Indian J. Hort. 67(2), June 2010: 147-151

ISSR and RAPD marker based DNA fingerprinting and diversity assessment of *Annona* spp. in South Andamans

Israr Ahmad*, S. Bhagat, T.V.R.S. Sharma, Krishna Kumar, P. Simachalam and R.C. Srivastava

Division of Field Crops, Central Agricultural Research Institute, Port Blair 744 101

ABSTRACT

Annona **spp. is one of the most important wild fruit tree having great medicinal value. The present study was aimed to assess the inter-relationships among four** ***Annona*** **species collected from various parts of south Andaman ecosystem. A set of 30 ISSR and 20 RAPD primers were taken for DNA fingerprinting, among them 14 ISSR and 14 RAPD primers produced 31 and 48 amplicons out of which 18 and 25 amplicons were polymorphic having 58 and 52% polymorphism, respectively. The maximum discriminating band was obtained from primer ISSR 7 and ISSR 9. Cluster analysis of ISSR divided the four species into three clusters while with RAPD it was two clusters. An assessment of genetic diversity among four** ***Annona*** **species would assist in planning for future germplasm collection, conservation and domestication programmes.**

Key words: RAPD, ISSR, *Annona* spp., genetic diversity.

INTRODUCTION

Annona spp. is wild fruit tree plants utilized by tribals and local people of Andaman and Nicobar Islands. *Annona* belongs to Annonaceae family which is one of the largest tropical and sub-tropical plant families with about 2,300 species of trees, shrubs and lianas. However, in island ecosystem of Andaman, only four species of *Annona* are reported, *i.e. A. squamosa, A. muricata, A. reticulata* and *A. glabra. Annona* is one of the most important wild indigenous fruit trees of Andaman (Sreekumar *et al*., 13). The fruit of *A. squamosa* is an important part of diet for tribals and most indigenous populations in islands of Andaman. Dried, powdered leaves are regarded as purgative and as a remedy for mucous diarrhoea. Venereal diseases and intestinal disorders are treated with preparations of the roots (Sofowora, 12).

The island ecosystem of Andaman and Nicobar is highly prone to natural calamities like Tsunami and earthquakes. In this regard knowledge of genetic diversity of different populations is important to form a basis for conservation, genetic tree improvement and promotion or domestication of populations with desirable traits. Since the extent of genetic diversity is not known, it is imperative to have an elaborate strategy aimed at evaluating genetic diversity of Andaman populations of *Annona* spp. Studies on use of morphological traits and isozyme markers have been reported on members of Annonaceae especially *Annona cherimola* (Perfectti and Pascual, 9). However, most morphological traits are highly influenced by environmental conditions or vary with development stage of plant and isozymes are limiting due to low levels of polymorphisms (Asha *et al*., 2). Consequently, DNA based techniques such as RAPD, ISSR and micro-satellites or simple sequence repeats (SSRs) are effective in assessing genetic diversity of plant species because they provide unlimited potential markers to reveal differences at molecular level (Gafoor *et al*., 5; Asha *et al*., 2). Compared to other classes of markers DNA based markers, often carry high numbers of alleles at very low frequencies (Vinod *et al*., 14). This greatly contributes to the assessment of genetic relationships among and within populations.

The present study was undertaken in order to identify and evaluate the genetic diversity present among the four species of *Annona*, *i.e. A. squamosa*, *A. muricata*, *A. reticulata*, and *A. glabra* reported in South Andamans using DNA (ISSR, RAPD) based marker. This is the first attempt to study the genetic diversity of *Annona* spp. in island ecosystem of India using ISSR marker system.

MATERIALS AND METHODS

Altogether 17 genotypes (Table 1) representing four different *Annona* species were collected from different parts of south Andaman Islands. The total genomic DNA was extracted from fresh young leaf by CTAB method (Murray and Thomson, 8) with slight modification. The quantitation of DNA in RNA-free sample was done using UV spectrophotometer.

Polymerase chain reaction (PCR) was performed in final volume of 20 µl containing 10x assay buffer, 2.5 mM dNTPs, 0.5 unit of *Taq* DNA polymerase, 10

*Corresponding author's E-mail: israr15ahmad@gmail.com

Table 1. Different *Annona* genotypes of collected from different part of South Andaman Islands.

Annona spp.	Genotype code	Location
A. squamosa	S-1	Gene garden
A. squamosa	S-2	Harpatabad
A. squamosa	S-3	CARI Residential area
A. squamosa	S-4	Bambooflat
A. squamosa	S-5	Wandoor
A. squamosa	S-6	Mount Harriate
A. muricata	S-7	Harpatabad
A. muricata	S-8	Gene garden
A. muricata	S-9	School lines
A. muricata	S-10	Bloomsdale
A. muricata	S-11	Wandoor
A. muricata	S-12	Bloomsdale
A. muricata	S-13	Ograbraj
A. reticulata	S-14	Wandoor
A. reticulata	S-15	Gene garden
A. glabra	S-16	Gene garden
A. glabra	S-17	Wandoor

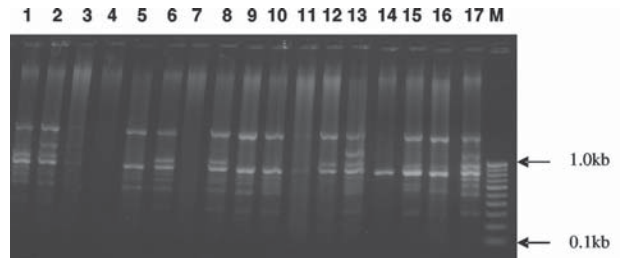

Fig. 1. PCR profile of *Annona* spp. using ISSR Primer 9. Lane 1-17 are genotypes as described in Table 1.

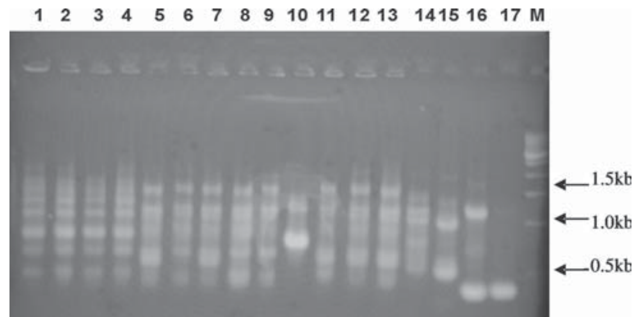

Fig. 2. PCR profile of *Annona* spp. using RAPD primer OPA 2. Lanes 1-17 are genotypes as described in Table 1.

pmols/reaction ISSR/ RAPD primer and 100 ng of templet DNA. ISSR was done with 30 primer of ISSR series and RAPD was done with 20 primer of OPA, OPE, OPF, OPX series. The PCR was performed by initial denaturation at 94°C for 5 min. followed by 45 cycles of denaturation at 94°C for one min., annealing at 37°C for one min., extension at 72°C for two min. and final elongation at 72°C for 7 min. For ISSR analysis the annealing temperature were taken as suggested by manufacturing/ synthesising company. The PCR products were run on 1% agarose gel prepared in 1 × TAE buffer containing 0.5 µg/ml of the ethidium bromide at 100 V for 2.0 h. The gel was photographed under UV-transilluminator.

All the genotypes were scored for presence and absence of the ISSR and RAPD bands. The 0/1 matrix was used to calculate similarity as Jaccard's coefficient using SIMQUAL subroutine in similarity routine. The resultant similarity matrix was employed to construct dendrogram using SAHN based UPGMA to deduce genetic relationship (Rohlf, 11)

RESULTS AND DISCUSSION

A total of 30 ISSR and 20 RAPD primers were used to study the genetic diversity among four different *Annona* species. Among 30 ISSR primers used, 14 produced amplification and a total of 31 amplicons of which 18 were found to be polymorphic (58%). Primers ISSR7 and ISSR9 produced maximum number of amplicons, while primer ISSR8 produced minimum number of amplicons. No single primer produced unique banding pattern for all the four species. Only combination of primers (ISSR7 & ISSR9) could identify all the four species.

The dendrogram generated by UPGMA (Fig. 3) could differentiate among all the four species of *Annona* at 35% similarity. Dendrogram could be clearly divided into three different clusters, *i.e.* cluster I had all the genotype of *A. muricata* (S7, S8, S9, S11, S12, S13) and two genotypes of *A. squamosa* (S1 & S6), showing high similarity of 84 and 81% with *A. muricata*. Cluster II had all the genotype of *A. squamosa* (S2, S3, S4, S5) differentiating at 73% similarity coefficient. However, two genotype of *A. squamosa* (S2, S3) showed 100% similarity with each other. Cluster II also had two genotype (S14, S15) of *A. reticulata*, and one genotype (S10) of *A. muricata* showing very high degree of similarity between these two species. Cluster III had the two genotypes (S16, S17) of *A. glabra* with 74% similarity.

Similar result were obtained with 14 RAPD primers which produced 48 amplicons of which 25 were polymorphic and the level of polymorphism was 52%. Dendrogram generated by UPGMA (Fig. 4) could differentiate between all the four species at 31% similarity, and divided them into two major clusters. Cluster I had four genotype of *A. squamosa* and two genotypes of *A. glabra*. Cluster II had all the genotypes of *A. muricata* and two genotypes of *A. reticulata*.

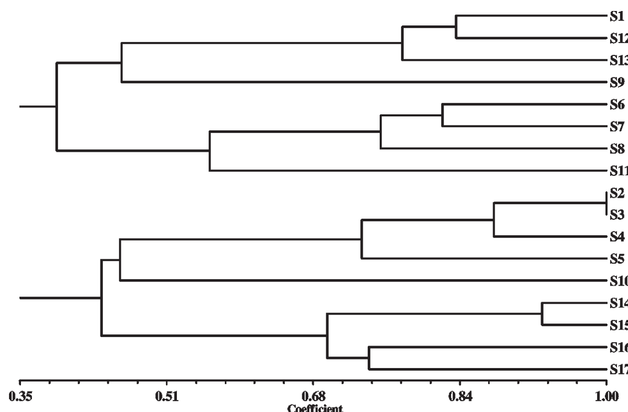

Fig. 3. Dendrogram showing genetic diversity amongst *Annona* genotypes by ISSR primers.

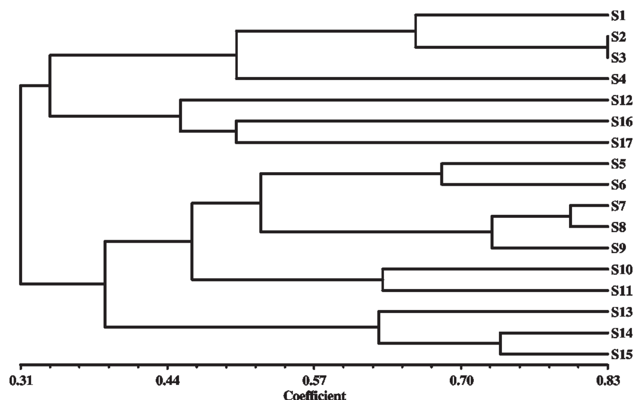

Fig. 4. Dendrogram showing genetic diversity amongst *Annona* genotypes by RAPD primers.

Plant genetic resources are an important component of biodiversity and provide the basic genetic variability that allows new and improved cultivars to be developed (Powel *et al.*, 10). Assessment of genetic diversity is an essential component in agriculture and other planting material germplasm characterisation and conservation. Classical breeding affects genetic diversity within breeding programme. Selection increases the efficiency of those alleles or allelic combinations which is having favourable effect at the expense of the other (Cao *et al.*, 3). Presently, RAPD and ISSR marker systems are routinely being used in ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences (Iqbal *et al.*, 6; Escribano *et al.*, 4). Overall comparison of ISSR and RAPD was indicative of greater efficiency of ISSR and RAPD markers for identification and diversity assessment. ISSR and RAPD profiling could identify within and among all the four species with a combination of 2 to 3 primers.

This low genetic difference among four species of *Annona* suggests that there was more gene flow within agro-ecological zones (Kingdom *et al.*, 7). The high gene flow may be due to random mating with very little selection (Ahmed *et al.*, 1; Kingdom *et al.*, 7). The advantage of species with great genetic diversity like *A. squamosa* and *A. muricata* is that it can easily adapt and conform to a wide range of environmental conditions in comparison to non-genetically diverse species as those of the domesticated crops. The species with highest genetic diversity, *viz. A. squamosa* (genotypes S1, S6) and *A. muricata* (genotype S10)

Table 2. ISSR primer used and polymorphism given by them.

ISSR primer No.	Sequence (5'-3')	Tm (°C)	Polymorphic band (s)	Polymorphism (%)
7	AGAGAGAGAGAGAGAGT	48.9	3	75
8	AGAGAGAGAGAGAGAGC	51.3	0	—
9	AGAGAGAGAGAGAGAGG	51.3	4	80
10	GAGAGAGAGAGAGAGAT	49.8	0	—
11	GAGAGAGAGAGAGAGAC	51.3	1	50
12	GAGAGAGAGAGAGAGAA	48.9	2	66.6
13	CTCTCTCTCTCTCTCTT	48.9	1	50
18	CACACACACACACACAG	51.3	2	66.6
20	GTGTGTGTGTGTGTGTC	51.3	1	50
22	TCTCTCTCTCTCTCTCA	48.9	0	—
23	TCTCTCTCTCTCTCTCC	51.3	0	—
24	TCTCTCTCTCTCTCTCG	51.3	2	66.6
25	ACACACACACACACACT	49.8	2	66.6
28	TGTGTGTGTGTGTGTGA	49.8	0	—

Table 3. RAPD primers used in study and their polymorphism.

Primer No.	Sequence (5'-3')	%GC	Polymorphic band (s)	Polymorphism (%)
OPA2	TGCCGAGCTG	70	4	66.6
OPA4	AATCGGGCTG	60	3	60
OPA5	AGGGGTCTTG	60	1	50
OPA6	GGTCCCTGAC	70	1	33.3
OPA7	GAAACGGGTG	60	3	60
OPA9	GGGTAACGCC	70	2	66.6
OPE1	CCCAAGGTCC	70	1	33.3
OPE2	GGTGCGGGAA	70	2	50
OPF1	ACGGATCCTG	60	4	80
OPF2	GAGGATCCCT	60	0	-
OPF3	CCTGATCACC	60	1	33.3
OPF4	GGTGATCAGG	60	1	50
OPF5	CCGAATTCCC	60	0	-
OPX3	TGGCGCAGTG	70	2	66.6

need to be prioritized in terms of *in-situ* conservation in their species groups.

The results have demonstrated that both ISSR and RAPD markers are suitable for characterization and assessment across four *Annona* species and there is significant difference in the genetic diversity across the species. Analysis of overall diversity of *Annona* revealed the inter-specific diversity is more than of intra-species diversity. This low intra-specific diversity is due to high degree of gene flow in population through random mating without barrier. The high level of genetic diversity across the species can be attributed to high out breeding nature of *Annona* which is a typical characteristic of wild tree species (Kingdom *et al.*, 7). The close relationship across the species might be explained by either historical relationship in sharing common ancestor or more likely geographical proximity and large population size which favour genetic interchange. The genetically rich pool of *Annona* exhibited by genotypes S1 and S6 provide an opportunity for improvement and genetic conservation programme of tree as well as in making rational base decision regarding prioritasing population which require conservation.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR, New Delhi for financial support. Thanks are also due to Dr V. Jayakumar and Smt. Sheela Pal for their help rendered.

REFERENCES

1. Ahmed, M.S. 1986. Pollination and selection in *Annona squamosa* and *Annona cherimola*. *Ministry of Agric. Egypt Bull.,* 157 p.
2. Asha, K.I., Nair, G.M. and Nair, M.C. 2006. Inter-relationship among the species of *Dioscoria* revealed by morphological trait and RAPD marker. *Indian J. Plant Genet. Resources*, **19**: 40-46.
3. Cao, T., Duprez, E., Borden, K.L.B. and Etkin, L.D. 1998. Ret finger protein in normal component of PML nuclear bodies and interacts direct with PML. *J. Cell Sci*. **111**: 1319-29.
4. Escribano, P., Viruel, M.A. and Hormaza, J.I. 2004. Characterization and crossspecies amplification of microsatellite markers in cherimoya (*Annona cherimola* Mill. Annonaceae). *Mol. Ecol. Notes*, **4**: 746-48.
5. Ghafoor, S., Shah, M.M. and Ahmad, H. 2007. Molecular characterisation of *Ephedra* species found in Pakistan. *Genet. Mol. Res.,* **6**: 1123-30.
6. Iqbal, M.J., Aziz, N., Saeed, N., Zafar, A. and Malik, Y. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theor. Appl. Genet*. **94**: 139-44.
7. Kingdom, K., Weston, F.M., Kwapata, M.B., Bokasi, J.M. and Munyenyembe, P. 2007. Genetic diversity of *Annona selegalensis* Pers population as revealed by SSRs. *African J. Biotech.* **6**: 1239-47.
8. Murray, M.G. and Thomson, W.F. 1980 Rapid Isolation of high molecular weight DNA. *Nucleic Acids Res*. **8**: 4321-25.

9. Perfectti, F. and Pascual, L. 2004. Genetic diversity in a worldwide collection of cherimoya cultivars. *Genet. Res. Crop Evol.* **52**: 959-66.

10. Powell, W., Proven, J. and Waugh, R. 1995. Polymerase chain reaction-based assays for the characterisation of plant genetic resources. *Electrophoresis*, **16**: 1726-30.

11. Rohlf, F.J. 1998. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 2.02, Exeter Publications Setauket, New York.

12. Sofowora, E.A. 1993. *Medicinal Plants and Traditional Medicine in Africa*. 2nd edition. P.(85) **174**: 195-98.

13. Sreekumar, P.V., Singh, D.B. and Sharma, T.V.R.S. 1996. Occurrence of *Annona glabra* L. – A wild relative of custard apple in the Andaman Islands, India. *Malayan Nature J.* **50**: 81-83.

14. Vinod, K., Singh, G., Sharma, R. and Sharma, S.N. 2007. RAPD and protein profiles of cotton varieties. *Indian J. Plant Physiol.* **12**: 115-19.

Received: November, 2009; Revised: April, 2010;
Accepted : May, 2010