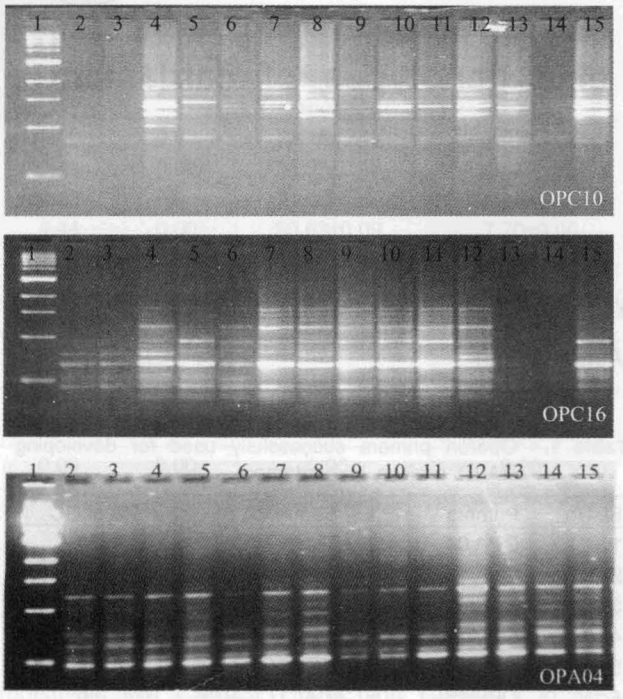

Fig. 1. RAPD polymorphism in the fourteen genotypes of cardamom analysed with the primers OPC 10, OPC 16 and OPA 04. 1. 1 Kb ladder; 2. ICRI 1; 3. ICRI 2; 4. MCC 12; 5. MCC 21; 6. MCC 40; 7. MCC 16; 8. MCC 73; 9. MCC-85; 10. MCC 260; 11. MCC 346; 12. MHC 24; 13. MHC 18; 14. MHC 26; 15. MHC 27.

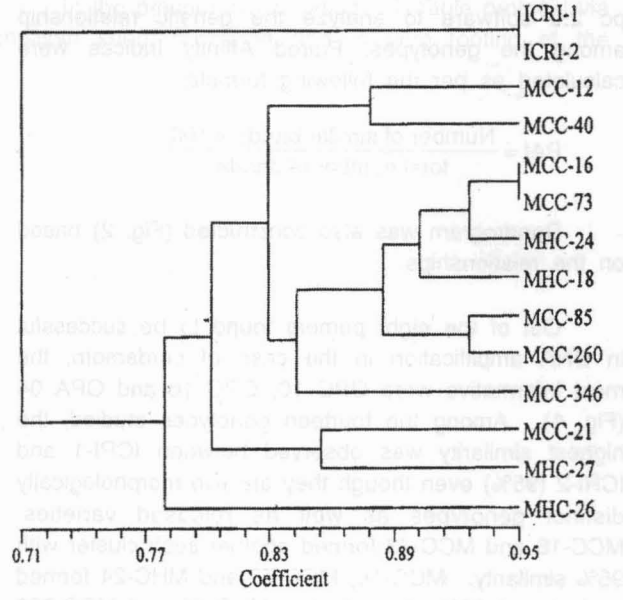

Fig. 2. Dendrogram of diversity in fourteen genotypes of cardamom studied for molecular characterization

However, five major clusters of genotypes were formed in the present study with ICRI-1 and ICRI-2 forming the first cluster; MCC-12 and MCC-40 forming the second cluster, MCC-16, MCC-73, MHC-24, MHC-18, MCC-85, MCC-260 and MCC-346 forming the third cluster, MCC-21 and MHC-27 forming the fourth cluster and MHC 26 forming a separate cluster.

Another interesting observation is the absence of RAPD polymorphism in the case of ICRI-1 and ICRI-2 in spite of their morphometric differences and it shows that there is high degree of genetic similarity between them. MHC-26 has been found to be distinct from the other genotypes. This genotype has already been reported to be genetically unique based on morphological characterization [2].

Morphometric characterization and assessment of variability have been attempted by earlier workers for the identification of high yielding clones of cardamom [3, 4, 5]. Molecular characterization can be used as a supplementary tool to analyze the genetic relationship among different genotypes of cardamom and the information can be used in crop improvement programmes. Since cardamom is highly heterogenous and heterozygous, vegetative propagation of selected clones has been suggested to produce true to type planting materials so as to increase production and productivity [6].

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