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Short communication

Diversity analysis in custard apple using RAPD analysis

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ABSTRACT

The present investigation was carried out on 12 custard apple genotypes. Isolated and purified DNA was subjected to PCR based marker (RAPD) for assessment of genetic diversity. Out of 15 RAPD primers, eight RAPD primers gave good amplified bands. They gave 32 scorable bands and 130 clear fragments. All 32 bands and fragments were polymorphic with 4.0 polymorphic bands per primer. Primers P-20 and C-04 were the most informative in present investigation. Nei and Li's dissimilarity coefficient varied from 0.14 to 1.00 and Jaccard's distance ranged from 0.25 to 1.00, respectively. Binary Squared Euclidean distance was ranged from 3 to 21. The dendrogram was constructed by Ward's method and clustering of genotypes was also done by using Tocher's method. Distribution of genotypes was similar in the both methods except two genotypes, *i.e.*, RCA111 and RCA053. The genotype RCA053 had the maximum genetic distance from the other genotypes. There was no relationship between genetic divergence and geographical distance.

Key words: RAPD, *Annona* spp., genetic diversity.

Custard apple [*Annona squamosa* (L.)] is an important fruit crop for wasteland because of its hardy nature. This plant species belonging to family Annonaceae is commonly found in India and Thailand and originates from the West Indies and south America. All parts of this plant, apart from its culinary uses, have great medicinal values (Mohamed *et al*., 7). The plants of *Annona* spp. are highly valued. Study of genetic divergence using traditional methods need uniform environment for all genotypes and required plenty of time and resources. Therefore, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity. It has been shown that different markers might reveal different classes of variation (Powell *et al*., 8; Russell *et al*., 10.). RAPD markers are simple, easy to use, abundant and economical (William *et al*., 12). RAPD has been used in analysis of genetic distance in different plant species and produce reliable results (Ahmad *et al*., 1; Colombo *et al*., 2; Lashermes *et al*., 6).

Eleven germplasm of custard apple collected from southern Rajasthan along with one released cultivar Balanagar (Table 2) were used in present investigation. The genomic DNA was isolated from leaf tissue using CTAB method (Doyle and Doyle, 3). DNA concentration and quality determined by UV-absorbance A_{260}/ A_{280}. PCR reactions were performed in final volume of 20 µl containing 10 X assay buffer, 1.0 unit of *Taq*DNA polymerase, 200 µM each of dNTPs, 0.5 µM /reaction of random primer's and 50 ng of template DNA. The Polymerase Chain Reaction (PCR) was performed by initial denaturation at 94ºC for 3 min. followed 45 cycles of denaturation at 94ºC for 1 min., annealing at 36ºC for 1 min. and extension at 72ºC for 2 min. Following the amplification, the PCR products were loaded on 1.6% agarose gel which was prepared in 1 x TAE buffer containing 0.5 µg/ml of the ethidium bromide. The amplified products were electrophoresed for 2.5-3 h at 50 V with cooling. After separation, the gel was analysed under gel documentation system.

The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored, but a major band corresponding to faint bands was considered for scoring. The record bands were arranged in a genotype (in row) X position of bands in different primers matrix and used to calculate similarity and dissimilarity as Nei and Li's coefficient and Jaccard's coefficient for statistical analysis. The genotype X band matrix was used to calculate the dendrograme using Wards linkage methods by SPSS software. Ward's (11) technique was used for grouping of genotsypes into different clusters. Tocher's method of clustering was also used for genotype clustering (Rao, 9).

Out of 15 primers screened 8 primers produced amplification, *viz*., C-04, P-02, P-05, P-10, P-13, P-20, Q-06 and OPA-18. These eight primers produced 32 bands and 130 fragments (Table 1) and showed polymorphism. No monomorphic band was seen for

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Table 1. Response of RAPD primers for polymorphism in custard apple.

Primer	Sequence (5'- 3')	Total No. of bands (a)	Total No. of polymorphic bands (b)	Total No. of amplicons	Polymorphism % (b/a × 100)
C-04	CCGCATCTAC	7	7	33	100
P-02	TCGGCACGCA	3	3	10	100
P-05	CCCCGGTAAC	4	4	13	100
P-10	TCCCGCCTAC	2	2	08	100
P-13	GGAGTGCCTC	3	3	06	100
P-20	GACCCTAGTC	5	5	27	100
Q-05	CCGCGTCTTG	NA	NA	NA	NA
Q-06	GAGCGCCTTG	4	4	19	100
Q-07	CCCCGATGGT	NA	NA	NA	NA
Q-08	CTCCAGCGGA	NA	NA	NA	NA
Q-11	TCTCCGCAAC	NA	NA	NA	NA
Q-12	AGTAGGGCAC	NA	NA	NA	NA
OPA-16	AGCCAGCGAA	NA	NA	NA	NA
OPA-17	GACCGCTTGT	NA	NA	NA	NA
OPA-18	AGGTGACCGT	4	4	14	100
	Total	32	32	130	-
	Average	2.13	2.13	8.66	100

NA: Not amplified

Table 2. Genotypes used for diversity analysis.

Cluster	Genotype	Sampling place
I	RCA 60	Chittoregarh fort
I	RCA 121	Chittoregarh fort
I	RCA 59	Chittoregarh fort
I	RCA 111	Kanjaleva
I	RCA 76	Shitlakhera
II	RCA 131	Bokano
II	RCA 53	Rawalia Kala
II	RCA 133	Merpur
III	RCA 81	Suanto ka Gudha
IV	RCA 107	Haldaraphali
V	RCA 115	Kumbhalgarh fort
VI	Balanagar	Hyderabad

any of the primers screened. Average number of bands per primer was 2.13 and the level of polymorphism was 100 per cent. All bands ranged between 100 to 3000 bp. The most informative primer was C-04 followed by P-20. These primers were having 33 and 27 clear and reproducible amplicons respectively. The minimum number of amplified amplicons was 6, observed in primer P-13. The variable number of bands and polymorphism were also reported by different workers. Ahmad *et al.* (1) studied 20 RAPD primers, among them 14 primers produced 48 amplicons out of which 25 were polymorphic having 42 per cent polymorphism. Khan *et al.* (5) used 45 RAPD markers to evaluate genetic diversity among 31 species, three sub-species and one inter-specific hybrid of cotton (*Gossypium* spp.). They observed 579 amplified bands, with 12.9 bands per primer. The polymorphism was 99.8 per cent.

The Nei and Li's similarity coefficient ranged from 0.00 to 0.86 (RCA081-RCA107). Maximum similarity coefficients followed by 0.84 (RCA133-RCA107), 0.82 (RCA133-RCA060 and RCA131-RCA076). The RCA053 was not closer to any genotype. Its similarity coefficients with all the genotypes were 0.0. The similarity coefficient higher than zero was between genotype RCA121-RCA060 (0.12). Minimum dissimilarity coefficient was 0.14 between RCA107-RCA081 and maximum was 1.0 recorded between genotype RCA053 and all other genotypes. The dissimilarity coefficient between RCA121-RCA060 was 0.88, which has maximum dissimilarity after distance of RCA053 from other genotypes.

The maximum Jaccard's distance 1.0 was observed between RCA053 and other all genotypes

followed by 0.94 between RCA060 and RCA121, 0.89 between RCA121-RCA081, RCA121-RCA107 and RCA060-RCA131. The minimum distance was 0.25 in between RCA081-RCA107. The maximum binary squared Euclidean distance was 21 between RCA131-RCA060 and RCA107-RCA081, and minimum distance was 3 between RCA133-RCA060, RCA107-RCA133 and RCA107-RCA081. The genotype RCA053 showed the maximum binary squared Euclidean distance 19 from genotype RCA131 and minimum distance 6 from RCA111. The relationship between different genotypes was presented in the dendrogram formed by Ward's method using Hierarchical cluster analysis (Fig. 1). The distance between different genotypes rescaled on 0.00 to 25.00 scale. All the genotype grouped in one cluster at 25 values and at 15 value genotypes divided into two clusters at 10 in three and at 5 in four clusters. At distance 1 all genotypes fall in different clusters. Clustering in both the methods was same. The first cluster had RCA081, RCA107, RCA133, RCA060 and RCA059 genotypes, second cluster had RCA076, RCA053 and RCA115 genotype and III, IV, V and VI one each, *i.e.*, RCA121, Balanagar, RCA111 and RCA053 genotype respectively. These indicate that clustering pattern of Wards and Tocher's methods was also similar except genotype RCA111 and RCA053. These two genotypes clustered together in Ward's method at rescaled values 2.5, falls in different clusters in Tocher's method.

Further the samples collected nearby places not fall in one cluster rather sample collected from different places clustered together (Table 2). Therefore, it is concluded that there is no relationship between genetic divergence and geographical distance amongst the germplasm under study. However, these findings are exclusive and no reports are available for comparative study in custard apple. However, most of the study on genetic diversity in various plant species showed positive correlation between genetic diversity and geographical distance (Ahmad *et al.*, 1; Colombo *et al.*, 3; Jayanthi and Senni, 4). Therefore, there is a need to undertake diversity analysis in more number of germplasm to bring some meaningful conclusion in this underutilized fruit species.

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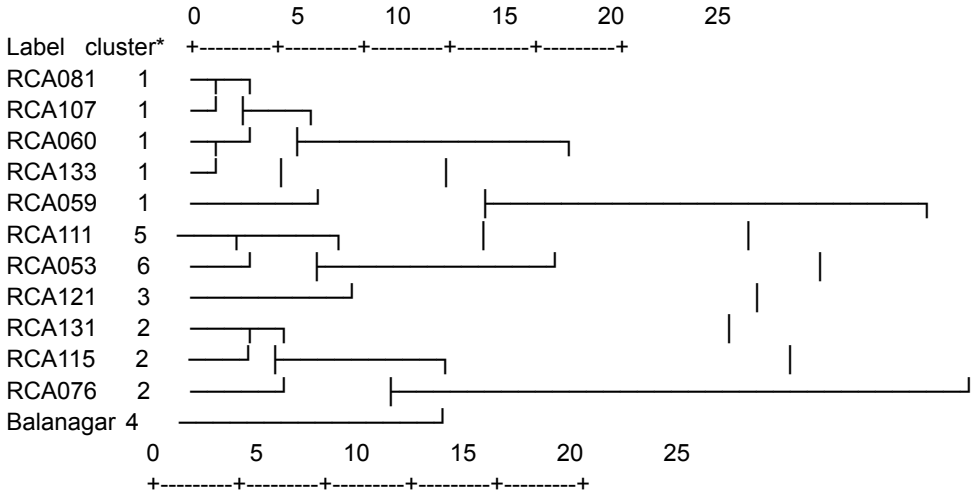

*clustering by Tocher's method.

Fig. 1. Dendrogram showing relationship among of custard apple genotypes.

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