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Molecular Studies in Papaya *Carica papaya* L. and *Carica Candamarcensis* L. and their F_1 & F_2 Progenies

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Abstract Papaya cultivated variety CO 2 (*Carcia papaya*) was crossed with its wild relative *Carica candamarcensis* which have the desirable character of cold tolerance. The parents and their progenies were analyzed at molecular level. RAPD was carried out to identify the genetic relationship among the parents, F_1 and F_2 progenies. Randomly Amplified Polymorphic DNA (RAPD) grouped the population (Parents, F_1 and F_2) into two clusters viz. A & B .and indicated that 16 genotypes exhibited the maximum similarity value to *Carica candamarcensis*. Protein profile of parents and progenies were compared to study the similarities and differences among them which indicated that the polypeptide bands with Rf value of 0.29, 0.43 and 0.48 were inherited from the male parents *Carica candamarcensis*.

Key words: *Carica papaya*, var.*CO 2, Carica candamarcensis, RAPD, SDS-PAGE*

INTRODUCTION

Randomly Amplified Polymorphic DNAs (RAPD) are produced by repeated cycles of DNA denaturation - renaturation-DNA replication in PCR equipment. The genomic DNA of selected strain is mixed with an excess of selected oligonucleotide and this mixture is then used for DNA amplification in a PCR. RAPD technique was applied to analyze genetic relationship among the cultivars of papaya (*Carica papaya*)[5]. SDS–PAGE analysis was done to identify the qualitative and quantitative differences of leaf protein present in parents and progenies.

MATERIALS AND METHODS

DNA from CO 2 (*Carica papaya*), *Carica candamarcensis*, F_1 and F_2 progenies were extracted by following the protocol[2]. A set of ten arbitrary oligonucleotide decamer primers were used.

Primer	Sequence (5-3)
OPZ 01	TCTCTGCCAC
OPZ 02	CCTACGGGGA
OPZ 03	CAGCACGCGA
OPZ 04	AGGCTGTGCT
OPZ 05	TCCCATGCTG
OPZ 06	GTCGCGTTCA
OPZ 07	CCACGAGGAC
OPZ 08	GGGTGGGTAA
OPZ 09	CACCCCAGTC
OPZ 10	CCGACAAACC

PCR amplified products were subjected to electrophoresis in 1.2% agarose gel in 1 x TBE buffer at 100 volts for 3.5 hr using Aplex submarine electrophoresis unit. Markers were scored for the presence and absence of the corresponding band among the parents and progenies. The data obtained by scoring the RAPD profiles of different primers were subjected to cluster analysis. Similarity matrix was constructed using Jaccard's coefficient and the similarity values were used for cluster analysis. Sequential agglomerative hierarchical non-over lapping (SAHN) clustering was done using unweighted pair group method with arithmetic averages (UPGMA) method. Data analysis was done using NTSYSC version 2.02[4].

SDS – PAGE: SDS – PAGE analysis was done[3,6]. A known quantity of shade dried leaf sample was homogenized in phosphate buffer (pH 7.0) in pre chilled pestle and mortar. The homogenate was squeezed through a muslin cloth and then was centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and used for analysis.

RESULTS AND DISCUSSIONS

RAPD: Out of 10 primers, eight primers had good amplification. A total of 67 markers were obtained. Using these markers, the similarity matrix and a graphic dendrogram were produced on the basis of unweighted pair group method and Arithmetic average (UPGMA). Two main clusters and three sub clusters were observed among parents, F_1 and F_2 progenies. The female parent CO 2 and male parent *Carica candamarcensis* were grouped into different

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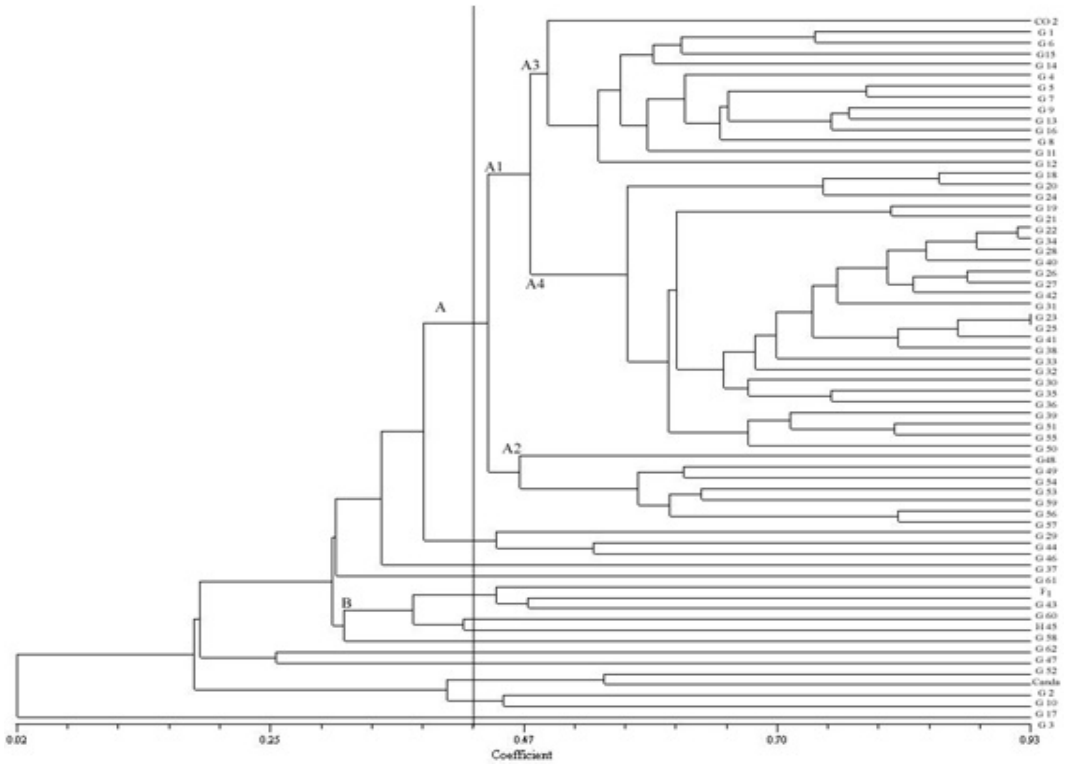

Fig 1: Dendrogram using Jaccard's similarity co-efficient for CO 2, carica candamarcensis and their F_1 and F_2 progenies based on data matrix from RAPD primers.

Table 1: Details of RAPD markers observed in CO 2, *Carica candamarcensis* and their F_1 and F_2 progenies

S. No.	Primer amplified in CO 2	Number of loci	Number of loci amplified in *Carica candamarcensis*	Number of loci amplified in F_1	Number of loci amplified in F_2	Total number of loci amplified
1	OPZ 03	1	1	2	6	6
2	OPZ 04	9	7	8	10	10
3	OPZ 05	7	8	6	9	9
4	OPZ 06	5	7	7	7	7
5	OPZ 07	4	1	3	7	7
6	OPZ 08	2	4	8	11	11
7	OPZ 09	5	3	7	8	8
8	OPZ 10	6	5	0	9	9
					Total	67

clusters viz., cluster A and cluster B respectively. In F_2 population, 16 genotypes exhibited the maximum similarly value (40 per cent) to the male parent *Carica candamarcensis.* It indicated that these 16 genotypes are more closely related to *Carica candamarcensis*.

SDS – PAGE: The leaf soluble protein of CO 2, *Carica candamarcensis* and their F_2 progenies were extracted and separated by SDS-PAGE method. The markers were also run simultaneously. Rf (Relative mobility) values for all the bands were computed and molecular weight of the bands was arrived. Two polypeptide bands with Rf (Relative mobility) value of 0.09 and 0.21 (corresponding molecular weight of 95 kDa and 70 kDa) were found in the female parents CO 2 where as, *Carica candamarcensis* had the polypeptide bands with Rf value of 0.29, 0.43 and 0.48 (Corresponding molecular weight of 56 kDa, 38 kDa and 35 kDa). These variations might be due to the genetic

difference between these two species. Among the F_2 progenies the polypeptide bands with Rf value of 0.29, 0.43 and 0.48 were found in 11, 5 and 1 progenies respectively. The advantage of SDS-PAGE of soluble proteins in differentiating the hybrids from their respective parents was explained[1]. The results of the present study have clearly indicated that the polypeptide bands with Rf value of 0.29, 0.43 and 0.48 were inherited from the male parents *Carica candamarcensis*.

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