Characterization of *Annona* Genotypes by ISSR and SSR Markers

Y.S. Saitwal[1], A.M. Musmade[1], A.A. Kale[2], V.S. Supe[1], V.R. Joshi[1], S.A. Ranpise[1], S.S. Mehetre[3]

10.18805/BKAP494

ABSTRACT

Background: *Annona* spp. are one of the underutilized fruit crop having great commercial and medicinal value and large diversity present in Maharashtra which is untapped. The present study conducted to characterize the *Annona* genotypes by ISSR and SSR markers for future germplasm conservation and crop improvement programme.

Methods: Ninety genotypes were collected from various regions of Maharashtra. These ninety genotypes and eleven varieties from All India Coordinated Research Project on Arid Zone Fruits, MPKV, Rahuri were screened for morphological and biochemical characters. After morphological and biochemical characterization twenty-two superior genotypes were characterized using ISSR and SSR markers.

Result: Among the 29 ISSR primers used, 20 were found polymorphic which produced a total of 171 reproducible amplicons, in which 109 amplicons (75.43%) were polymorphic. In 20 SSR primers used, 14 primers were found polymorphic which produced a total of 43 reproducible amplicons, in which 36 amplicons (83.72%) were polymorphic. Twenty unique amplicons produced by ISSR primer and eight unique amplicons produced by SSR in studied genotypes is useful in genotype/variety identification in future crop improvement. The dendogram generated based on UPGMA method of cluster analysis using ISSR and SSR marker data revealed little different but similar grouping of genotypes into two major clusters *viz*., cluster A and cluster B. The UPGMA based cluster analysis using dice similarity coefficient grouped *Annona* genotypes into two major clusters which differentiate *squamosa* and *atemoya* species. The distribution of the genotypes in the dendogram was mostly consistent with the known pedigree information, geographical locations and the morphological attributes. Genotypes collected from Purandar, Sawargaon, Pemgiri and Ajanta districts of Maharashtra formed cluster as per geographic collection. The close relationship across genotypes might be explained by either historical relationship to sharing common ancestor or more likely geographical proximity and large population size which favour genetic interchange. This low genetic difference among genotypes suggests that there was more gene flow through random mating without barrier within agro-ecological zones. ISSR and SSR markers gives direction in characterization of *Annona* genotypes but more areas need to be survey and explore for tapping genetic diversity to widen the gene pool. The screened material found superior need to be prioritized in terms of *in-situ* and *ex-situ* conservation for further evaluation and crop improvement.

Key words: *Annona*, ISSR, Polymorphism, SSR.

INTRODUCTION

Annona spp. is of tropical and subtropical fruit tree belonging to the Annonaceae family and is widely distributed in Asia, Africa and America. Annonaceae family has 40 to 50 genera and 119 species, of which only six species are of commercial importance (Geurts 1981). *Annona* spp. having important medicinal properties such as anti HIV activity (Wu *et al*., 1996) and anticancerous property.

Naturally a large intra- and inter-specific variability exists in Annonaceae family due to highly cross pollinated nature. *Annona* plantations owe their origin to vast populations of seedlings that have originated in nature from scattering of seed. As a result of this, they exhibit great diversity in quality and bearing tendency. This has offered ample scope for studying the genetic variation in *Annona*. This variability comprises of about 500 species (Popenoe 1974).

In custard apple, various cultivars are available but barring few, none of the available cultivars have got the status of commercial cultivar due to seed propagation, which results in considerable variability. To expedite the crop improvement programme, it is necessary to trap the natural variability through surveys and the variability should be conserved *ex situ* and *in situ* to utilize for further crop improvement programmes.

[1]Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Maharashtra, India.
[2]State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Maharashtra, India.
[3]Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Maharashtra, India.

Corresponding Author: Y.S. Saitwal, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Maharashtra, India. Email: yssaitwal3@gmail.com

How to cite this article: Saitwal, Y.S., Musmade, A.M., Kale, A.A., Supe, V.S., Joshi, V.R., Ranpise, S.A. and Mehetre, S.S. (2022). Characterization of *Annona* Genotypes by ISSR and SSR Markers. Bhartiya Krishi Anusandhan Patrika. 37(1): 43-49. DOI: 10.18805/BKAP494.

Submitted: 15-03-2022 **Accepted:** 04-05-2022 **Online:** 16-05-2022

For primary screening, the morphological characterization is effective and to trace true genetic variation, molecular characterization is essential. Molecular marker and DNA fingerprinting techniques are quick and accurate which excludes the managemental and environmental effects unless there are strong genetic mutations. Thus, truly divergent

genotypes could be further collected and utilized in crop improvement programmes. Studies on the use of morphological and molecular markers have been reported on members of Annonaceae (Escribano *et al.*, 2007; Kwapata *et al.*, 2007; Ahmad *et al.*, 2010; Pomper *et al.*, 2010 and Cota *et al.*, 2011).

There are limited reports on exploitation of molecular markers for diversity analysis in *Annonas*. Few of these markers are random amplified polymorphic marker (RAPD) marker (Bharad *et al.* 2009), amplified fragment length polymorphism (AFLP) markers and simple sequence repeat (SSR) markers (Escribano *et al.* 2004; Kwapata *et al.* 2007). On the other hand, information regarding nutritional value is of utmost important to select desired genotype for domestication in area of adaptation. Very little information is available on proximate analysis of *Annona* fruits (Kulkarni *et al.* 2013; Boake *et al.* 2014). Keeping in view about scanty information of *Annonas*, efforts have been made to study the genetic diversity using ISSR and SSR molecular markers and proximate analysis of fruits of selected accessions.

The varietal or genetic differences get masked confusing varietal identification. In this regards knowledge of genetic diversity of different varieties are important to form a basis for conservation, genetic tree improvement and promotion of domestication of population with desirable traits. Since the extent of reliable genetic diversity is not known, it is imperative to have an elaborative strategy aimed at evaluating genetic diversity of the *Annona* spp.

In the present investigation, characterization of *Annona* spp., which were found divergent on earlier morphological and biochemical analysis, were compared using ISSR and SSR analysis.

MATERIALS AND METHODS

Experimental site

Four districts of Maharashtra state of India (Ahmednagar, Aurngabad, Beed and Pune) were explored and ninety (90) elite types were collected. Eleven promising genotypes were selected from the existing germplasm available at AICRP on Arid Zone Fruits, Mahatma Phule Krishi Vidyapeeth, Rahuri. In all, 101 genotypes (90+11) were screened by morphological and biochemical parameters for 2 seasons during year 2011 and 2012 to select promising genotypes (Saitwal *et al*, 2015).

The molecular analysis of 22 genotypes (Table 1) was carried out by ISSR and SSR markers at State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in 2012.

Extraction of DNA

The isolation of genomic DNA from fresh young leaves of the third leaf stage of twenty two *Annona* genotypes was carried out as per procedure of Zaho Zhichang *et al.,* 2011 with some modifications in the procedure.

PCR amplification

PCR amplification was performed with ISSR and SSR primers. Amplification was performed in a 0.2 ml PCR tubes having 20 µl reaction volume as described by Escribano

Table 1: List of *Annona* genotypes and their pedigree used for molecular characterization.

Genotype	Pedigree	Source
Balanagar	Selection from Mahboobnagar	(AICRP on AZF)
Madhu	Selection from Balanagar at MPKV, Rahuri	(AICRP on AZF)
Purandar selection	Clonal selection from local types in Purandar	(AICRP on AZF)
Salem selection	Clonal selection from local types in Tamil Nadu	(AICRP on AZF)
Hyderabad selection	Clonal selection from local types in Andhra Pradesh	(AICRP on AZF)
APK-1	Clonal selection from local types in Tamil Nadu	(AICRP on AZF)
Arka Sahan	Interspecific hybrid between Island Gem (*A. atemoya*) and Mammoth (*A. squamosa*)	(AICRP on AZF)
Island Gem	Selection of *A. atemmoya* at Australia	(AICRP on AZF)
Pink Mammoth	Selection of *A. atemmoya* at Australia	(AICRP on AZF)
PT-13	Local collection from Pathardi, Dist. Ahmednagar	Pathardi
SG-8	Local collection from Sawargaon, Dist. Beed	Sawargaon
SG-12	Local collection from Sawargaon, Dist. Beed	Sawargaon
SG-21	Local collection from Sawargaon, Dist. Beed	Sawargaon
PD-5	Local collection from Purandar, Dist. Pune	Purandar
PD-14	Local collection from Purandar, Dist. Pune	Purandar
PD-16	Local collection from Purandar, Dist. Pune	Purandar
PD-23	Local collection from Purandar, Dist. Pune	Purandar
PG-4	Local collection from Pemgiri, Dist. Ahmednagar	Pemgiri
AJ-2	Local collection from Ajanta, Dist. Aurangabad	Ajanta
PT-1	Local collection from Pathardi, Dist. Ahmednagar	Pathardi
Red Sitaphal-10(A)	Selection at Australia	(AICRP on AZF)
Red Sitaphal-11(O)	Selection at Australia	(AICRP on AZF)

et al., 2007; Ahmad *et al.*, 2010 and Pomper *et al.*, 2010 with some modifications. The composition of PCR mixture is presented in Table 2.

The 20 µl reaction mixture was gently vortexed and made spinned down. The DNA amplification was carried out in a Thermal Cycler (Table 3).

Gel electrophoresis

PCR products were resolved on agarose gel electrophoresis (1.5% for ISSR and 2% for SSR) prepared by using 1x TBE buffer and stained with pre added ethidium bromide (0.5 µg/ml). The amplified PCR products were observed under UV transilluminater in gel documentation system (Flour Chem. ™ Alpha innotech, USA) and image was captured.

Data analysis

The clearly resolved PCR amplified ISSR and SSR bands of *Annona* genotypes were scored manually for their presence (1) and absence (0). Data were analyzed and similarity matrix was constructed from binary data with Dice similarity coefficients which are calculated as per model suggested by Nei and Li (1979). Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) was employed for cluster analysis. The analyses were carried out using the computer package NTSYSpc 2.02i (Rohalf, 1998).

RESULTS AND DISCUSSION

Characterization by ISSR markers

Among twenty nine ISSR primers used, twenty produced amplification. A total of 171 amplicons were produced of which 109 were found polymorphic and twenty were monomorphic. The per cent polymorphism of ISSR was 75.43% (Table 4). The amplicons generated by each primer varied from 4 (ISSR-821 and ISSR-856) to 15 (ISSR-815). The extent of polymorphism in the present study was higher as compared to the earlier reports by Ahmad *et al.* (2010) and Pomper *et al.* (2011) for ISSR primers and Sadaphal (2009) for RAPD primers.

In ten genotypes, unique amplicons were produced by ISSR primers which was useful in genotype/variety identification to be used in future crop improvement (Table 5).

Cluster analysis based on ISSR

The UPGMA based cluster of 22 *Annona* genotypes grouped into 2 major clusters *viz.,* A and B (Fig 1). Cluster B had maximum number of genotypes (19) and cluster A had only 3 genotypes.

Cluster A had 3 genotypes namely Arka Sahan, Island Gem and Pink Mammoth which were *atemoya* species of *Annona*. Within A cluster, genotype Arka Sahan formed a separate sub-cluster, which is F_1 of *atemoya* species. The cluster B was grouped into 9 sub-clusters. Out of these 9 sub-clusters, cluster B3 was formed based on the colour of fruit. This sub-cluster had red-fruited genotypes *viz*. Red Sitaphal (10 A), Red Sitaphal (11 O) and PT-1. Another sub-cluster B5 and B6 were formed based on their geographic locations. The sub-cluster B5 consisted genotypes from Purandar (Pune) *viz*. PD-5, PD-14, PD-16 and PD-23. The sub-cluster B6 consisted genotypes from Sawargaon (Beed) location *viz*. SG-12 and SG-21. The sub-cluster B7, B8 and B9 consisted genotypes selected from southern parts of India. Balanagar, Hyderabad selection, APK-1 and Salem selection genotypes were selection from southern parts of India and formed the clusters close to each other.

Table 2: Composition of PCR mixture for ISSR and SSR primers.

PCR reaction component	Initial concentration	Volume collected (ISSR)	Volume collected (SSR)
Genei *Taq* DNA Polymerase Buffer F	10x	2 µl (1x)	2 µl (1x)
$MgCl_2$	15 mM	2 µl (1.50 mM)	2 µl (1.50 mM)
dNTP mix (Geni)	2.5 mM each	2 µl (0.25 mM)	2 µl (0.25 mM)
Primer	25 picomoles	4 µl	1 µl (25 pico) Primer F 1 µl (25 pico) Primer R
Genei *Taq* DNA Polymerase	3 units/µl	0.33 µl (1 unit)	0.33 µl (1 unit)
Sterilized distilled water	-	8.67 µl	10.67 µl
Template DNA	50 ng/µl	1 µl (50 ng)	1 µl (50 ng)
Total volume	20 µl	20 µl	

Table 3: Thermocycler profile for ISSR and SSR primers.

Name of the steps followed	Temperature	Time (ISSR)	Time (SSR)	Cycles
Initial denaturation	94°C	5 min.	5 min.	1 cycle
Denaturation	94°C	1 min.	1 min.	40 cycles
Annealing	Table-4 Table-6	1 min.	1 min.	
Extension/elongation	72°C	1 min.	2 min.	
Final extension	72°C	10 min	10 min	1 cycle
Final hold	04°C	Till retrieval	Till retrieval	-

Table 4: Polymorphic ISSRs used to analyze *Annona* genotypes.

Primer	Sequence (5′-3′)	T_m (°C)	Total amplicons	Polymorphic amplicon (s)	Polymorphism (%)
ISSR-807	AGA GAG AGA GAG AGA GT	42.4	12	11	91.67
ISSR-809	AGA GAG AGA GAG AGA GG	42.4	9	5	66.67
ISSR-810	GAG AGA GAG AGA GAG AT	42.8	13	8	61.54
ISSR-812	GAG AGA GAG AGA GAG AA	43.3	10	9	100.00
ISSR-813	CTC TCT CTC TCT CTC TT	43.3	10	9	90.00
ISSR-815	CTC TCT CTC TCT CTC TG	43.3	15	12	80.00
ISSR-821	GTG TGT GTG TGT GTG TT	45.8	4	1	50.00
ISSR-823	TCT CTC TCT CTC TCT CC	45.8	10	2	40.00
ISSR-826	ACA CAC ACA CAC ACA CC	45.8	7	3	71.42
ISSR-827	ACA CAC ACA CAC ACA CG	48.2	6	3	66.67
ISSR-834	AGA GAG AGA GAG AGA GYT	45.0	7	2	71.42
ISSR-841	GAG AGA GAG AGA GAG AYC	46.0	5	2	80.00
ISSR-854	TCT CTC TCT CTC TCT CRG	48.0	5	4	80.00
ISSR-856	ACA CAC ACA CAC ACA CYA	48.0	4	2	75.00
ISSR-857	ACA CAC ACA CAC ACA CYG	48.0	11	7	63.64
ISSR-859	TGT GTG TGT GTG TGT GRC	44.4	10	7	80.00
ISSR-860	TGT GTG TGT GTG TGT GRA	51.0	6	1	50.00
ISSR-873	GAC AGA CAG ACA GAC A	44.4	12	9	91.66
ISSR-878	GGA TGG ATG GAT GGA T	52.0	6	4	83.33
ISSR-890	VHV GTG TGT GTG TGT GT	50.8	9	8	100.00

Single letter abbreviation for mixed base positions:
Y=C, T; H=A, C, T; V= A, C, G; R=A, G.

Table 5: Unique ISSR amplicons amplified in *Annona* genotypes.

Annona genotypes	Primer	Unique ISSR amplicons size (kb)
Balanagar	ISSR-812	0.29
Madhu	ISSR-809	0.81
	ISSR-827	0.55
	ISSR-841	1.75
	ISSR-873	0.48
Salem Selection	ISSR-834	1.45
Arka Sahan	ISSR-878	0.82
Island gem	ISSR-821	1.53
	ISSR-841	1.25
Pink Mammoth	ISSR-826	1.19
	ISSR-860	0.65
	ISSR-873	0.43
Red Sitaphal (10 Acute)	ISSR-834	0.79
	ISSR-834	1.88
	ISSR-859	0.75
Red Sitaphal (11 Obtuse)	ISSR-823	1.00
	ISSR-823	1.11
PD-14	ISSR-856	0.37
PD-23	ISSR-890	0.48
AJ-2	ISSR-826	0.75

The genotype Purandar selection formed distinct sub-cluster from genotypes collected from Purandar region which may be due to highly cross pollinated nature of this crop and there may be high out-breeding in this genotype. The sub-cluster B1 consisted genotypes PT-13 and SG-8, which having more similar inflorescence and fruit morphology as well as geographical proximity. Pomper *et al.* (2011) assessed the utility of ISSR markers for evaluating the genetic relationship among the *Annnona* species.

Characterization by SSR

Among twenty SSR primers used, fourteen produced amplification. A total of 43 amplicons were produced of which 28 were found polymorphic and eight were unique. The per cent polymorphism of SSR was 83.72%. Maximum numbers of amplicons (9) were produced by primer LMCH-33. The primers LMCH-1, LMCH-3, LMCH-4, LMCH-5 LMCH-6 and LMCH-9 showed highest percentage of polymorphism *i.e.* 100 per cent (Table 6). Escribano *et al.* 2007, Escribano *et al.* 2009 and Kwapata *et al.* 2007 reported the results for *Annona* spp. using SSR primers. Five genotypes have unique amplicons which is the uniqueness for particular genotype (Table 7). Kavya *et al.* (2019) reported that SSR markers effectively segregated the Jackfruit genotypes based on different pulp colours and can be used for both diversity analysis and in breeding applications.

Table 6: Polymorphic SSRs used to analyze *Annona* genotypes.

Locus	Sequence (5′-3′)	GeneBank accession no.	T_m (°C)	Total amplicons	Polymorphic amplicon (s)	Polymorphism (%)
LMCH1	F: CTCTTCAAAGGTACGACTTC	AY685388	55	2	2	100.00
	R: TTGAGAAAAGGATAAGGATT					
LMCH2	F: CATTAACAGAGCATCAAAAT	AY685389	55	1	0	0.00
	R: AGATTGAGAAGTCGTACCTT					
LMCH3	F: TCTGTGAAAATACTCTCGTA	AY685390	55	2	2	100.00
	R: TCTCCACTGAATAATCTTTAAT					
LMCH4	F: ATTAGAACAAGGACGAGAAT	AY68s5391	55	2	2	100.00
	R: CCTGTGTCTTTCATGGAC					
LMCH5	F: CCCACTCTTCTACCCTCAAC	AY685392	55	2	2	100.00
	R: CAAGTCCCTGTAAGAATCAGA					
LMCH6	F: GGCATCCTATATTCAGGTTT	AY685393	55	4	4	100.00
	R: TTAAACATTTTGGACAGACC					
LMCH7	F: ATCACCAACACTGAATCTTA	AY685394	55	1	0	0.00
	R: AATTTTTACCTGTAGACGTG					
LMCH8	F: AATTACGCAGATCACAGTAGC	AY685395	55	1	0	0.00
	R: CATCTTGCCTTGCTCTCTAC					
LMCH9	F: TCAAACACGTATAGAAAACC	AY685396	55	6	5	100.00
	R: TATGTGAAAGATCAAAAAGAG					
LMCH10	F: TTCTTGTTGGGAAGTATAGA	AY685397	55	3	2	100.00
	R: GAAATCAATGTAGGTGTGAC					
LMCH11	F: TACCTCTCGCTTCTCTTCCT	AY685398	55	5	2	80.00
	R: GATGATTAGACACAAGTGGATG					
LMCH29	F: GTACCATCTTTTAGGAAATC	DQ923748	45	2	1	50
	R: TGCAATCTATGTTAGTCAC					
LMCH33	F: AAGAAATGGGAGTAAATAGTG	DQ923749	50	9	5	88.88
	R: ACGGTTGTGAATAGTTGAGT					
LMCH34	F: ATTTGACGGTGTTAAGGTGGT	DQ923750	50	3	1	66.67
	R: TATGTAGGAAATGACCAGGCTA					

Table 7: Unique ISSR amplicons amplified in *Annona* genotypes.

Annona genotypes	Primer	Unique SSR allele size (kb)
Arka Sahan	LMCH-9	0.14
	LMCH-10	0.19
Red Sitaphal (11 Obtuse)	LMCH-33	0.39
	LMCH-33	0.42
SG-21	LMCH-11	1.15
PD-23	LMCH-33	0.50
PG-4	LMCH-11	1.68
	LMCH-34	0.80

Cluster analysis based on SSR

The selected twenty two genotypes showed two major clusters *viz*. A and B (Fig 2). Sub-cluster A1 had genotypes which were *atemoya* species of *Annona viz*. Arka Sahan, Island Gem and Pink Mammoth. Within sub-cluster A1 of *atemoya* species, genotype Arka Sahan formed another sub-cluster indicated its indicated it's closely relatedness with Island Gem and Mammoth. The sub-cluster B was grouped into 7 sub-cluster on the basis of geographical locations and morphological characters. Out of these 7 sub-clusters, cluster B1 and B2 were formed based on the colour of fruit. The sub-cluster B4 consisted genotypes collected from Purandar (Pune) *viz*. PD-5, PD-16, PD-14-8 and PD-23.

In cluster B, Red Sitaphal (11 obtuse) formed distinct cluster. Red Sitaphal (10 acute) and genotype PT-1 were similar to each other indicated that they were siblings or selections. Close similarity coefficient and same cluster of Balanagar and Madhu showed that they were less divergent. Pedigree of Madhu (Table 1) also supported the similar results.

Genotypes collected from Purandar, Sawargaon, Pemgiri and Ajanta formed cluster as per geographic collection. The close relationship across genotypes might be explained by either historical relationship to sharing common ancestor or more likely geographical proximity and large population size which favour genetic interchange. This low genetic difference among genotypes suggests that there was more gene flow through random mating without barrier within agro-ecological zones.

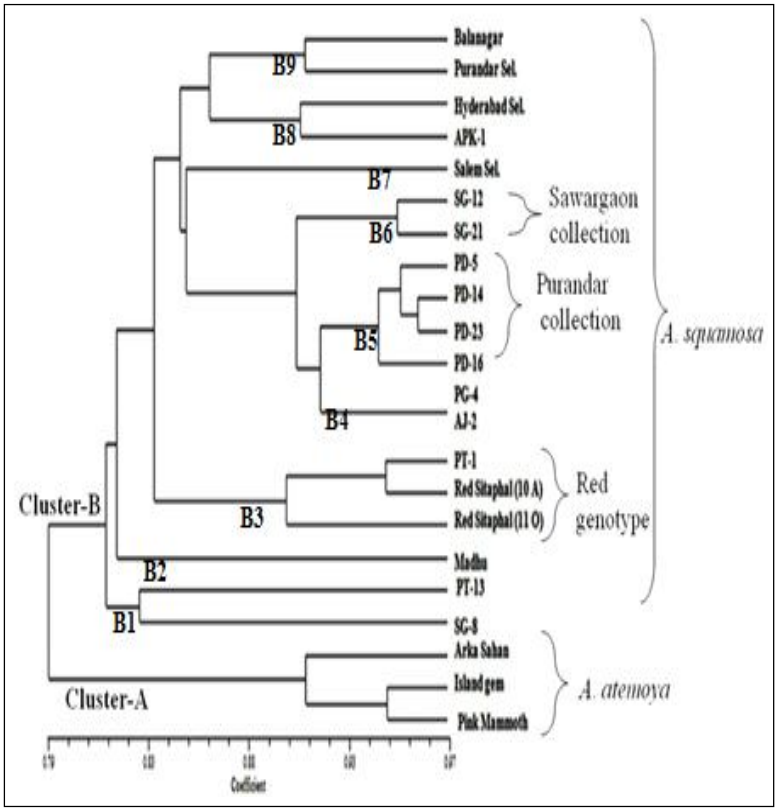

Fig 1: Dendogram of 22 *Annona* genotypes using inter simple sequence amplicons.

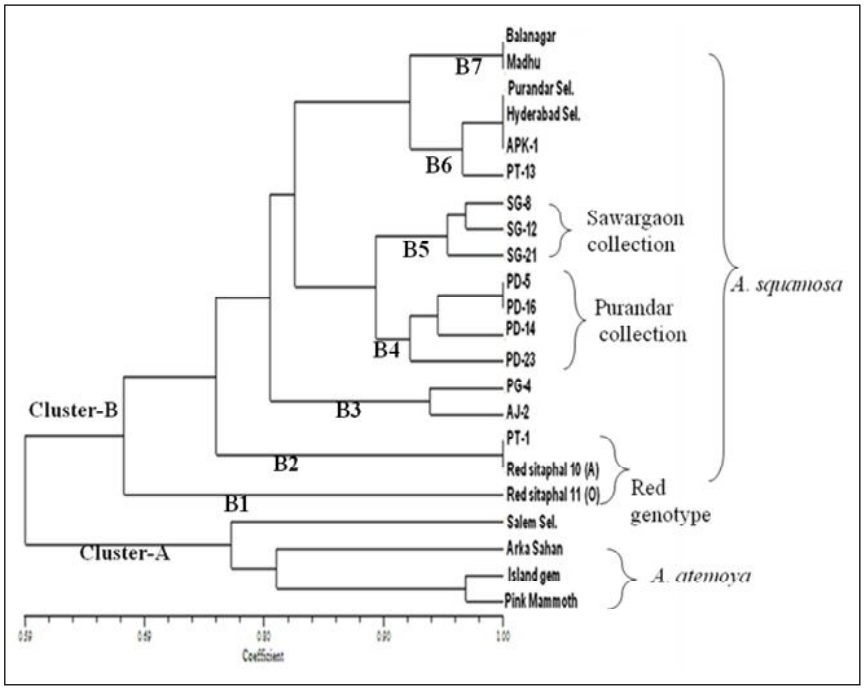

Fig 2: Dendogram of 22 *Annona* genotypes by simple sequence repeat profiling.

Escribano *et al.* (2007) reported that UPGMA cluster analysis based on SSR primer indicated the relationships among the analyzed *cherimoya* collections, showed some specific groups related to their geographic origins.

CONCLUSION

The average percent polymorphism was 75.43% and 83.72% produced by ISSR and SSR primers, respectively. The unique amplicons of the genotypes produced by ISSR and SSR is helpful in identification of genotype/variety.

The dendogram obtained by UPGMA cluster analysis in *Annona* genotypes based on ISSR and SSR primers was about more similar and formed clusters which differentiate genotypes from each other like different species. The distribution of the genotypes in the dendogram was mostly consistent with the known pedigree information, geographic locations and the morphological attributes. But SSR primers based dendogram discriminate genotypes more clearly as compare to ISSR and allowed to distinguish synonymies and homonymies. The UPGMA cluster analysis based on ISSR and SSR analysis showed that variety Arka Sahan, Island Gem and Pink Mammoth have independent cluster and totally different from studied genotypes. The low genetic difference within geographic region suggests that there was more gene flow within these zones and great genetic diversity between various regions may be due to high out breeding nature of *Annona* spp. SSR markers are excellent co-dominant markers to study diversity *Annona* germplasm and most useful in crop improvement programme.

REFERENCES

Ahmad, I., Bhagat, S., Sharma, T.V.R.S., Kumar, K. and Simachalam, P. (2010). ISSR and RAPD marker based DNA fingerprinting and diversity assessment of *Annona* spp. in South Andaman. Indian J. Hort. 67(2): 147-151.

Bharad, S.G., Kulwal, P.L., Bagal, S.A. (2009). Genetic diversity study in *A. squamosa* by morphological, biochemical and RAPD markers. Acta Hort. 839: 615-623.

Boake, A.A., Faustina, D., Jacob, K., Ibok, O. (2014). Dietary fibre, ascorbic acid and proximate composition of tropical underutilized fruits. Afr. J. Food Sci. 8: 305-310.

Cota, L.G., Vieira, F.A, Melo, Junior, A.F., Brandão, M.M., Santana, K.N.O., Guedes, M.L. and Oliveira, D.A. (2011). Genetic diversity of *Annona crassiflora* (*Annonaceae*) in northern Minas Gerais State. Genet. Mol. Res. 10 (3): 2172-2180.

Escribano, P., Viruel, M.A. and Hormaza, J.I. (2007). Molecular analysis of genetic diversity and geographic origin within an ex situ germplasm collection of *cherimoya* by using SSRs. J. Amer. Soc. Hort. Sci. 132(3): 357-367.

Escribano, P., Viruel, M.A. and Hormaza, J.I. (2009). Establishment of a core collection to optimize the conservation of cherimoya (*Annona cherimola* Mill.) genetic resources using SSR information. Acta Hort. 814: 67-70.

Geurts, F. (1981). Annonaceous Fruits. Royal Tropical Institute, Amsterdam, the Netherlands., 16 pp.

Jaccard, P. (1908). Nouvelles researches sur la distribution florale. Bulletin de la Société Vaudoise Des Sciences Naturelles. 44: 223-270.

Kavya*, K., Shyamalamma, S. and Gayatri, S. (2019). Morphological and molecular genetic diversity analysis using SSR markers in Jackfruit (*Artocarpus heterophyllus* Lam.) genotypes for pulp colour. Indian J. Agric. Res. 53(1): 8-16.

Kulkarni, S., Joshi, S., Kamthe, P., Tekale, P. (2013). Proximate analysis of peel and seed of *Annona squamosa* (*Custard apple*) fruit. Res. J. Chem. Sci. 3: 92-94.

Kwapata, K., Mwase, W.F., Bokosi, J.M., Kwapata, M.B. and Munyenyembe, P. (2007). Genetic diversity of *Annona* senegalensis Pers. populations as revealed by simple sequence repeats (SSRs). Afr. J. Biotechl. 6(10): 1239-1247.

Nei, M., Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases, Proceeding of National Academy of Sciences. U.S.A. 76: 5269-5273.

Pomper, K.W., Jeremiah, D., Lowe, Li Lu, Sheri, Crabtree, B. and Shandeep, D. (2010). Characterization and Identification of Pawpaw Cultivars and Advanced Selections by Simple Sequence Repeat Markers. J. Amer. Soc. Hort. Sci. 135 (2): 143-149.

Pomper, K.W., Jeremiah, D., Lowe, Li Lu, Sheri, Crabtree, B. and Kochnar, T.S. (2011). Assessment of the Utility of ISSR Markers for Evaluating Genetic Relationships among Members of *Assimina* and *Annona*. Land Grant Programme, Kenttucky State University, FL-33435.

Popenoe, J. (1974). Status of *Annona* Culture in South Florida. proc. Florida State Hort. Soc. 87: 342-344.

Rohalf, F.J. (1998). NTSYSpc. Numerical Taxonomy and Multivariate Analyses, Version 2.02i. Exeter Software, New York, N.Y.

Sadaphal, S.V. (2009). Assessment of molecular diversity in custard apple (*Annona squamosa*). M.Sc. (Agri.) thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri, (Maharashtra), India.

Saitwal, Y.S, Musmade, A.M., Supe, V.S., Joshi, V.R. and Nimbalkar, C.A. (2015). Physiochemical profiling for selection of promising *Annona* genotypes. Indian J. Dryland Agric. Res. and Dev. 30(1): 89-93.

Wu, Y.C., Hung, Y.C., Chang F.R., Cosentino, M., Wang, H.K. and Lee, K.H. (1996). Identification of Ent-16, 17-dihydroxykauran -19-oic acid as anti-HIV principle and isolation of the new diterpeniods *Annona* squamosins A and B from *Annona* squamosa. Journal of Natural Products. 59(6): 635-637.

Zhao Zhichang, Hu Guibing, Ouyang Ruo, Liu Yunchun, Chen Yeyuan and Luo Shirong. (2011). Studies of the genetic diversity of seven sweetsop (*Annona squamosa* L.) cultivars by amplified fragment length polymorphism analysis. Afr. J. of Biotechnol. 10(35): 6711-6715.