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Characterization of *Annona* cultivars under Northern dry zone of Karnataka using molecular markers

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Abstract
The current study was conducted at the College of Horticulture in Bagalkot, Karnataka, between 2017 and 2019. A Randomized Complete Block Design was used to set up the experiment, and twelve cultivars were duplicated three times. Horticulturists have traditionally used morphological and physiological descriptors to identify different varieties of fruit crops. Using the allele sizes of nine SSR markers acquired from previously described *Annona* species, the genetic diversity of the 12 *Annona* cultivars was evaluated (Larranaga and Hormaza, 2015). Nine microsatellite markers were used to identify a total of 21 alleles from the nine SSR manufacturers that were evaluated across 12 *Annona* cultivars in the current investigation. In the current study, a total of 11 RAPD markers that were evaluated across 12 *Annona* cultivars amplified a total of 28 loci. Additionally, to determine how closely related these 12 cultivars were genetically, they were subjected to phylogenetic analysis using the pair-wise genetic matrix generated by RAPD markers. The results were presented in Figure 2 using an unrooted neighbour joining tree and the Mega7.0 software. The findings of the molecular analysis had shown that the genetic diversity of cultivars varied. Twelve *Annona* cultivars under investigation have been successfully characterized using the SSR and RAPD markers. The findings showed that SSR markers are co-dominant and highly reproducible. The significance of SSR markers in the molecular characterisation of *Annona* cultivars is indicated by this.

Keywords: Molecular markers, *Annona* species, dominant, genetic diversity

Introduction
Annona belongs to the family *Annona*ceae, is one of the largest living families of primitive angiosperms of Magnoliales in the clade Magnolid (Gupta *et al.*, 2015) [5]. The genus *Annona* consists of 166 species of which six species produce edible fruits *viz.*, *Annona squamosa* L., Sitaphal, Sharifa, Sugar apple, Sweet sop), *A. reticulata* (Bullock's Heart, custard apple), *cherimoya* (*A. cherimola*), *A. muricata* (Guanabana or sour sop), *A.atemoya* (a natural hybrid of *A. squamosa x A. cherimola*), pond apple (*A. glabra*) (Larranaga and Hormaza, 2015) [9]. Most of the *Annona* species are diploid in nature with chromosome number 2n = 2x = 14 and 16, except *A. glabra*, which is reported be the tetraploid species with 2n = 4x = 28. According to Gupta *et al*. (2015) [5], *Annona* is a member of the family *Annona*ceae, which is one of the largest extant groups of primitive angiosperms in the clade Magnolid. Six of the 166 species in the *Annona genus*, Sitaphal, Sharifa, Sugar apple, Sweet sop, cherimoya (*A. cherimola*), guanabana or sour sop, pond apple (*A. glabra*), and *A. reticulata*, produce edible fruits (Larranaga and Hormaza, 2015) [9]. Except for A. glabra, which is said to be a tetraploid species with 2n = 4x = 28, the majority of *Annona* species are diploid in nature and have chromosome numbers 2n = 2x = 14 and 16.
The *Annona* is a very abundant source of significant therapeutic substances (Pinto *et al*., 2005) [10]. The *Annona* fruit is a healthy fruit in and of itself, rich in beneficial fatty acids, vitamins, and minerals (Gyamfi *et al*., 2011) [6]. It also has a high calorific content. Due to its richness, it could be a fruit crop that can help with the problems caused by malnutrition. However, due to a lack of high producing cultivars, *Annona* cultivars yield very little in India when compared to other nations. That is generally attributable to heavy fruit drop, which is primarily linked to moisture stress, inadequate nutrition, temperature changes, hormonal imbalance, greater wind speeds, insect pests and illnesses, etc. India is said to be *Annona squamosa's* secondary place of origin. This allows for more *Annona* species diversity. Additionally, certain cultivars have been introduced in order to increase genetic diversity and variability.

The development of various DNA markers such as Random Amplified Polymorphic DNA, microsatellites, also referred to as simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) has increased the range of characterization of existing genetic resources of diverse crop species more effectively and reliably. Since custard apple is one of the orphan crops of the tropics, there have been very few attempts to develop molecular markers (Yoshida *et al*., 1998; Kwapata *et al*., 2007; Escribano *et al*. 2009; Larranaga and Hormaza *et al*., 2015) [13, 8, 4, 9] or use of 'omics' tools (Gupta *et al*., 2015) [5]. Due to their relative lack of commercial value and the paucity of studies on genetic improvement of cultivars, the genetic resources of dry fruit crops have not received the attention they deserve. Furthermore, thorough characterization and evaluation of the genetic resources for various growth, developmental, and economic features, either at the phenotypic or genotypic levels, or both, makes it feasible to comprehend the significance of their richness. Therefore, in the activities of plant breeding, characterization and evaluation of germplasm material are given top priority.

One of the effective molecular approaches, RAPD has been used to assess genetic diversity, assess genetic links between accessions or cultivars, and estimate relatedness in a variety of crops. Microsatellite markers (SSRs), among other particular molecular markers, have shown to be extremely helpful for genetic diversity research in a variety of plants because of their high polymorphism, abundance, dispersion throughout the entire genome, and co-dominant inheritance. Furthermore, these markers are the go-to ones for quick and accurate selection of suitable clones due to their great reproducibility and reliability. In order to find the potential cultivars/species for economic features, the current study was conducted to thoroughly evaluate the 12 cultivars that correspond to four *Annona* species for morphological and biochemical traits. Utilizing several molecular markers, such as RAPD and SSR, to characterize these cultivars was another goal of the work.

Materials and Methods

The current study was carried out in 2017–19 at the University of Horticultural Sciences' fruit orchard in Bagalkot, Karnataka. It was 610 meters above sea level and situated at 16°12′N and 75°45′E. With an average annual rainfall of only 518 mm, the climate is hot and dry all year long and is classified as semi-arid tropical. In the current investigation, twelve unique genotypes originating from *Annona squamosa, A. atemoya, A. cherimoya*, and *A. reticualta* were employed. There were noted significant morphological and biochemical characteristics. The cultivars were also examined in a natural field setting for pest infestation (mealy bug, fruit fly, myrid insect), disease (dry rot), and physiological abnormalities (percent of mummified fruits).In the current study, 28 RAPD markers were screened across the cultivars of *Annona* to explore the genetic linkages among the cultivars of *Annona* involving different species based on the twelve SSR markers. This was done for the molecular diversity and cluster analysis. Using the UPGMA method of cluster analysis, a dendrogram was created, and the software Mega 7.0 was used to create an unrooted neighbor joining tree. The clusters were grouped using the bootstrap value of 10,000. The process for molecular marker profiling includes the steps listed below.

1. Collection of samples and DNA extraction

The chosen cultivars' leaves were collected from the field, wrapped in aluminum foil, and labeled appropriately. The samples were then brought to the lab in an ice basket and kept there until extraction at a temperature of 80 °C. With modest changes, Doyle and Doyle's (1987) [3] cetyltrimethyl ammonium bromide (CTAB) technique was used to extract DNA.

2. Qualitative and Quantitative estimation of DNA

A Nanospectrometer was used to measure the absorbance at 260 and 280 nm, and the ratio of the absorbance at 260 to 280 nm (A260/A280) was used to quantify the DNA's purity. Pure DNA had a ratio of 1.8 or slightly less. The extracted genomic DNA samples were run on a 0.8% agarose gel with 0.5X TAE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) to perform a qualitative analysis of the DNA. The gels were stained with ethidium bromide and examined using the Gel Documentation system's UV fluorescence.

3. Genotyping and Amplification

In this work, eleven microsatellites—four universal markers and twelve RAPD markers—were employed. From stock DNA with a known concentration (quantified with a nanospectrometer), working DNA with a concentration of 50 ng was made. 16 polymorphic SSR markers (mentioned in Table 3) were diluted from the stock to a concentration of 10 Pico moles/l for the polymerase chain reaction. The specifics of the components of the 10 l reaction mixes used for the PCR, together with their concentrations, and the PCR procedure used are provided below.

4. Electrophoresis

Prior to size separation on 3.5% to 4.0% Agarose for polymorphism analysis, PCR products were verified for amplification on 1.5% Agarose. According to allele sizes, the bands were graded for each allele. Only polymorphic markers were scored.

5. Marker parameters and allelic diversity estimation

In order to estimate basic marker diversity parameters, such as the number of alleles per locus (Na), the number of effective alleles (Ne), the Shannon Information Index (I), the polymorphic information content (PIC), the observed heterozygosity (Ho), and the expected heterozygosity (He), as well as the occurrence of unique, rare, and common alleles, statistical analysis was performed on the data set across 12 microsatellites, on 12 *Annona* cultivar Similarly, using the software program GenAlex (6.503), the allelic richness parameters were applied to the binary scores of 12 RAPD markers distributed over 30 loci across 12 cultivars of *Annona*.

6. Phylogenetic analysis

In order to determine the molecular relatedness of these 12 *Annona* cultivars, binary data including 12 microsatellites and 12 RAPD markers across 12 cultivars was treated to unrooted neighbour joining tree construction. The Dendrogram was built using a bootstrap value of 10,000.

Results and Discussion

SSR and SNP markers are found to be effective, robust, and reliable markers for molecular characterisation, mapping,

map-based cloning, or marker aided breeding programs among the different molecular markers discovered over the past few decades. A few arbitrary SSR markers, like RAPD, were used in the current experiment to define the 12 cultivars because they were genomically less informative. Additionally, based on the three target sites of chloroplast DNA, including universal Matk, rbcla, and *Annona squamosa* specific Matk, these 12 cultivars were subjected to multiple sequence alignment in order to understand the evolutionary relatedness and genetic variation across the species at the single nucleotide level. This subheading provides more information on earlier findings related molecular markers and the characterisation of *Annona* cultivars.

All eukaryotes have simple sequence repeats (SSRs), commonly referred to as microsatellites, in their genomes. Due to their abundance, these DNA markers are perfect for genetic mapping and population studies. As an alternative to RAPD and RFLP markers, sequence tagged sites markers are now being developed more and more. These are tandemly organized repetitions of mono-, di-, tri-, tetra-, penta-, and other nucleotide types with varying repeat lengths (such as A, T, AT, GA, AGG, AAC, etc.). Based on variations in the amount of tandemly repeating units at a locus, these repeats are extensively spread across the genomes of plants and animals, which exhibit high levels of genetic variation. When the sequence of the flanking region is known, locus-specific flanking region primers are used to identify these SSR length polymorphisms at individual loci by PCR (Tautz, 1989; Bhat *et al*., 2010) [12, 2]. The co-dominant mode of inheritance of microsatellite type markers, as opposed to dominant marker type RAPD, which necessitates the creation of a new map for each cross, allows for the simple transfer of markers between genetic maps of various crosses.

SSRs were used by Escribano *et al*. (2007) [4] in their study of the genetic diversity of cherimoya germplasm. In this study, 279 cherimoya accessions from a global ex-situ field germplasm collection were examined for genetic polymorphisms using 16 SSR loci. With 16 pairs of SSR primers, a total of 79 amplification fragments were produced, with each SSR producing an average of 4.9 bands. The distribution of genetic variation among the 148 accessions gathered from presumed cherimoya origin regions in Ecuador and Peru was examined using analysis of molecular variance (AMOVA), which revealed that the largest variations happened within valleys in each nation. The outcomes supported the value of microsatellites in determining the genetic diversity and place of origin of cherimoya.

Four *Annona* species were gathered from diverse locations within the South Andaman environment, and evaluated the relationships between them. The findings showed that there are notable differences in the genetic diversity among spp. and that both ISSR and RAPD markers are suitable for characterizing and evaluating across four *Annona* species. According to an analysis of *Annona*'s overall diversity, inter-specific diversity outweighs intra-species variability. This high degree of gene flow in the population caused by unrestricted random mating is the cause of the low intra-specific diversity. A historical connection to a shared ancestor or, more likely, geographic closeness and a large population size that encourage genetic exchange could account for the tight relationships between species. Nine SSR markers that were derived from previously described *Annona* species were used in the current study to determine the genetic diversity of the 12 *Annona* cultivars (Larranaga and Hormaza, 2015) [9]. Observed heterozygosity (Ho) and other marker diversity metrics like Na (number of alleles), Ne (number of effective alleles), and Ho. He predicted both Shannon's In formativeness (I) and heterozygosity or gene diversity.

Nine microsatellite markers were used to identify a total of 21 alleles from the nine SSR manufacturers that were evaluated across 12 *Annona* cultivars in the current investigation. Table 12a lists the results of the calculation of the Fixation index (F) based on the frequency of alleles at each locus in order to examine the informative potential of molecular markers and genetic diversity among cultivars, including the effective number of alleles, number of alleles per marker (Na), number of effective alleles (Ne), Shannon Index (I), observed heterozygosity (Ho), and expected heterozygosity (He). SSR There were two alleles in markers like LMCH-6, LMCH-16, LMCH-29, LMCH-79, and LMCH-112. LMCH-10 had four alleles, but LMCH-4 only had one allele and was thought to be monomorphic. However, the total number of effective alleles discovered varied from 1.00 for the single-allele LMCH-4 to 3.769 for the four-allele LMCH-10 (Table 2).

The markers LMCH-10 (1.352), LMCH-33 (1.061), and LMCH-3 (0.950) were found to have the highest Shannon's Information Index (I), demonstrating their utility in cultivar identification. While LMCH-4 (0) and LMCH-79 (0.349) received the least support from me. Additionally, observed heterozygosity (He) was found to be lowest for the markers LMCH-79 (0.198) with an expected frequency of 0.209 and LMCH- 6 (0.420) with an expected frequency of (0.44) (Table 2). The highest observed heterozygosity (He) was found for marker LMCH-10 (0.735) with an expected frequency of 0.791, followed by LMCH-33 (0.642) with an expected frequency of 0.680.

Using RAPD markers, Huang *et al*. (2003) [7] developed a molecular characterisation of cultivated pawpaw. Fruit like custard is pawpaw. Using 71 RAPD markers, 34 extant pawpaw cultivars and advanced selections that make up a sizable component of the farmed pawpaw gene pool were examined to determine genetic identities and assess genetic relatedness. The genetic identities of pawpaw cultivars and advanced selections can be determined using the consensus fingerprint profile using the genetically established RAPD markers. Additionally, it turned out to be a more effective discriminating tool for determining genetic relatedness and variety than isozyme markers.

RAPD markers reportedly showed a stronger capacity for discriminating, according to Sadaphal (2009) [11]. The highest level of polymorphism displayed by particular primers indicates their capacity to amplify the less conserved DNA sequences. The RAPD analysis demonstrated that the types utilized in the experiment exhibited variability and may thus be employed in an early inbreeding effort for custard apples.

Eleven RAPD markers in all were used in this work to screen across 12 *Annona* cultivars, amplifying a total of 28 loci. The Shannon's Informative index (I), gene diversity (h), number of alleles per locus (Na), number of effective alleles per locus (Ne), frequency of each locus' alleles (p), frequency of each band's absence from the corresponding band (q), number of alleles per locus (Na), and Shannon's Informative index (Ne) were all estimated using the GenAlex 6.502 software.

RAPD markers like RAPD-9_240, RAPD-9_340, RAPD-9_520, RAPD-9_690, RAPD-9_1200, RAPD-9_1500,

RAPD_4_165, RAPD-4_248, RAPD-4_400, RAPD-4_960, RAPD-4_1500, RAPD-16_400, RAPD-16_1500, RAPD-12_800, RAPD-23_200, RAPD-23_400, RAPD-23_100, RAPD-36_200, RAPD-36_500, RAPD-24_243 and RAPD-24 _ 277 had two alleles and other markers like RAPD-36_400, RAPD-34_300,RAPD-34_400, RAPD-13_200, RAPD-13_300, RAPD-27_200, RAPD-27_300, RAPD-27_400 had one allele (Table 3).The marker RAPD_690 had the highest Shannon's information index (0.693), followed by RAPD-16_400, RAPD-7_600, RAPD-400, and RAPD_500 (0.679), while the markers RAPD_500, RAPD_400, RAPD_960, RAPD_100, and RAPD_36_200 had the lowest Shannon's index values (0.287 and 0.451, respectively).Diversity was found to be lowest for the markers RAPD-4_400, RAPD-4_960, RAPD-16_1500, RAPD-23_100, RAPD_23_200, and RAPD_36_200 (0.153), and highest for RAPD-9_690 (0.500), followed by RAPD_7_600, RAPD-23_400, and RAPD-27_500 (0.486).

Higher gene diversity markers (h) and Shannon's information index (I) show their value in differentiating cultivars by the appropriate markers. Additionally, to determine how closely related these 12 cultivars were genetically, they were subjected to phylogenetic analysis using the pair-wise genetic matrix generated by RAPD markers. The results were presented in Figure 2 using an unrooted neighbour joining tree and the Mega7.0 software. The cultivars were divided into two main clusters, clusters I and II, as well as two smaller clusters that combined to produce sub- and sub-sub-clusters (Fig. 1). Only Ramphal (*Annona* reticulata) made up the first cluster (Group A), and the other 11 cultivars made up the second cluster (Group B). Cluster B was divided into two additional subclusters (Fig. 1). Chance seedling and Pink mammoth were placed in subcluster 1, while the remaining nine cultivars—Red and Pink, Atemoya, Atemoya x Balanagar, Arka Sahan, No. 1, Balanagar, Island Gem, Red Sitaphal, and No. 2—were placed in the second subcluster. Therefore, it was able to show the genetic relatedness of these *Annona* species in the current study using the phylogenetic analysis.

It was feasible to determine the molecular diversity of these cultivars from a total of 37 marker loci using RAPD and SSR markers, including 28 loci from 12 RAPD markers and 9 loci from 9 SSR markers. Even though there weren't many alleles found in this study, it was to be expected for a perennial species that is propagated by cloning. The findings of the molecular analysis had shown that the genetic diversity of cultivars varied. Twelve *Annona* cultivars under investigation have been successfully characterized using the SSR and RAPD markers. The findings showed that SSR markers are co-dominant and highly reproducible. The significance of SSR markers in the molecular characterisation of *Annona* cultivars is indicated by this.

Table 1: List and source of collection of different *Annona* cultivars utilized in the present study

Sl. No.	Cultivar	Description	Breeding method
1	Balanagar	One of the best custard apple varieties, originated in India. It is used as a pollen parent for Arka Sahan. It belongs to *Annona squamosa* L.	Clonal selection
2	Arka Sahan	Released from IIHR Bangalore. Hybrid between *Annona atemoya cv.* Island Gem × *Annona squamosa cv.* Mammoth	Hybridization
3	No. 1	Seedling selection released as a cultivar at Solapur by farmer Mr. N. M. Kaspate (*A. squamosa* cultivar)	Selection
4	No. 2	Seedling selection released as a cultivar at Solapur by farmer Mr. N. M. Kaspate (*A. squamosa* cultivar)	Selection
5	*Atemoya*	Commonly called Lakshman phal. *Annona Squamosa* L. *x Annona cherimoya* (Sitaphal x Hanuman phal)	Hybridization
6	Red Sitaphal	Cultivar of India,originated as a Chance seedling (*A. squamosa* L.)	Selection
7	*Atemoya* x Balanagar	Hybrid between Lakshman phal and Balanagar.	Hybridization
8	Island Gem	Originated from Australia.It belongs to *Atemoya* spp. Arka Sahan is the progeny of Island gem.	Selection
9	Red and Pink	Cultivar of India. It belongs to *Annona squamosa* L.	Selection
10	Chance Seedling	Mother plant for clonal selection (*A. squamosa* L.)	Selection
11	Pink Mammoth	Variety introduced from Australia. Originated as a bud sport variety of *Atemoya*	Selection
12	Ramphal	Also known as Bullock's Heart, originated in India. It is a tropical fruit not grown commercially in India. *Annona reticulata* L. mostly used as a rootstock	Wild species/Landrace

Table 2: Genetic parameters for (9) SSR markers used across (12) *Annona* cultivars

Sl. No.	Locus	N	Na	Ne	I	Ho	He	uHe	F
1	LMCH-3	5	3.00	2.27	0.950	0	0.560	0.622	1
2	LMCH-4	12	1.00	1.00	0.00	0	0	0	0
3	LMCH-6	10	2.00	1.72	0.611	0	0.420	0.442	1
4	LMCH-10	7	4.00	3.77	1.352	0	0.735	0.791	1
5	LMCH-16	7	2.00	1.96	0.683	0	0.490	0.527	1
6	LMCH-29	8	2.00	2.00	0.693	0	0.500	0.533	1
7	LMCH-33	9	3.00	2.79	1.061	0	0.642	0.68	1
8	LMCH-79	9	2.00	1.25	0.349	0	0.198	0.209	1
9	LMCH-112	8	2.00	1.88	0.662	0	0.469	0.5	1
	Mean	8.333	2.333	2.07	0.71	0	0.446	0.478	1
	SE	0.667	0.289	0.28	0.13	0	0.075	0.081	0

Na=Number of alleles; Ne: Number of effective alleles; I=Shannon's Information Index; Ho: Observed heterozygosity, He: Expected Heterozygosity, uHe: unbiased Heterozygosity; F=Fixation index

Table 3: Genetic parameters for eleven RAPD markers used across 12 *Annona* cultivars

Marker Name	Locus_Band_ Size	Band Freq.	p	q	N	Na	Ne	I	H
RAPD-9	RAPD-9_240	0.17	0.17	0.83	12.00	2.00	1.39	0.45	0.28
	RAPD-9_340	0.25	0.25	0.75	12.00	2.00	1.60	0.56	0.38
	RAPD-9_520	0.17	0.17	0.83	12.00	2.00	1.39	0.45	0.28
	RAPD-9_690	0.50	0.50	0.50	12.00	2.00	2.00	0.69	0.50
	RAPD-9_1200	0.67	0.67	0.33	12.00	2.00	1.80	0.64	0.44
	RAPD-9_1500	0.67	0.67	0.33	12.00	2.00	1.80	0.64	0.44
RAPD-4	RAPD-4_165	0.25	0.25	0.75	12.00	2.00	1.60	0.56	0.38
	RAPD-4_248	0.25	0.25	0.75	12.00	2.00	1.60	0.56	0.38
	RAPD-4_400	0.92	0.92	0.08	12.00	2.00	1.18	0.29	0.15
	RAPD-4_960	0.92	0.92	0.08	12.00	2.00	1.18	0.29	0.15
	RAPD-4_1500	0.67	0.67	0.33	12.00	2.00	1.80	0.64	0.44
RAPD-16	RAPD-16_400	0.58	0.58	0.42	12.00	2.00	1.95	0.68	0.49
	RAPD-16_1500	0.08	0.08	0.92	12.00	2.00	1.18	0.29	0.15
RAPD-7	RAPD-7_600	0.42	0.42	0.58	12.00	2.00	1.95	0.68	0.49
	RAPD-7_1200	0.33	0.33	0.67	12.00	2.00	1.80	0.64	0.44
RAPD-12	RAPD-12_800	0.67	0.67	0.33	12.00	2.00	1.80	0.64	0.44
Marker Name	Locus_Band_ Size	Band Freq.	p	q	N	Na	Ne	I	H
RAPD-23	RAPD-23_200	0.08	0.08	0.92	12.00	2.00	1.18	0.29	0.15
	RAPD-23_400	0.58	0.58	0.42	12.00	2.00	1.95	0.68	0.49
	RAPD-23_100	0.08	0.08	0.92	12.00	2.00	1.18	0.29	0.15
RAPD-36	RAPD_36_200	0.92	0.92	0.08	12.00	2.00	1.18	0.29	0.15
	RAPD-36_400	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
RAPD-34	RAPD_34_300	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
	RAPD-34_400	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
RAPD-13	RAPD-13_200	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
	RAPD-13_300	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
RAPD-27	RAPD-27_200	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
	RAPD-27_300	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
	RAPD-27_400	1.00	1.00	0.00	12.00	1.00	1.00	0.00	0.00
	RAPD-27_500	0.42	0.42	0.58	12.00	2.00	1.95	0.68	0.49
RAPD-24	RAPD-24_243	0.33	0.33	0.67	12.00	2.00	1.80	0.64	0.44
	RAPD-24_277	0.17	0.17	0.83	12.00	2.00	1.39	0.45	0.28
	Mean				12.00	1.74	1.44	0.39	0.26
	SE				0.00	0.08	0.07	0.05	0.04

P=frequency of presence of allele across 12 cultivars; q=frequency of absence of allele; N=Number of cultivars used; Na: Number of alleles, Ne: Number of effective alleles; I: Shannon's Informative index; h=heterozygosity/gene diversity

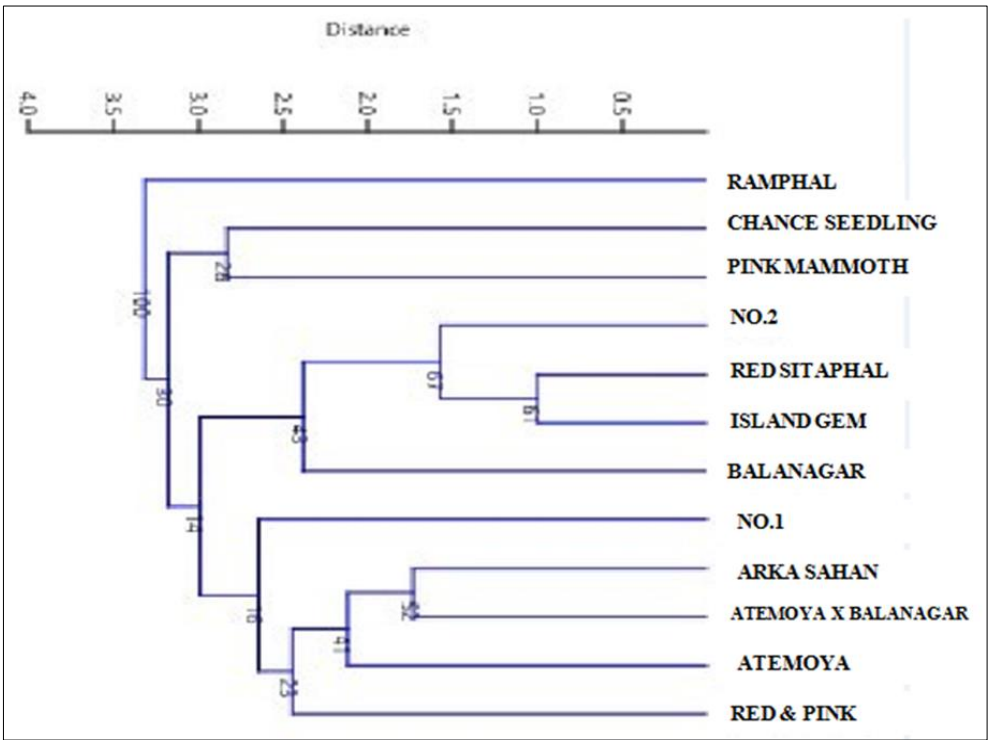

Fig 1: Uprooted neighbour joining tree based on pair-wise genetic distance matrix obtained from 28 locus of 11 RAPD markers across 12 *Annona* cultivars

Conclusion

It was feasible to determine the molecular diversity of these cultivars from a total of 37 marker loci using RAPD and SSR markers, including 28 loci from 12 RAPD markers and 9 loci from 9 SSR markers. Even though there weren't many alleles found in this study, it was to be expected for a perennial species that is propagated by cloning. The identified variants at Matk and Asq Matk will be used to develop species and cultivar specific primers using the 3'end of SNP variant to subject various cultivars of *Annona squamosa, A. Atemoya, A. chrimola and A. reticulata* cultivars to DNA finger printing in future. Hence it is possible to accurately identify the clones of each of these cultivars at the seedling stage itself especially in the segregating populations or clones for their varietal and species integrity can be confirmed with precision.

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Declaration

There is no conflicts of interest among authors.

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