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Phenology and Molecular Characterization of *Annona* Genotypes

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ABSTRACT

The experiment was conducted during July, 2020–January, 2021 at Agroforestry Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India. *Annona* belongs to the family Annonaceae. The Annonaceae fruits are morphologically uniform but each type has a unique taste, flavour pulp colour and texture. The study revealed three types of leaf shapes (ovate, elliptic and lanceolate), leaf apex (acute, rounded and obtuse), leaf base (acute, rounded and acuminate), fruit shape (cordate, rounded and irregular), medium and late time of harvest maturity, overlapping and smooth segmentation of the surface and only one type for fruit at peduncle end i.e., inflated. The biochemical analysis of *Annona* species revealed the ranges of TSS (18.39 to 28.32 °B), TA of pulp (0.18–0.80%), reducing sugar (15.02–19.44%), non-reducing sugar (2.77–5.92%), total sugar (17.79–23.70%), ascorbic acid (27.55–43.10 mg 100 g^{-1}) and phenol (0.18–0.36%). The DNA was amplified using fourteen simple sequence repeats primers and twenty-six alleles were found with an average of 1.86 alleles $loci^{-1}$. The polymorphism information content value of the SSR markers was in the range of 0.09 to 0.38. A dendrogram was developed based on the cluster analysis, which revealed similarity index values varied from 0.58 to 1.00 with an average of 0.83. Based on the dendrogram the genotypes were clustered into two main clusters viz., A and B. Cluster A has sixteen genotypes and cluster B has two genotypes belonging to different *Annona* species. These results concluded that SSR markers could be efficiently used to study divergence among *Annona* genotypes.

KEYWORDS: *Annona* genotypes, morphological traits, biochemical traits, SSR, PIC

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Annona squamosa L. of the family Annonaceae [commonly known as sugar apple, sweetsop and Sharifa is a native of the tropical region of the West Indies. In India, the custard apple is the most important and favourite fruit of the annonaceous family. The Annonaceous fruits originated from tropical America and are widely distributed in the tropics and subtropical regions (Ghosh, 2019). *Annona* means year's harvest and *squamosa* means scaly, referring to the scale-like structure on the fruit surface. It is a cross-pollinated crop with wide variability in fruit size and colour of the fruit. *Annona atemoya* Hort. (a hybrid of *Annona squamosa* and *Annona cherimola*) is a commercially significant and economically important fruit. Custard apple is considered delicious and has high nutritional value. Custard apple is heart shaped weighing about 150 g, with very bumpy skin and their pulp is slightly, creamy yellow or white, sweet with a good flavour and low acidity (Cheng et al., 2018). Custard apple is rich in sugar (14.5%), proteins (0.8–1.5%), minerals (0.7%), etc. The nutrient value of 100 g ripe pulp estimated as a good source of carbohydrates 20.0–25.2 g, calcium 17.6–27 mg, phosphorus 14.7–32.1 mg, iron 0.42–1.14 mg, carotene 0.007–0.018 mg, thiamine 0.075–0.119 mg, riboflavin 0.086–0.175 mg, niacin 0.53–1.19 mg, ascorbic acid 15.0–44.4 mg and nicotinic acid 0.5 mg (Ghosh, 2019).

Morphology and fruit quality a complex and polygenic traits that are highly influenced by several environmental factors or vary with various developmental stages of plants. Hence, the extensive natural diversity within the custard apple global germplasm can be harnessed to discover superior genotypes. These genotypes hold the potential as valuable genetic resources for advancing nutri-agricultural research in the future. The most crucial factor in crop improvement is estimating genotype genetic variation. *A. squamosa* L. was mostly employed for biochemical analysis according to studies on the proximate fruit composition of genotypes, which allows fruit breeders the opportunity to develop the other *Annona* species (Anuragi et al., 2016).

Molecular markers provide information on DNA polymorphism, to study genetic diversity within/between populations. Simple Sequence Repeats (SSR) [Escribano et al., 2004 (*Annona cherimola* Mill); Kwapata et al., 2007 (*Annona senegalensis* pers.); Escribano et al., 2008 (*Annona cherimola* Mill) and Anuragi et al., 2016 (*Annona cherimola* Mill, *Annona reticulata, Annona muricata* L., *Annona atemoya* and *Annona squamosa* L.) had been used to characterize *Annona* genotypes. Microsatellites, also known as SSR, are among the molecular markers that provide good tools for genotypes because of their unique characteristics, such as high polymorphism, high repeatability, codominant nature, and often high levels of allelic variation at various loci. Microsatellites are currently the most used marker because they are highly polymorphic even among closely related lines, need little DNA, can be automated, are quickly shared between laboratories, and are highly transferrable between populations. According to Kwapata et al. (2007), SSRs frequently carry large numbers of alleles at extremely low frequencies. The most crucial factor in choosing desired *Annona* genotypes for domestication in the domain of adaptation is the knowledge of nutritional value. Fruits of particular genotypes were examined using SSR molecular markers to explore genetic diversity (Anuragi et al., 2016). Hence, the present study was designed to extensively characterize the 18 *Annona* genotypes belonging to two *Annona* species for genetic diversity for morphological, biochemical traits and molecular analysis to detect the promising genotypes.

2. MATERIALS AND METHODS

Eighteen promising genotypes were selected from the existing germplasm available at Agroforestry Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujrat, India planted in 1996 years back. Among all these, Sr. no. 1 to 17 belongs to *Annona squamosa* L. while, Island Gem belongs to *Annona atemoya* Hort. In all, 18 genotypes were screened

Table 1: List of genotypes used for present study

Sl. No.	Genotype	Source/Collection from
1.	Selection-1	Shamji Patel, Junagadh
2.	Selection-2	Janagadh Uparkot Jungle, Junagadh
3.	Selection-3	Dharaji, Waste land
4.	Selection-4	Waste land, Junagadh
5.	Selection-5	Waste land, Bhayavadar, Rajkot
6.	Selection-6	Waste land, Junagadh
7.	Selection-7	Waste land, Vanthali, Junagadh
8.	Selection-9	Waste land, Junagadh
9.	Selection-10	Uparkot, Junagadh
10.	Selection-11	Waste land, Junagadh
11.	Selection-12	Waste land, Bhayavadar
12.	Selection-13	Waste land, Junagadh
13.	Selection-14	Waste land, Vanthali, Junagadh
14.	Selection-15	Waste land, Vanthali, Junagadh
15.	Sindhan	Agroforestry Research Station, SDAU, Sardarkrushinagar
16.	Mammoth	Aruppukottai, Tamil Nadu
17.	Washington	Aruppukottai, Tamil Nadu
18.	Island Gem	Aruppukottai, Tamil Nadu

by morphological and biochemical parameters for the *kharif* seasons (July–January) during the year 2020–21 to select promising genotypes (Table 1).

2.1. Morphological traits

In order to determine criteria such as leaf shape, leaf base, leaf apex, fruit shape, fruit at peduncle end, time of harvest, and segmentation of fruit surface, the material was assessed for eight morphological traits based on Anonymous (2011) descriptors. From every two plants, mature fruits and completely formed, healthy leaves were randomly chosen for observational qualities.

2.2. Biochemical traits

Five fully ripened mature fruits from similar and comparative stages were collected from the 18 fruit genotypes of *Annona* that had their varied biochemical compositions determined. Fruit pulp was removed from seeds, homogenized, and subjected to biochemical examination for this purpose. Total soluble sugar (TSS) (°Brix), titrable acidity (TA) (%), TSS to titrable acidity ratio, reducing sugar (%) (Nelson, 1944), total sugar (%) (Miller, 1972), ascorbic acid (mg 100 g^{-1}) (Anonymous, 1990), and phenol content (%) (Bray and Thorpe, 1954) are the seven biochemical traits.

2.3. Molecular diversity

Eighteen *Annona* genotypes genomic DNA was isolated from fresh, tender leaves using the modified CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method reported by Doyle and Doyle in 1987. Simple sequence repeat (SSR) marker was used for genotyping. The amplification was set up, to begin with a 94°C denaturation for 4 minutes, followed by 35 cycles of 94°C denaturation for 30 seconds, an annealing temperature for the primer for 30 seconds, and an extension temperature of 72°C for 1 minute. A final extension step lasting 7 minutes at 72°C brought the cycling session to an end. On 3% agarose gels, electrophoresis was used to separate the amplification products from a common 100 bp DNA marker.

2.4. Statistical analysis

SSR markers were amplified and assessed in a present (1) or absent (0) manner. An easy text file was used to store the data. A data matrix was created by assembling all of these profiles. The numerical taxonomy and multivariate analysis system for personal computers, NTSYS-pc version 2.20 (Exeter Software), developed by Rohlf (1998), was used to read the data matrix. SIMQUAL (Similarity for Quantitative Data), a program, was used to analyze the data matrix using Jaccard's similarity coefficient (Jaccard, 1908). The polymorphic information content (PIC) was calculated, according to the method of Miks and Binkowski (2018).

$$PIC = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2 P_i^2 P_j^2$$

P_i, P_j are the frequency of the i^{th} and j^{th} allele (float format), I–Number of alleles

3. RESULTS AND DISCUSSION

3.1. Morphologically traits

Ovate, elliptic, and lanceolate varieties of leaves were observed, making three different leaf shape types. There are fifteen genotypes, namely Selection-1, Selection-2, Selection-3, Selection-4, Selection-5, Selection-6, Selection-7, Selection-9, Selection-10, Selection-11, Selection-12, Selection-13, Selection-14, Selection-15 and Selection-16. The remaining two genotypes, Washington and Island Gem, had elliptic leaf shapes (11.12%) while Mammoth had lanceolate leaf shapes (5.56%), with Sindhan having the highest percentage of ovate leaves (83.40%). This revealed that the majority of the genotypes examined had ovate leaves. These results were confirmed by the findings of Anuragi (2015) and Jyolsna (2016).

Three types of leaf bases were observed acute, rounded and obtuse types. Fifteen genotypes viz., Selection-1, Selection-2, Selection-3, Selection-4, Selection-5, Selection-6, Selection-7, Selection-9, Selection-10, Selection-11, Selection-12, Selection-13, Selection-14, Selection-15 and Sindhan had (83.40%) acute leaf base, remaining two genotypes viz., Washington and Island Gem had (11.12%) rounded leaf base, whereas Mammoth had (5.56%) obtuse leaf base. Similar variations observed in the present investigation were reported by Jyolsna (2016).

Three types of leaf apex observed were acute, rounded and acuminate. Fifteen genotypes viz., Selection-1, Selection-2, Selection-3, Selection-4, Selection-5, Selection-6, Selection-7, Selection-9, Selection-10, Selection-11, Selection-12, Selection-13, Selection-14, Selection-15 and Sindhan had (83.40%) acute leaf apex, remaining two genotypes viz., Washington and Island Gem had (11.12%) rounded leaf apex, while Mammoth had (5.56%) acuminate leaf apex. The results of visual selection were confirmed with the results reported by Jyolsna (2016) .

Sindhan Mammoth Washington Iceland Gem

Figure 1: Variation in leaf morphology among different *Annona* genotypes

The fruit shape variability in the different genotypes varied from cordate, rounded and irregular shapes. Out of eighteen genotypes, fifteen genotypes viz., Selection-1, Selection-2, Selection-3, Selection-4, Selection-5, Selection-6, Selection-9, Selection-11, Selection-12, Selection-13, Selection-14, Selection-15, Sindhan, Mammoth and Washington had (83.40%) cordate fruit shape, Selection-7 and Selection-10 recorded (11.13%) rounded fruit shape and irregular shape (5.56%) in Island Gem. The results observed in the present investigation conformed with those of Anuragi (2015) and Jyolsna (2016) .

Sindhan Mammoth Washington Island Gem

Figure 2: Variation in fruit morphology among different *Annona* genotypes

The fruit could be harvested when it is physiologically mature. Harvesting at the time of optimum maturity will produce the best quality fruit with a good shelf life. Thirteen genotypes namely, Selection-1, Selection-2, Selection-3, Selection-4, Selection-6, Selection-9, Selection-10, Selection-11, Selection-12, Selection-13, Selection-14, Selection-15 and Sindhan were (72.28%) medium in harvesting while, the five genotypes viz., Selection-5, Selection-7, Mammoth, Washington and Island Gem were (27.80%) late in harvesting. The results were in agreement with those reported by Bhatnagar et al. (2012) who obtained November time of harvest and Girwani et al. (2011) who reported from September onwards, October and November.

The fleshy fruit dispersed as inflated at the peduncle end. All the eighteen genotypes possessed inflated fruit at the peduncle end. None of the studied genotypes recorded flattened or depressed fruit at the peduncle end. Seventeen genotypes, except Island Gem, reported overlapping segmentation of the surface. The result suggested that variable traits among the various genotypes might be due to influencing gene expression and genetic interactions (Figure 3).

3.2. Biochemical traits

Several biochemical traits of a few *Annona* genotypes showed significant variance, which is shown in Table 2. When assessing a fruit for consumer acceptance, a breeder is more interested in perceived sweetness than just soluble solids alone. Perceived sweetness is strongly influenced by the relative quantities of total soluble solids and acids in the fruits. All of the soluble solids found in fruits are included in the total soluble solids. Customers favor fruits with the ideal

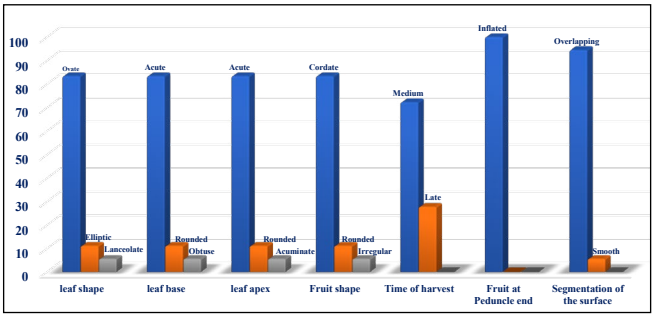

Figure 3: Morphological characterization of different *Annona* genotypes

sugar-acid balance in desert fruits like custard apples. The types of organic acids present and their concentration level have a significant impact on how a fruit tastes. High acidity typically results in superior blends and flavors. Total soluble solids were at their highest for the genotype Selection-9 (28.32°B). The range of variance across all genotypes was 18.39 to 28.32°B, with a mean of 25.51°B (Table 2). Various factors, including climatic conditions and genetic traits of the genotype, may contribute to variations in TSS values.

Since fruit acidity affects the flavor, appearance, and microbiological stability of fruit juice, it can be inferred that Annona fruits may be more palatable to consumers. In freshly matured fruit, titrable acidity ranged from 0.18 to 0.80%, with a mean value of 0.36%. Titrable pulp acidity was noticeably higher for Washington (0.80%), which is listed in Table 3. For acidity mean performance Saitwal et al. (2015) reported 0.29% titrable acidity in Island Gem, 0.21% in Washington, and 0.32% in Island Gem.

The ratio of sugar to acid in a fruit mixture is one of the key factors that affect the fruit's flavour. Fruit flavour and taste are affected by both the TSS and acidity of the fruit. For all genotypes, ranging from 23.02 to 153.09, a significant difference was noted. A high TSS TA^{-1} ratio of 153.09 was seen in the genotype Selection-9 (Table 3).

The main byproduct of photosynthesis, sugars play a variety of roles in plants, including those of energy and carbon transport molecules, hormone-like signaling agents, and building blocks for proteins, polysaccharides, lipids, and woody materials. Additionally, it is in charge of the biotic stress resistance mechanism. Sugars are essential for osmotic adjustment and for protecting against a variety of stressors. The sole element that affects how sweet the pulp is is the fruit's sugar concentration.

For reducing sugar, a range of variance between 15.02 and 19.44% was noted. Selection-3 (19.44%) and Selection-2 (18.51%) had the highest levels of reducing sugar, respectively. Washington (15.02%), Mammoth (15.15%), and Selection-7 (16.47%) were the genotypes with the lowest reducing sugar levels. According to the

Table 2: Biochemical characters of different *Annona* genotypes

Sl. No.	Genotypes	Total soluble solids (°B)	Titrable acidity (%)	TSS/ TA ratio	Reducing sugar (%)	Nonreducing sugar (%)	Total sugar (%)	Ascorbic acid (mg/100g)	Phenol (%)
1.	Selection – 1	24.74	0.46	54.45	17.92	4.44	22.36	27.55	0.23
2.	Selection – 2	25.73	0.36	72.41	18.51	3.55	22.57	30.75	0.20
3.	Selection – 3	27.53	0.27	102.18	19.44	4.27	23.70	29.45	0.23
4.	Selection – 4	27.21	0.28	98.98	17.06	5.92	22.96	33.10	0.23
5.	Selection – 5	25.75	0.35	73.67	17.67	3.98	21.96	36.95	0.25
6.	Selection – 6	25.32	0.41	63.51	16.98	5.22	21.20	36.65	0.19
7.	Selection – 7	27.62	0.33	85.73	16.47	4.54	21.01	34.70	0.22
8.	Selection – 9	28.32	0.18	153.09	18.21	5.17	23.38	43.10	0.19
9.	Selection – 10	24.45	0.39	63.02	16.12	5.29	21.42	33.65	0.20
10.	Selection – 11	26.63	0.30	89.22	17.60	5.04	22.64	38.30	0.21
11.	Selection – 12	26.49	0.24	112.85	17.60	5.55	23.05	35.25	0.23
12.	Selection – 13	25.62	0.26	98.54	15.74	4.94	20.69	38.85	0.20
13.	Selection – 14	26.69	0.23	116.07	16.54	4.64	21.18	37.15	0.27
14.	Selection – 15	26.62	0.28	98.18	18.42	3.87	22.28	39.80	0.18
15.	Sindhan	28.06	0.18	124.76	17.77	4.17	21.95	35.00	0.27
16.	Mammoth	19.51	0.68	28.80	15.15	3.56	18.71	29.70	0.25
17.	Washington	18.39	0.80	23.02	15.02	2.77	17.79	30.55	0.19
18.	Iceland Gem	24.52	0.47	52.22	16.53	5.18	21.72	32.60	0.36
General mean		25.51	0.36	83.93	17.15	4.56	21.75	34.62	0.23
Range		18.39-28.32	0.18-0.80	23.02-153.09	15.02-19.44	2.77-5.92	17.79-23.70	27.55-43.10	0.18-0.36
SEm±		1.24	0.03	6.09	0.72	0.36	0.63	2.36	0.02
CD (p=0.05)		3.69	0.08	18.18	2.16	1.07	1.88	7.04	0.07
CV%		6.86	11.23	10.27	5.96	11.12	4.10	9.64	14.69

aforementioned data, *Annona* genotypes have a significant amount of reducing sugar, which could serve as a source of resistance to biotic stress (Anuragi et al., 2017). These results were in coherence with Saitwal et al. (2015) who recorded reducing sugar in Island Gem (21.01%).

The genotype, Seletion-4 (5.92%) recorded higher non-reducing sugar followed by Selection-12 (5.55%) and Selection-10 (5.29%) whereas, the lowest non-reducing sugar was recorded in Washington (2.77%) followed by Selection-2 (3.55%) and Mammoth (3.56%). These results conformed with Idate et al. (2019). Looking at the average performance of various genotypes, Saitwal et al. (2015) in Island Gem (2.53%) which differs from the present study.

With a mean of 21.75%, all the genotypes demonstrated a significant difference ranging from 17.79 to 23.70%. The genotypes with the highest total sugar were Selection-3 (23.70%), Selection-9 (23.38%) and Selection-12 (23.05%) (Table 3). These results conformed with Saitwal et al. (2015) obtained total sugar in Island Gem (23.54%).

The potential use of *Annona* as an excellent source of ascorbic acid is indicated by the fruit's high ascorbic acid levels. The recommended daily intake (RDI) for ascorbic acid is 17 mg day^{-1} for children and 30 mg day^{-1} for adults. As a result, these fruits could be regarded as excellent providers of ascorbic acid for the needs of human nutrition. With a mean variance for ascorbic acid of 34.62 mg 100 g^{-1} across all genotypes, ascorbic acid variation varied from 27.55 to 43.10 mg 100 g^{-1}. Among the genotypes, the Selection-9 genotype had the highest ascorbic acid concentration (43.10 mg 100 g^{-1}), followed by the Selection-15 and Selection-13 genotypes (39.80 mg 100 g^{-1} and 38.85 mg 100 g^{-1}, respectively) in Table 3. These results by Yadav et al. (2017) found a range between 18.31 to 38.24 mg 100 g^{-1}.

The average concentration of phenol was 0.23%, with a

Table 3: Summary of SSR marker analysis in *Annona* genotypes

Sl. No.	Locus name	No. of amplified bands	Molecular size range (bp)	Total no. Alleles	Polymorphism information content
1.	LMCH2	20	178 - 216	2	0.30
2.	LMCH9	19	181- 216	2	0.17
3.	LMCH10	15	203 - 251	2	0.20
4.	LMCH33	19	238 - 255	2	0.17
5.	LMCH42	18	219 - 250	1	0.00
6.	LMCH43	18	252 - 312	2	0.10
7.	LMCH53	18	105 - 112	1	0.00
8.	LMCH69	19	178 - 192	2	0.09
9.	LMCH78	19	155 - 184	2	0.17
10.	LMCH93	30	241 - 268	2	0.36
11.	LMCH112	21	164 - 177	2	0.21
12.	LMCH114	20	164 - 172	2	0.16
13.	LMCH119	18	179 - 205	2	0.10
14.	LMCH127	36	173 to 186	2	0.38

range of 0.18 to 0.36%. The amount of phenol was highest on island Gem (0.36%), then in Sindhan (0.27%), as shown in Table 3. The results above showed that *Annona* genotypes, which had a substantially greater phenol content, could potentially be a source of disease resistance as well as be used for medicinal therapeutic purposes. The disparity between the stated results per se performance and that of the current study could be attributed to the influence of the environment and reference population.

3.3. Molecular diversity-SSR marker

3.3.1. Number of alleles and molecular size range

The 20 SSR markers employed across 18 *Annona* genotypes discovered a total of 26 alleles (Table 4). The average number of alleles per locus ranged from 1 to 2, with the total number of alleles being 1.86. Eleven polymorphic markers produced two alleles each, and three monomorphic markers produced one allele each. This finding, which showed that all of the primers displayed distinctive polymorphisms among the *Annona* genotypes under investigation, demonstrates the capacity of microsatellites to identify polymorphism. In this study, amplicon size ranged from 152 bp to 497 bp which is similar to results obtained in *A. cherimola* using 15 SSRs by Escribano et al., 2004 and Escribano et al., 2008. A similar result is obtained in *A. senegalensis* (Kwapata et al., 2007) and 152 bp to 497 bp in *Annona* species (Anuragi et al., 2016). PCR amplification profile of 18 *Annona* genotypes using SSR markers ASSSR_5 and ASSSR_16 are presented in the plates (Plate 1 and 2).

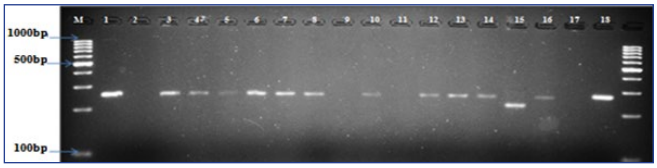

Plate 1: SSR profile with LMCH10

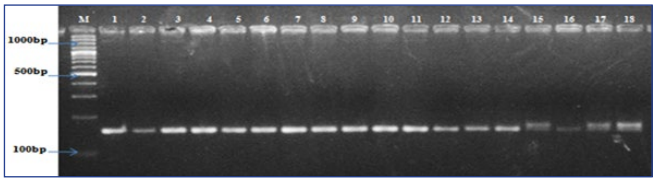

Plate 2: SSR profile with LMCH112

3.3.2. Polymorphism information content

These SSR markers have polyporphism information content (PIC) values ranging from 0.09 to 0.38, with a mean of 0.17. LMCH127 has the highest PIC value (0.38), followed by LMCH93 (0.36) and LMCH2 (0.30). The SSR markers utilized in this investigation were not extremely informative because only PIC values higher than 0.5 indicate high polymorphism, whereas these primers obtained a mean PIC value of 0.17. Anuragi et al. (2016) analyzed genetic diversity among twenty genotypes of *Annona* species using twelve SSR primers and reported a mean PIC of 0.339 ranging from 0.169 to 0.694. The details of the amplification product size, total number of alleles and PIC value of each are given in Table 3.

Table 4: Similarity matrix for Jaccard's coefficient for eighteen *Annona* genotypes based on microsatellite markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Selection-1	1.00																	
Selection-2	0.86	1.00																
Selection-3	0.86	0.77	1.00															
Selection-4	1.00	0.86	0.93	1.00														
Selection-5	0.83	0.86	0.82	0.88	1.00													
Selection-6	1.00	0.86	0.88	1.00	0.83	1.00												
Selection-7	1.00	0.86	0.88	1.00	0.83	1.00	1.00											
Selection-9	1.00	0.86	0.88	1.00	0.83	1.00	1.00	1.00										
Selection-10	0.88	0.85	0.87	0.93	0.82	0.88	0.88	0.88	1.00									
Selection-11	1.00	0.86	0.88	1.00	0.83	1.00	1.00	1.00	0.88	1.00								
Selection-12	0.94	0.86	0.81	0.93	0.78	0.94	0.94	0.94	0.93	0.94	1.00							
Selection-13	0.94	0.92	0.81	0.93	0.88	0.94	0.94	0.94	0.81	0.94	0.88	1.00						
Selection-14	0.94	0.86	0.81	1.00	0.78	0.94	0.94	0.94	0.81	0.94	0.88	0.88	1.00					
Selection-15	1.00	0.86	0.88	1.00	0.83	1.00	1.00	1.00	0.88	1.00	0.94	0.94	0.94	1.00				
Sindhan	0.70	0.65	0.68	0.72	0.75	0.70	0.70	0.70	0.78	0.70	0.74	0.65	0.65	0.70	1.00			
Mammoth	0.94	0.92	0.81	0.93	0.88	0.94	0.94	0.94	0.81	0.94	0.88	1.00	0.88	0.94	0.65	1.00		
Washington	0.67	0.67	0.57	0.65	0.64	0.67	0.67	0.67	0.68	0.67	0.70	0.70	0.62	0.67	0.61	0.70	1.00	
Island Gem	0.63	0.60	0.61	0.64	0.67	0.63	0.63	0.63	0.64	0.63	0.58	0.65	0.58	0.63	0.58	0.65	0.83	1.00

3.3.3. Clustering of annona genotypes

To investigate the evolutionary links between the various *Annona* genotypes, a cluster analysis based on UPGMA and Jaccard's similarity coefficients was carried out. After doing a cluster analysis, a dendrogram was created that showed similarity index values ranging from 0.58 to 1.00. Between Selection-1, Selection-4, Selection-6, Selection-7, Selection-9, Selection-11 and Selection-13, the greatest similarity index values of 1 were displayed. In contrast, the least similarity value of 0.58 between Sindhan, Selection-15, and Selection-14 indicates minimal genetic diversity (Table 4). The *Annona* genotypes were divided into A and B, the two main clusters, based on the dendrogram. One significant cluster A contained sixteen genotypes. Selection-1, Selection-4, Selection-6, Selection-7, Selection-9, Selection-11, Selection-15, Selection-14, Selection-13, Mammoth, Selection-10, Selection-12, Selection-2, and Selection-11 were among the *Annona* genotypes contained

in Sub-cluster A1. Genotype Sindhan is part of Subcluster A2. The genotypes Washington make up sub-cluster B1, while Island Gem differs from the individuals clustered in cluster B2 (Figure 4). SSR markers gave sufficient precision to distinguish between crop accessions and could be used as a tool to identify and characterize genetically dissimilar accessions from multiple sources. The genotypes under study showed a range of low to high levels of similarity, which suggested a significant level of genetic variety as well as the potential for using these genotypes in breeding programs aimed at enhancing premium *Annona* varieties. Breeders could choose several distantly related *Annona* genotypes for crossing from various clusters to produce hybrid varieties with the maximum heterosis and combining ability for the variables examined in the current study.

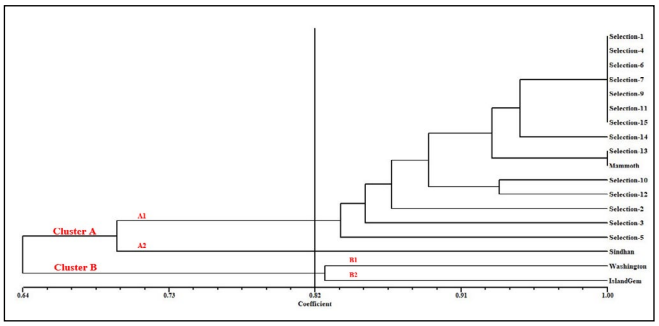

Figure 4: Dendrogram showing clustering of eighteen *Annona* genotypes based on molecular analysis

4. CONCLUSION

Annona fruit contained vital amounts of antioxidant and high amount of nutrient that played an eminent role in people's daily diet. The variability in the morphological and biochemical traits was mostly found to be useful for improving the quality of *Annona* and thus helpful for the exploitation of heterosis. The molecular analysis concluded that the information of genetic diversity among the genotypes could be easily assessed by using SSR markers.

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6. REFERENCES

Anonymous, 1990. AOAC: Official methods of analysis. 15th Ed. Virginia, 838–841. Available from AOAC: Official Methods of Analysis : AOAC International : Free Download, Borrow, and Streaming : Internet Archive. Accessed on 20th December 2019.

Anonymous, 2011. Guidelines for the conduct of test for Distinctiveness, Uniformity, and Stability on Custard apple/Sugar apple (*Annona squamosa* L.). protection of plant varieties and farmer's Rights Authority, Government of India, 1–13. Available from draftcustardapple.pdf (plantauthority.gov.in). Accessed on 24th November 2019.

Anuragi, H., 2015. Morphological, biochemical, and molecular characterization of different genotypes of *Annona* species. M.Sc. (Agri.) Thesis (Unpublished). Anand Agriculture University, Anand.

Anuragi, H., Dhaduk, H.L., Jain, B.T., Kumar, S., 2017. Genetic association studies for fruit yield and its components and qualitative phytochemical screening in promising *Annona* genotypes. Research Journal of Agricultural Sciences 8(1), 222–227.

Anuragi, H., Dhaduk, H.L., Kumar, S., Dhruve, J.J., 2016. Molecular diversity of *Annona* species and proximate fruit composition of selected genotypes. 3 Biotech 6, 204.

Bhatnagar, P., Singh, J., Jain, M.C., Singh, B., 2012. Evaluation of landraces of custard apple (*Annona squamosa* L.). Plant Archives 12(2), 1045–1048.

Bray, H.G., Thorpe, W.V., 1954. Analysis of phenolic compounds of interest in metabolism. Methods Biochemistry Analytic 52, 1–27.

Cheng, Z., Zhou, W., Gong, X., Wei, X., Li, J., Peng, Z., 2018. Physicochemical changes of custard apple at different storage temperatures. IOP Conference Series: Materials Science and Engineering 392, 052013.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11–15.

Escribano, P., Viruel, M.A., Hormaza, J.I., 2004. Characterization and cross-species amplification of microsatellite markers in cherimoya (*Annona cherimola* Mill. *Annonaceae*). Molecular Ecology Notes 4, 746–748.

Escribano, P., Viruel, M.A., Hormaza, J.I., 2008. Development of 52 new polymorphic SSR markers from cherimoya (*Annona cherimola* Mill.): transferability to related taxa and selection of a reduced set for DNA fingerprinting and diversity studies. Molecular Ecology Notes 8, 317–321.

Ghosh, N., 2019. Crop improvement and varietal wealth, tropical and subtropical fruits crops. Jaya Publishing House, New Delhi, 581–618.

Girwani, A., Madhavi, A., Kumar, T.S., Reddy, G.S., 2011. Evaluation of custard apple hybrids for fruit yield and quality attributing characters. Acta Horticulture 890, 251–254.

Jaccard, P., 1908. Nouvelles recherché sur la distribution florate. Bulletin de la Societevaudoise des Sciences Naturelles 44, 223–270.

Jyolsna, M., 2016. Assessment of variability in *Annona* spp. M.Sc. (Horti.) Thesis (Unpublished). Kerala Agricultural University, Vellanikkara, Thrissur.

Kwapata, K., Mwase, W.F., Bokosi, J.M., Kwapata, M.B., Munyenyembe, P., 2007. Genetic diversity of *Annona senegalensis pers*. populations as revealed by simple sequence repeats (SSRs). African Journal of Biotechnology 6(10), 1239–1247.

Miks, S., Binkowski, J., 2018. Gene-calc [computers software]. Available from: www.gene-calc.pl.

Miller, G.L., 1972. Use of dinitrosalicyclic acid reagent for determination of reducing sugars. Analytical Chemistry 31, 426–428

Nelson, D.H., 1944. A photometric adaptation of the Somogyi's method for determination of the glucose. Journal of Biological Chemistry 153, 373–380.

Rohlf, F.J., 1998. NTSYS-pc Version.2.20e, numerical taxonomy and multivariate analysis system. Applied Biostatistics Inc., Exeter Software, New York.

Saitwal, Y.S., Musmade, A.M., Supe, A.M., Joshi, V.R., Nimbalkar, C.A., 2015. Physicochemical profiling for selection of Promising *Annona* genotypes. Indian Journal of Dryland Agricultural Research and Development 30(1), 89–93.

Yadav, V., Singh, A.K., Singh, S., Apparao, V., 2017. Variability in custard apple (*Annona squamosa*) genotypes for quality characters from Gujarat. Indian Journal of Agricultural Sciences 87(12), 1627–1632.