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Short communication

Genetic diversity of carambola in North East India

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ABSTRACT

Present research investigation was aimed to determine the genetic diversity of carambola found in North East Region of India using morphological and RAPD markers. Results revealed that genetic variability was observed among the selected genotypes in morphological and yield traits. RAPD molecular marker also showed considerable variation in which out of the 20 primers used 15 primers are amplified which generated 92 total numbers of band and 64 polymorphic bands with a mean of 4.26 bands per primer and 69.34% polymorphism among the selected genotypes. Results showed the usefulness of morphological and RAPD molecular marker as a tool for screening variability of carambola genotypes.

Key words: *Averrhoa carambola*, morphological, RAPD marker.

Carambola (*Averrhoa carambola* L.) is an underutilized attractive fruit of the family Oxalidaceae. It is a subtropical evergreen tree, usually 6 to 9 m in height. The fruit has distinctive ridges running down its sides which in cross section appear in form of a star hence called as 'Star fruit' having light to dark yellow in colour and smooth with a waxy cuticle while the flesh is light yellow, translucent, crisp and very juicy, with or without fiber (Margen, 2). Arunachal Pradesh has a rich diversity of carambola having different fruit shape, size and fruit weight ranges from 83.5g to 300 g / fruit and sweetness ranges from 5.0 to 14.9 °brix (Padun and Singh, 3). It is rich source of reducing sugars, ascorbic acid and minerals, such as K, Ca, Mg and P. There is a great demand for preserved product notably pickles, jam, jelly, preserved, drink etc. and increased in popularity as a fresh fruit in tropical countries. The leaves are antipyretic, anthelmintic and are also helpful for curing scabies, fractured bones and various types of poisoning, intermittent fevers and elimination of intestinal worms (Kirtikar *et al.*, 1). However, in India there is no report for the exploration of this underutilized fruit using molecular marker for the screening of elite mother plant. As a result, the farmers have been using planting materials of seedling origin with unknown yield potential and fruit quality. Looking at the importance of this underutilized fruit crop and high price value, the demand for its planting material is also increasing for the sweet genotype for fresh consumption. Therefore, there is a need to study the genetic diversity of carambola using morphological and molecular marker to screen the superior genotype of carambola found in the North East Region of India.

The experimental materials for the study comprised of 20 collected genotypes ($P_1$to P_2 from Manipur, P_3 from Garo Hill Meghalaya, P_4 to P_7 from Assam and P_8 to P_{20} from Arunachal Pradesh) of carambola maintained by the Department of Fruit Science, College of Horticulture & Forestry, CAU, Pasighat, Arunachal Pradesh. Fresh young leaves were collected and brought to the laboratory in ice box and stored at -20°C freezer. Leaves were ground using motor and pestle until they become fine powder. Time to time the addition of liquid nitrogen facilitate the grinding process of the samples as it help in removal of moisture. Resulted powder was stored in a sterile falcon tube at -20°C until use for DNA extraction. Fruits are collected from the selected genotypes in laboratory for physico-biochemical analysis to analyze the existing variability between them and components of variance for individual character was carried out as per analysis of variance using OPSTAT and Web Based Agricultural Statistics Software Package (WASP). Extraction of DNA was done through modified CTAB method by grinding plant tissue to a fine paste in CTAB buffer with the help of liquid Nitrogen (-196°C) which was transfer CTAB mixture to a microcentrifuge tube and mixed with 75µl of 10%SDS and 3µl of ß -mercaptoethanol and incubated for about 15 min-20mins at 65°C in a recirculating water bath then mixed with 200µl of potassium acetate. To each tube Chloroform: Iso Amyl Alcohol (24:1) was added and spun at 12000

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rpm for 10 mins. After the upper aqueous phase was transferred to a clean microcentrifuge tube, 0.6 volume of ice cold Isopropanol was added to precipitate the DNA and stored at -20°C or overnight. Following precipitation, the DNA has been extracted by spinning the tube at 13000 rpm for a minute or 10 minutes to form pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol and allow the DNA pellet to dry. The DNA was resuspended in sterile DNase free water and RNaseA (10 µg/ml) has been added to the water prior to dissolving the DNA to remove RNA in the preparation and stored at -20°C. The screening of carambola genotypes was done using twenty decamer primers (S97, S103, S122, S123, S126, S197, S199, S5, S 29, S 33, RPI 4, RPI 7, RPI 10, RPI 11, RPI 14, RPI 16, RPI 17, RPI 21, RPI 23 and RPI 24) for genetic diversity analysis. Each 25µl of PCR mixture consisted of 2 µl of template DNA, 1 µM of 10mM each deoxynucleotide triphosphate, 1 µl of decanucleotide primers, 2.5 µl of 1X Taq buffer with MgCl2 (supplied with the Taq Polymerase enzyme), 0.5µl of 5U/µl Taq DNA polymerase. Amplifications were performed in Master Cycler. The PCR mixtures were heated at an initial step of 94°C for 3 min and then subjected to 40 cycles of following programme: 94°C for 1min, 36°C for 1 min, 72°C for 1 min. After the last cycle temperature was maintained at 72°C for 10 min. Amplified products were resolved on a 1% agarose gel containing 0.5 mg/ml ethidium bromide in 1x Tris Acetate EDTA (TAE) buffer in which electrophoresis was carried out for 1 hour at 70 Volt and visualized under UV light. Presence or absence of the band was scored as 1 for presence and 0 for absence, obtaining the molecular identification profile for each individual. The binary matrix was used to calculate the Simple Matching coefficient. Cluster analyses were done by UPGMA method, and the corresponding dendrogram was constructed where data analysis was done using the NTSYSpc software (Rohlf, 5).

Fig. 1. Variation in fruit shape and size of carambola found in North East region of India.

A large variability was recorded among morphological characters of fruits of the collected carambola genotypes (Fig. 1). The fruit weight ranged from 24 g (P_{10}) to 117g (P_{16}) and mean value of 72.24g, average weight of 10 seeds among the different genotypes varied from 0.51g (P_{10}) and 0.73 g (P_{11}) with the average value of 0.62 g, ridge depth/ width was recorded to range from 1.90cm (P_1) to 0.60cm (P_8) with a mean value 1.32cm, juice content of the different genotypes was recorded to range from 12.00ml (P_{18}) to 41.20ml (P_{16}) with a mean value 24.04ml, length of the mature fruit also showed significant difference that varied from 6.10cm (P_{10}) to 12.16cm (P_{16}) and mean length 8.51cm and the fruit breadth ranged from 3cm (P_{18}) to 7.10cm (P_{16}) with mean value 4.95cm. The number of fruits/tree/year different genotypes ranged from 480 (P_6) to 1250 (P_9) with mean yield 399.37 fruits/tree/year (Table 2). Similar finding was also reported by Rathod *et al*. (4) and Padun and Singh (3).

Out of the 20 RAPD primers used in the study, 15 primers could produce amplified products and 5 primers were not reproducible. The 15 RAPD primers generated a total of 92 RAPD bands and the number of amplification products or bands for each primer varied from 4 (RPI-7) to 9 (RPI-23) with a mean of 6.133 (Fig. 2). The RAPD primers

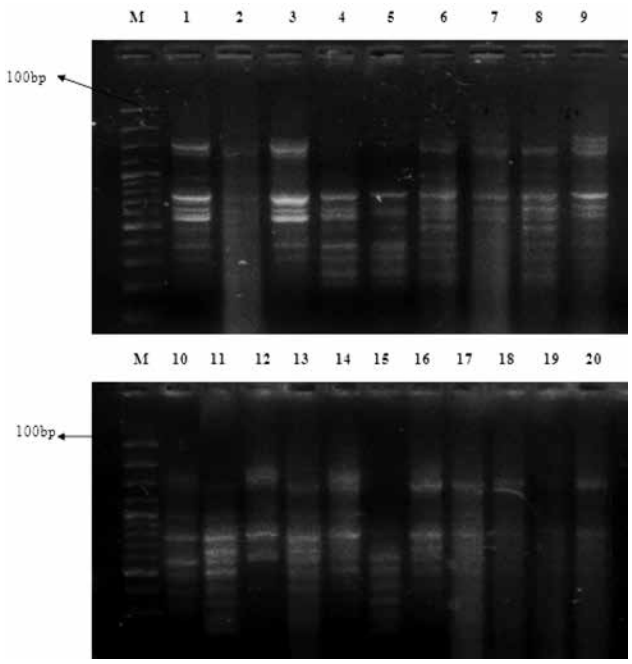

Fig. 2. Amplification profile of 20 genotypes of carambola using RAPD marker RPI-23 Lane M: 100bp molecular marker. Genotypes P_1-P_2 from Manipur, P_3 from Garo Hill Meghalaya, P_4 to P_7 from Assam and P_8-P_{20} from Arunachal Pradesh.

Table 1. Primers used for amplification and their polymorphic percentage.

Sl. No.	Primers	Sequences	Total no. of bands	Polymorphic bands	Polymorphic percentage (%)
1	S-123	CCTGATCACC	7	6	85.7
2	RPI-23	CCAGCAGCTA	9	7	77.8
3	S-122	GAGGATCCCT	7	4	57.1
4	RPI-21	CACGAACCTC	5	4	80.0
5	RPI-7	ACATCGCCCA	4	2	50.0
6	S-199	GAGTCAGCAG	5	4	80.0
7	S-126	GGGAATTCGG	6	5	83.3
8	RPI-14	ACTTCGCCAC	6	4	66.7
9	S-33	CAGCACCCAC	6	4	66.7
10	S-103	AGACGTCCAC	5	3	60.0
11	RPI-24	CCAGCCGAAC	7	4	57.1
12	RPI-11	ACGGAAGTGG	8	5	62.5
13	RPI-16	AGGCGGCAAG	6	5	83.3
14	RPI-4	AATCGCGCTG	6	3	50.0
15	RPI-10	ACGATGAGCG	5	`4	80
Total			92	64	1040.2
Mean			6.133	4.26	69.34

Table 2. Physical parameters of 20 genotypes of carambola found in North East Region of India.

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	No.of seeds/ fruit	Average weight of 10 seeds (g)	Fruit ridge depth (cm)	Fruit juice content (ml)	Fruit volume (ml)	Yield (No. of fruits per tree per year)
P_1	79.50	7.50	4.80	2.00	0.66	1.90	24.00	80.00	600.00
P_2	87.00	8.40	5.90	2.00	0.55	1.86	28.50	87.10	800.00
P_3	63.60	8.10	4.70	2.00	0.68	1.43	20.10	64.30	1150.00
P_4	45.60	7.60	4.30	4.30	0.61	1.23	15.00	46.60	740.00
P_5	50.00	9.50	5.50	5.00	0.59	1.30	16.20	50.00	785.00
P_6	70.00	7.30	5.00	5.00	0.67	1.09	25.00	71.00	480.00
P_7	115.60	11.10	4.90	3.00	0.71	1.40	36.00	115.30	780.00
P_8	86.60	9.30	5.10	4.30	0.64	0.63	26.50	86.60	790.00
P_9	100.00	9.20	5.50	3.30	0.63	0.93	34.67	100.60	1250.00
P_{10}	24.00	6.10	3.80	1.33	0.51	1.70	12.80	24.00	960.00
P_{11}	82.20	9.60	5.30	5.00	0.73	1.50	22.67	81.00	505.00
P_{12}	100.00	8.10	5.00	2.00	0.67	1.41	32.50	101.60	510.00
P_{13}	36.90	7.25	4.20	5.00	0.52	1.20	22.00	37.30	720.00
P_{14}	106.00	9.70	5.90	1.30	0.70	0.86	30.00	106.60	664.50
P_{15}	30.10	8.70	3.70	5.00	0.64	1.22	16.20	31.00	870.00
P_{16}	117.00	12.16	7.10	5.00	0.66	1.40	41.20	116.60	1150.00
P_{17}	38.50	7.00	4.20	5.00	0.52	1.37	19.50	40.00	750.00
P_{18}	30.10	6.60	3.00	4.30	0.50	1.60	12.00	30.00	650.00
P_{19}	72.20	8.90	4.50	5.00	0.68	1.10	20.00	73.30	690.00
P_{20}	110.00	8.10	6.60	2.30	0.59	1.31	26.00	109.00	789.00
Mean	72.24	8.51	4.95	3.60	0.62	1.32	24.04	72.59	781.65
SEm±	3.24	0.49	0.47	0.19	0.05	0.13	0.61	7.72	15.61
CD 5%	9.32	1.41	1.36	0.55	NA	0.38	1.76	22.20	44.86

also generated a total of 64 polymorphic bands with a mean of 4.26 as a result the 15 polymorphic RAPD markers showed polymorphism percentage of 69.34% (Table 1) which ranges from 50% (RPI-7 and RPI-4) to 85.7% (S-123). From the investigation it is concluded that the there is variability of carambola genotypes found in found in the North East region of India using morphological and RAPD markers which can be used for the crop improvement work for this underutilized fruit crop for the selection of elite mother plant in the future.

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