Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 2729-2738

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.332

Evaluation of Genetic Diversity of Carambola (*Carambola averrhoa* L.) in Arunachal Pradesh, India

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Keywords

Carambola, Physico-chemical character and SDS-PAGE

Article Info

Accepted:
24 January 2018
Available Online:
10 February 2018

A B S T R A C T

An extensive survey was conducted in different District (Upper Siang, West Siang and East Siang) of Arunachal Pradesh during 2015-17 to study the genetic diversity of carambola and to identify superior genotypes having sweet in taste based on the descriptor of NBPGR carambola. The experimental result showed that individual fruit weight ranged from 83.53 g (P_{20}) to 300.00g (P_{14}); fruit diameter 4.07 cm (P_5) to 9.13 cm (P_{14}); fruit length 6.83 cm (P_4) to 15.93 cm (P_{14}); juice content 25.00 ml (P_4) to 61.67 ml (P_{14}); seed/fruit 2.00 (P_1) to 4.67 (P_{12}); fruit yield 84.33 kg (P_4) to 185.00 kg/tree (P_8); TSS 5.03 (P_{12}) to 14.97 ^{0}Brix (P_{11}); oxalic acid 0.01% (P_2, P_3, P_5, P_7, P_8, P_{14} and P_{18}) to 0.35% (P_9); ascorbic acid 25.00 (P_{17}) to 68.00 mg/ 100g (P_9); acidity 0.08 % to 1.22 %; reducing sugar 3.20 % (P_3) to 11.93 % (P_{11}); total sugars 3.40 %(P_3) to 12.80% (P_{11}) and shelf life 10.67 days (P_{10}) to 14 days (P_{17}). Based on the results obtained from the present investigation, it can be concluded that, selected carambola genotypes exhibited noticeable variation in the morphological, biochemical and yield characters. SDS-PAGE analysis also showed considerable variation in band number of protein which ranged from 14-38. Thus, these findings illustrated the usefulness of physic-chemical characterization and biochemical marker as a tool for the genetic diversity evaluation in carambola.

Introduction

Carambola (*Averrhoa carambola* L.), also known as star fruit, is a curious attractive underutilized fruit of warm and sub-tropical areas of the world which belong to the family Oxalidaceae. The fruit has distinctive ridges running down its sides (usually five but can sometimes vary); in cross-section, it resembles a star, hence its name. The entire fruit is edible and is usually eaten as raw. They may also be used in cooking as substitute of tamarind (Singh *et al.*, 1990). It is believed that it may have originated from Sri Lanka or Moluccas, Indonesia, but has been cultivated in the Indian Subcontinent and South-East Asian countries for hundreds of years. They are cultivated commercially in Southeast Asia, Southern China, Taiwan and Florida. They are also grown in Nicaragua, Costa Rica, Panama, Colombia, Ecuador, El Salvador, Peru, Brazil, Jamaica, Haiti, the Dominican Republic, Puerto Rico, Trinidad, Mexico, Guyana and parts of Africa (Morton, 1987). In India, they

are mainly confined in the homestead garden (Chadha, 2013). The fruit is about 5 to 15 centimeters (2 to 6 inches) in length and is oval shaped. It usually has five prominent longitudinal ridges, but in rare instances it can have as few as four or as many as eight. In cross section, it resembles a star. The skin is thin, smooth and waxy. It turns a light to dark yellow when ripe having climacteric nature. The flesh is translucent and light yellow to yellow in color. Each fruit can have 10 to 12 flat light brown seeds having about 6 to 13 mm (0.25 to 0.5 in) in width and enclosed in a gelatinous aril. Once removed from the fruit, they lose viability within a few days as they are recalcitrant in nature (Crane, 1994). The fruit of carambola is a rich source of reducing sugars, ascorbic acid and minerals such as K, Ca, Mg and P (Haick, 1952). Ripe fruits of sweet form of carambola contain both oxalic acid (0.16%) and malic acid (0.06%), whereas fruits of sour form contain only oxalic acid in quantities ranging from 1.0% in unripe fruits to 0.51% in ripe ones. Sugars present in both type consist largely of glucose with moderate quantities of fructose and traces of sucrose and vitamin C content is also high (Lewis *et al.,* 1954). Samson (1986) stated that the fruits contain several amino acids including serine, glutamic acid and alanine. It is also a potent source of both primary and secondary polyphenolic antioxidants (Shui and Leong, 2004). Besides, Singh *et al.,* (2014) also reported that ripe fruit pulp of carambola along with little common salt is eaten against jaundice, bleeding piles and for washing utensils. Also, the crushed leaves have also been for curing chicken pox, ring worm and scabies and its root extract is used as an antidote for poisoning.

Arunachal Pradesh has a rich diversity of carambola and there is availability of different size and fruit quality having sour and sweet taste. The superior genotypes of carambola developed through selection from countries like Thailand and Malaysia are sweet in taste and have TSS ranging from 8-15 ^{o}Brix (Ray, 2002). Similar superior genotypes of sweet type carambola are also available in Arunachal Pradesh but still unexploited as they are grown in the homestead garden or wild form in the forest. Locally sweet genotypes known as '*Rohodoi*' and sour genotypes as '*Kordoi*' are grown in homestead garden. However, research in diversity of carambola in this region for the evaluation of superior genotype has not yet been documented. Besides, there is lack in the crop improvement for this important underutilized fruit crop in India. Further, there is a need for multiplication of the superior genotypes of sweet type of carambola found in this region as it might be liable to extinction if there is no documentation and multiplication for such superior genotypes. Looking at the importance of this fruit, the demand for its planting material is also increasing due to its medicinal and nutritional properties. Moreover, documentation of the genetic diversity to evaluate promising genotypes found in Arunachal Pradesh has also not been attempted. Therefore, keeping in view of the above facts, the present experiment was carried out in three districts of Arunachal Pradesh (East, West and Upper Siang district) for the exploration of superior genotypes of carambola having sweet taste and heavy bearing habit.

Materials and Methods

The survey, selection and identification were specifically carried out in East Siang, Upper Siang and West Siang districts of Arunachal Pradesh. The geographical location of this area is situated in between N 27°56.629` - N 28°10.402` latitude and E 095°07.233`- E 095°26.520` longitudes with an altitude of 132-393 meter above the mean sea level. The experimental materials for the present investigation comprised of 20 selected

genotypes of carambola having sweet taste to evaluate the superior genotype from it. The sources of 20 carambola were P_1, P_{10}, P_{11}, P_{17} from Pasighat, East Siang district, P_2, P_5, P_6, P_7, P_8 from Ngorlung, East Siang distrct, P_3 and P_4 from Sille, East Siang, P_9, P_{12}, P_{13}, P_{15} P_{16} from Geku, Upper Siang, P_{14} from Kiyit, East Siang and P_{19}, P_{20} from Aalo, West Siang. Survey work was carried out in different districts of Arunachal Pradesh. Selection and identification of superior genotype were done following the NBPGR carambola descriptor. The basic parameters and physico-chemical characters of carambola were recorded based on the NBPGR carambola descriptor for identification of superior genotypes**.** Estimation of protein was done as Lowry's method (1951) and Polyacrylamide gel electrophoresis (PAGE) in presence of denaturing agent (SDS) was carried out as per procedure described by Laemmli (1970) with some modifications with the 10% Acrylamide Separating Gel for the diversity analysis among the selected genotypes of carambola.

Results and Discussion

Variability in physical characters

There were wide variations among different carambola genotypes with respect to physical characters of the fruit. The fruit length parameter varied from 6.83 cm in P_4 to 15.93 cm in P_{14} and the average fruit length was 8.81 cm. In contrast Chadha (2013) obtained more narrow range of fruit length (12-15 cm). In this regard Narain *et al.,* (2001) found that the average length of the fruit was 7.92 cm. In respect of fruit diameter, significant variations were seen among the fruit of different genotypes. The observed values ranged from 4.07 cm in P_5 to 913 cm in P_{14}. Average fruit diameter observed by Narain *et al.,* (2001) was 5.24 cm whereas the average fruit diameter of present finding is 5.51cm. The data obtained on fruit rib thickness showed significant differences among different genotypes. The thinness rib was recorded in P_7 (1.3 cm) while the thickest rib was in P_5 (1.83 cm) and P_{14} (1.83 cm). The average fruit rind thickness was 1.53 cm. Similarly, Narain *et al.,* (2001) reported that the fruit rib thickness varies from 0.19-1.69 cm.

The data obtained on fruit rib length showed significant differences among different genotypes. The fruit rib length range from 1.40 cm in P_{18} to 2.33 cm in P_{14}.The average fruit rib length was 1.92 cm. The fruit weight varied significantly and ranged from 83.53 g to 300 g. The fruit of genotype P_{14} recorded the maximum fruit weight (300g) followed by P_2 (170.03 g). On the other hand, the minimum fruit weight (83.53 g) was recorded in genotypes P_{20} followed by P_3 (90.03 g) and P_5 (90.03 g). The mean value was 122.46 g. Narain *et al.,* (2001) reported that fruit weight ranged from 20.26-57.32 g which is contrast to the present investigation.

The fruit yield of different carambola genotypes ranged from 84.33 Kg per year per plant to 185 Kg per plant per year with the average yield of 123.5 Kg per plant per year. Genotype P_8 was found to be highest (185 Kg per plant per year) followed by P_9 (165 Kg per plant year) and lowest was recorded in P_4 (84.33 Kg per plant per year). Similarly, Goenaga (2007) also found that maximum yield was 36,060 Kg per ha and minimum yield was recorded 23,490 Kg per ha in the carambola tree.

Investigation reported that there were two flushes of flowering in a year, a number of fruits per plant were observed among the selected carambola. The maximum number of fruit per tree of 2250 was recorded by P_8, which was followed by P_9 (2000).The least number of fruit per tree of 400 was recorded in P_{14}. Goenaga (2007) reported that number of fruit per ha was 258761 fruit per ha.

The number of seeds per fruit significantly differed among the genotypes and ranged from 2 to 4.67 with the average seeds content of 3.42. Minimum number seed was found in P_1 (2). Narain *et al.,* (2001) reported that number of seeds per fruit ranged from 1.83 to 4.97 which are almost similar to the present finding. Besides, Bhaskar and Shantaram (2013) also reported that number of seed per fruit range from 0.87 to 5.35. The seed colour of all selected genotypes of carambola was found to be brown. Similarly, Gheewala *et al.,* (2012) also reported that seed colour of the fruit is brown. The average weight of 10 seeds among the different genotype ranged between 0.56 g in P_1 and 0.76 g in P_{17} with the average value of 0.66 g. In this regard, Narain *et al.,* (2001) found that average weight of 10 seeds range from 0.37-0.70 g which is almost similar to present findings.

The fruit pedicel of selected genotypes of carambola was found to be brown in colour. The data obtained on fruit pedicel length showed significant differences among different genotypes. The pedicel length range from P_{16} (3.87 cm) to P_2 (5.13 cm) followed by P_8 (4.93 cm). The average fruit pedicel length was 4.36 cm. Pedicel diameter of the selected genotypes of carambola was found to be insignificant. With regard to the fruit juice content, it significantly varied among different genotypes and ranged from 25 ml to 61.67 ml with the mean value of 36.39 ml. The highest fruit juice content recorded was in P_{14} with 61.67 ml followed by P_{18} (49.67ml) and P_8 (47.33 ml). Juice content of P_4 was recorded lowest (25 ml) (Table 1).

Variability in chemical characters of fruit

The observed data of different genotypes showed significant variation for TSS. The observed value ranged from 5.03 ^{0}Brix in P_{12} to 14.97 ^{0}Brix in P_{11} with the mean value of 7.28 ^{0}Brix. In this regard, Watson *et al.,* (1988) estimated that the TSS content of the carambola ranged from 5-13^{0}Brix.

The oxalic acid content of different selected genotypes of carambola, Oxalic acid content showed varied significance which ranged from 0.01% (P_2, P_3, P_5, P_7, P_8, P_{14} and P_{18}) to 0.35% (P_9). In this regard, Watson *et al.,* (1988) reported that the oxalic acid content of carambola ranged from 0.04-0.07% in 100 g and Arrexels *et al.,* (2001) also reported that oxalic acid content is less in ripe fruit (Table 2).

Fig.1 SDS-PAGE banding pattern among 20 genotypes of carambola

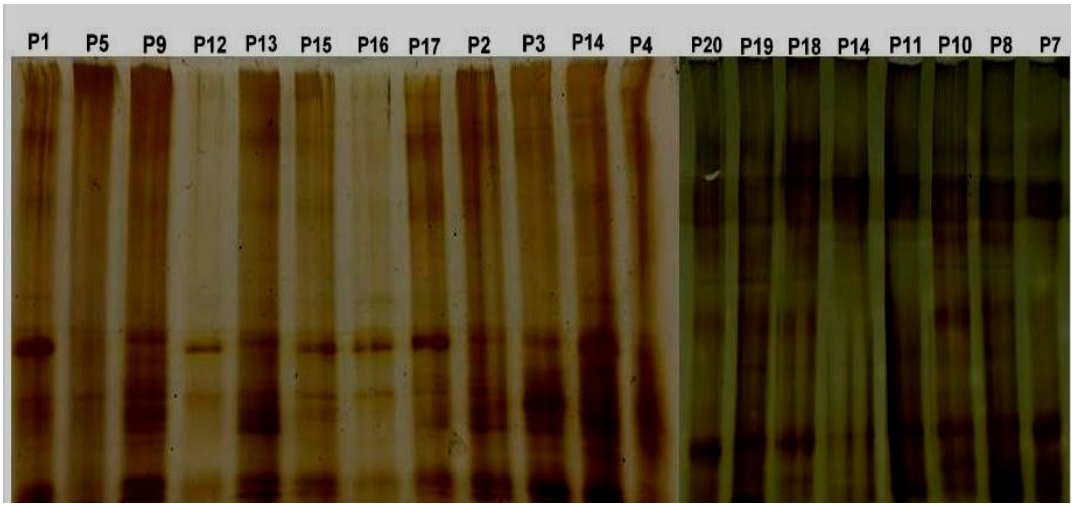

Fig.2 UPGMA of twenty carambola genotypes based on total seed protein profiles obtained by SDS-PAGE

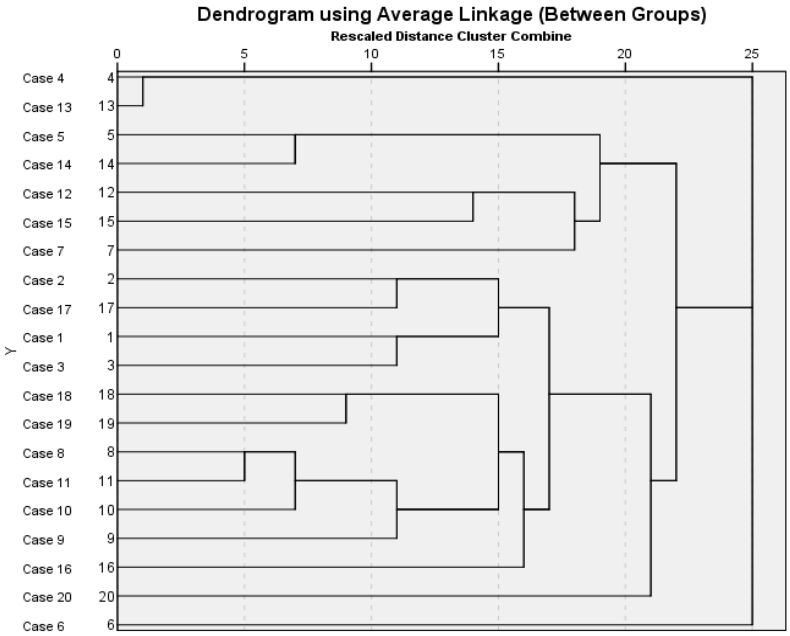

Fig.3 Diversity of carambola found in Arunachal Pradesh

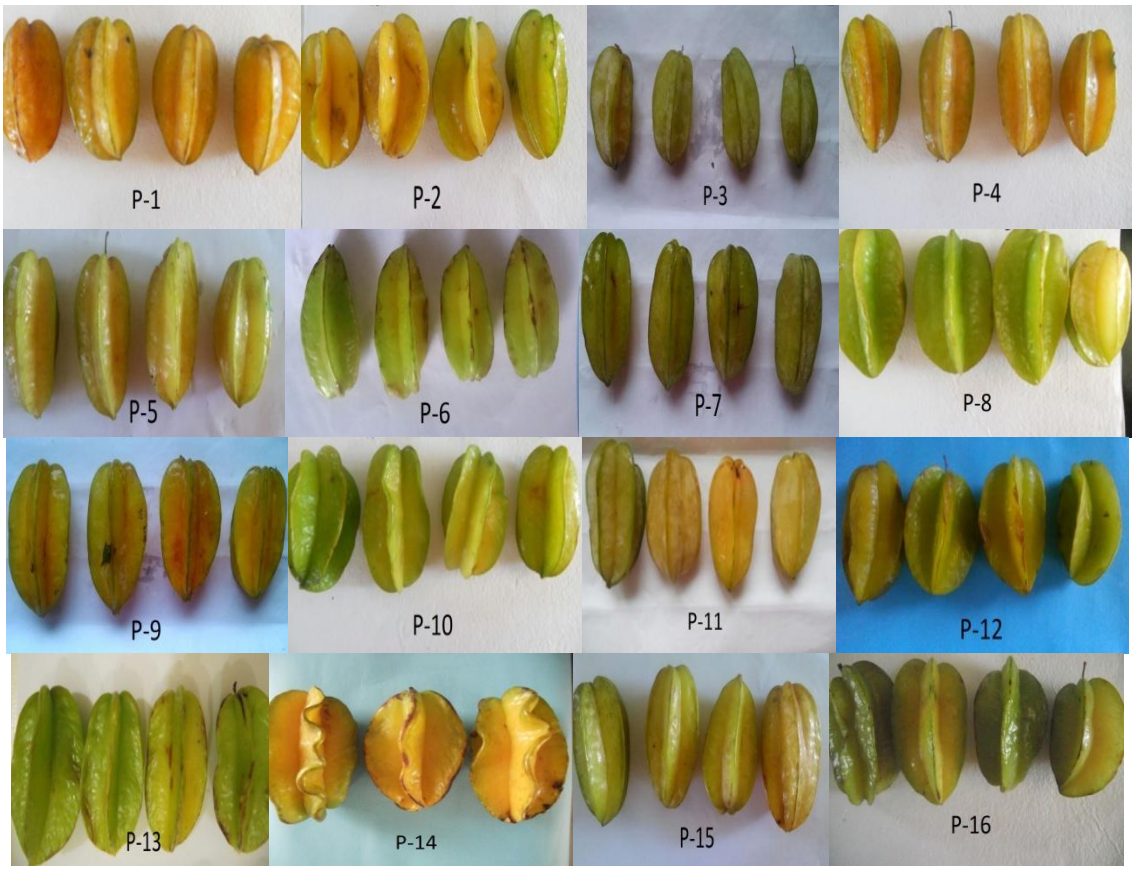

Table.1 Physical parameters among the selected genotypes based on descriptors

Treatment	Fruit Weight (g)	Fruit Diameter (cm)	Fruit Length (cm)	Fruit rib length (cm)	Fruit rib thickness (cm)	No. of Seeds/ Fruit	Average Weight of 10 Seed (g)	Pedicel Length (cm)	Pedicel diameter(cm)	Fruit juice content per Fruit (ml)	Productivity (yield per plant per year)
P_1	100.00	4.93	7.90	1.80	1.40	2.00	0.56	4.17	0.25	32.00	125.00
P_2	170.03	6.90	11.10	1.87	1.50	3.00	0.63	5.13	0.24	36.37	155.00
P_3	90.03	5.40	8.13	2.00	1.43	3.33	0.67	4.40	0.22	30.00	108.33
P_4	109.67	4.93	6.83	2.10	1.60	4.00	0.64	4.60	0.23	25.00	84.33
P_5	90.03	4.07	6.87	2.10	1.83	4.33	0.73	4.37	0.23	25.67	95.00
P_6	105.00	4.17	8.30	1.93	1.57	3.00	0.68	4.77	0.22	35.00	111.67
P_7	112.00	5.03	10.27	2.10	1.30	3.67	0.63	4.87	0.21	32.00	115.00
P_8	120.00	7.37	9.77	2.03	1.50	4.00	0.67	4.93	0.20	47.33	185.00
P_9	99.77	5.37	8.20	2.20	1.73	3.33	0.60	3.90	0.18	34.67	165.00
P_{10}	115.00	6.63	8.10	2.20	1.40	2.67	0.68	4.10	0.21	38.00	110.33
P_{11}	130.03	4.13	10.27	2.30	1.57	3.33	0.73	4.07	0.19	43.33	115.00
P_{12}	121.00	5.10	8.20	2.10	1.37	4.67	0.62	4.27	0.22	32.67	111.67
P_{13}	118.13	5.37	8.30	1.73	1.70	2.67	0.69	4.53	0.23	34.33	111.00
P_{14}	300.00	9.13	15.93	2.33	1.83	3.67	0.73	4.57	0.26	61.67	124.33
P_{15}	130.97	6.13	7.07	1.53	1.40	2.67	0.60	4.00	0.24	41.67	112.33
P_{16}	112.93	5.13	6.90	1.90	1.57	4.00	0.64	3.87	0.22	41.00	112.67
P_{17}	152.90	5.60	11.30	1.80	1.53	3.33	0.76	4.03	0.23	33.00	118.33
P_{18}	90.07	6.17	7.60	1.40	1.40	2.67	0.67	4.17	0.21	49.67	95.00
P_{19}	98.00	4.43	7.40	1.50	1.53	4.00	0.62	4.20	0.18	26.00	162.67
P_{20}	83.53	4.20	7.73	1.50	1.50	4.00	0.66	4.23	0.22	28.50	152.33
Mean	122.46	5.51	8.81	1.92	1.53	3.42	0.66	4.36	0.26	36.39	123.50
SEm±	1.38	0.07	0.07	0.04	0.02	0.20	0.01	0.06	-	0.48	1.69
CD 5%	3.94	0.21	0.19	0.11	0.07	0.56	0.02	0.17	-	1.38	4.85

Table.2 Quality parameters of fruit based on descriptor of carambola

Treatment	TSS (°Brix)	Fruit Oxalic acid (%)	Fruit titratable acid (%)	Vitamin C (mg/100g)	pH	Non-reducing Sugars (%)	Reducing sugars (%)	Total Sugars (%)	Fruit firmness (kgf)	Shelf life In days
P_1	6.20	0.02	0.57	48	3.73	0.09	6.23	6.63	2.20	12.00
P_2	7.33	0.01	0.27	42	4.63	0.08	8.50	9.07	1.63	12.00
P_3	8.07	0.01	0.37	47	4.50	0.07	3.20	3.40	1.59	12.00
P_4	6.03	0.03	0.88	51	2.53	0.07	4.30	4.57	2.27	13.33
P_5	5.23	0.01	0.37	41	3.60	0.07	7.30	7.70	1.51	12.00
P_6	5.07	0.02	0.49	45	3.57	0.07	9.73	10.50	1.56	13.33
P_7	7.30	0.01	0.30	41	2.43	0.08	8.67	9.10	1.53	11.33
P_8	8.50	0.01	0.32	31	4.03	0.07	7.60	8.23	2.43	12.00
P_9	8.53	0.35	0.94	68	4.10	0.07	5.70	5.97	1.49	12.67
P_{10}	5.83	0.16	0.38	30	3.47	0.05	5.00	5.23	2.35	10.67
P_{11}	14.97	0.16	0.20	47	2.60	0.07	11.93	12.80	2.30	11.33
P_{12}	5.03	0.03	0.78	42	3.80	0.06	5.93	6.30	2.27	13.33
P_{13}	6.07	0.03	0.08	38	3.80	0.07	3.60	3.97	2.31	12.67
P_{14}	9.50	0.01	0.38	30	2.40	0.03	8.53	9.27	1.66	12.00
P_{15}	6.13	0.02	0.57	45	2.50	0.08	7.93	8.23	1.78	12.67
P_{16}	5.73	0.03	0.75	51	3.53	0.07	4.60	4.73	2.29	13.33
P_{17}	6.10	0.05	1.22	25	2.73	0.14	7.47	8.00	2.38	14.00
P_{18}	8.03	0.01	0.40	38	3.73	0.06	9.07	9.17	2.29	11.33
P_{19}	7.87	0.02	0.48	50	3.37	0.05	8.73	8.93	1.45	12.67
P_{20}	8.03	0.10	0.25	33	3.20	0.08	8.40	3.97	1.55	11.33
Mean	7.28	0.05	0.50	63	3.41	0.08	7.12	7.29	1.94	12.30
SEm±	0.08	0.0034	0.0012	0.17	0.03	-	0.10	0.07	0.03	0.36
C.D. 5%	0.16	0.0096	0.0034	0.01	0.08	-	0.27	0.14	0.08	1.01

On content of titratable acidity the minimum acidity was recorded in P_{13} (0.08%) whereas maximum titratable acidity was observed in P_{17} (1.22 %). In this regard, Das and Bal (2010) reported that acidity ranged from 0.47-0.87%. Besides, Chadha (2013) also reported that acidity of carambola ranged from 0.4% to 1%. The observed values on firmness of fruit varied significantly among different genotypes. P_{17} was recorded highest (2.43 kgf) followed by P_8 (2.38 kgf) whereas lowest was recorded in P_{19} (1.45 kgf) with the average value of 1.94 kgf. Omar and Matjafri (2013) reported that firmness of carambola ranged from 1.5-8 kgf. Different selected genotypes showed significant variation for fruit pH value and the observed value varied from 2.4 to 4.63. Minimum pH value was found in P_{14} (2.4) and maximum in P_2 (4.63) with average mean value 3.41. In this regard Watson *et al.,* (1988) found that the pH content of carambola ranged from 2.5-5.0 which is in consonance with the present finding.

Different selected genotypes showed significant variation for ascorbic acid content and varied from 25 mg/ 100 g in P_{17} to 68 mg/ 100 g in P_9. Genotype P_{19} (50 mg/100 g) was statistically at par with both P_4 and P_{16} (51mg/ 100 g).The vitamin C content of genotype P_9 was found to be highest. In this regard Lim and Lee (2013) recorded that the vitamin C contents of carambola was 1.56 g per 100g which is a contrast to the present finding. Ali and Jafaar (2013) also reported that vitamin C content of carambola was 38- 40.2 mg per 100g in 13 weeks of fruit set. The observed data on total sugar content of fruit revealed significant variation among genotypes and varied from 3.4 % in P_3 to 12.8 % in P_{11} with an average value of 7.29 %. Genotypes P_{11} recorded to have the highest content of total sugar followed by P_6 (10.5%), P_{14} (9.27%), P_{18} (9.17%) and P_7 (9.1%). Watson *et al.,* (1988) found out that total sugar content ranged from 3.5-11% in 100g which is in consonance with the present study. The observed values on reducing sugar content of fruit varied significantly which ranged from 3.2 % in P_3 to 11.93 % in P_{11} with an average of 7.12 %. P_{11} is followed by P_6 (9.73%), P_{18} (9.07%) and P_{18} (8.73%). Das and Bal (2010) reported reducing sugar carambola fruit ranged from 2.79-4.58%.

The observed values on non-reducing sugar content of the fruits were non-significant among genotypes in which average reducing sugar ranged from 0.03- 0.14%. Das and Bal (2010) reported that non- reducing sugar of the carambola ranged from 2.25-2.36%. At room temperature shelf life of selected genotypes of carambola varied significantly which ranged from 10.67 days in P_{10} to 14 days in P_{17} with the average of 12.30 days. Rathod (2011) reported that carambola treated with 200 gauge highly density polyethylene without ventilation can store upto for 21 days.

Genotype characterization through seed protein profiles

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Fufa *et al.,* 2005; Iqbal *et al.,* 2005). Seed protein variants have been observed to be the most widely used biochemical genetic markers during the last quarter century. Its success depends upon the polymorphism of seed and seedling proteins and the fact that these proteins represent primary gene products and are largely unaffected by the environmental interactions (Smith and Smith, 1992). Caution should be taken in interpreting darkness and thickness as the kind of variation may be due to the lack of separation on the gels of several proteins having similar migration rates and studies are required to estimate the number of

genes causing quantitative variation in seed protein bands (Ladizinsky and Haymowitz, 1979). SDS-PAGE was carried out to determine the protein banding patterns of 20 carambola genotypes. The genotypes showed considerable variation in band number of protein in present investigation which ranged from 14-38. Among the genotypes P_3 showed maximum number (38) of protein bands while the minimum number (14) of bands was present in genotype P_6 and P_{12}. Cluster analysis utilizing SDS-PAGE banding pattern produced a dendrogram depicting clear separation of genotypes. Difference index generated by SDS-PAGE analysis in the germplasm under study range from 0.00 to 0.95. Values of difference index coefficient matrix suggested least difference index of genotype P_4 with P_{13} (0.11) while genotype P_4 was found to have maximum difference index with genotype P_6 as evident by coefficient value 0.95. Thus, SDS-PAGE marker data provided more sub groupings and revealed higher amount of diversity as compared to morphological data analysis. It is evident from the present study that genetic relationship estimated from protein banding pattern enhanced the resolution of diversity and thus provided a better picture of variability as compared to morphological markers. Although SDS-PAGE analysis could show discrete variation among few genotypes of carambola under study, this protein marker should be applied in future to more number of genotypes to arrive at a reasonable conclusion. Therefore, seed protein electrophoresis could be proved to be a successful technique in certain cases to distinguish morphologically indistinguishable genotypes (Fig. 1–3).

Based on the results obtained from the present investigation, it can be concluded that, selected carambola genotypes exhibited noticeable variation in the morphological and biochemical characteristics. The genotypes P_8 gave maximum yield (185 kg per tree per year), P_{11} highest TSS (14.95^0Brix), P_1 lowest seed (2), P_9 highest vitamin C (68mg/100g) and P_{14} gave highest fruit weight (300g). Thus, these findings illustrated the usefulness of physic-chemical characterization and biochemical marker as a tool for the genetic diversity evaluation in carambola.

Acknowledgements

The authors like to render gratefulness to the College of Horticulture & Forestry, Central Agricultural University for allowing the research work for this under ultized fruit crop.

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How to cite this article:

Rebica Padun and Singh, S.R. 2018. Evaluation of Genetic Diversity of Carambola (*Carambola averrhoa* L.) in Arunachal Pradesh, India. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 2729-2738. doi: https://doi.org/10.20546/ijcmas.2018.702.332