Genetic diversity analysis in Jackfruit selections of Kuttanad region using RAPD technique

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Abstract- *Artocarpus heterophyllus* Lam (Jackfruit) which belongs to the *Moraceae* family , is believed to be indigenous to the south western rain forest of India. There exists a lot of variability among jack trees since most of them are raised from seedlings. A survey has been conducted in Kuttanad region to find out promising jack types during 2010-12 and selected six superior trees based on physico-chemical characters and organoleptic properties. Hence a study was undertaken to realize the genetic relationship among these jackfruit selections during 2012-2014, using RAPD technique. DNA was isolated from young leaves of six *A. heterophyllus* selections . Out of the thirty RAPD primers used for the analysis only ten produced maximum reproducible polymorphic bands (OPA-1, OPA-2, OPA-4, OPA9, OPC7, OPD19, OPN-05, OPM- 16, OPG-03 and OPG-10) . The primer OPA-1 gave the maximum number of bands and OPN-05 produced least. An UPGMA dendrogram was constructed and the statistical analysis was done using NTSYSPC-2.02i.

Index Terms- Jackfruit, RAPD , genetic diversity

I. INTRODUCTION

Artocarpus heterophyllus Lam. (Jackfruit) is a member of the family Moraceae. It is considered to be a native species of the rainforests of the western Ghats of India . It comprises nearly 60 species with jackfruit as the most cultivated one. Jackfruit being an important component of homestead gardens in Kerala , there exists a lot of variability since most are raised from seedlings.Different marker systems are currently available for monitoring and assessing genetic diversity. Random amplified polymorphic DNA (RAPD) markers established by Williams et al. (1990) are DNA fragments from PCR amplification of random segments of genomic DNA with a single primer of arbitrary nucleotide sequences, which are able to differentiate between genetically distinct individuals. This technique is simple to use, and does not need any sequence information. The RAPD marker becomes one of the fewer molecular techniques for assessing genetic variation in Moraceae species ie; in Jackfruit (Prasad et al 2014, Pushpakumara and Harris 2007), in fig (Saleh 2013) and in Mulberry (Srivastava et al 2004). The present work was performed to investigate the genetic diversity among the various superior Jackfruit sp of Kuttanad region using RAPD.

II. MATERIALS AND METHODS

Sample collection

An extensive survey has been conducted in Kuttanad region to find out promising jack types during 2010-12 and selected six superior trees based on physico-chemical characters and organoleptic properties. All these six genotypes were firm flesh types and regular bearing. Sampling and the molecular characterization was conducted during 2013-2014. Random sampling strategy was followed for collection of leaf samples. The leaf samples were collected in sterile plastic covers and brought to the laboratory in ice buckets. About five healthy and undamaged young, but fully developed leaves were picked from each of the trees. The leaves were washed gently with distilled water to remove all surface particles, air dried and placed in sterile plastic covers with labels and stored in -80◦c .

DNA extraction

The veins of the leaves were removed prior to the isolation. The genomic DNA was extracted using the CTAB method slightly modified after the protocol described by Doyle and Doyle (1987). A 10% CTAB isolation solution was used for the extraction. A 250ml CTAB working solution which contained 50ml 10% CTAB, 70ml 5M NaCl, 10ml 0.5M EDTA, 25ml 1M Tri HCl and 95ml distilled H_2O.About 7ml CTAB isolation buffer was preheated to 60°C in a water bath and 0.3% β-mercaptoethanol was added. About 1 gm of the deveined leaf tissue was ground well into powder after freezing with liquid nitrogen. Preheated CTAB isolation buffer was added to the ground tissue and the slurry was transferred to centrifuge tubes. The tubes were incubated at 60°C for 45min with intermittent shaking. Equal volume of chloroform:isoamyl alcohol (24:1) was added to each tube, mixed gently and centrifuged at 7500rpm for 10min at 15°C. The supernatant was transferred to a fresh tube and the centrifugation was repeated 4-5 times using chloroform-isoamylalcohol. Equal quantity of choloroform: isoamylalcohol was added again and centrifuged at 7500rpm. The supernatant was transferred to a fresh tube and equal volume phenol: chloroform (1:1) was added, mixed by inversion and centrifuged at 7500rpm. The resultant supernatant was slightly yellowish in color. 600µl chloroform was added to it and centrifuged again at 7500rpm and icecold isopropanol (0.6 volume) was added gently to the supernatant through the sides of the tube. 1/3rd volume 5M sodium chloride was also added to the tubes. The DNA was precipitated and kept overnight at 4°C for complete precipitation. The tubes were taken next day, mixed gently and centrifuged at 10000rpm for 5min at -20°C.The supernatant was drained out and the pellet was washed with 75% ethanol 3-4 times to remove

the final contaminants. 20-25µl Rnase A was added , mixed by inversion and incubated for 30min at 37°C. The pellet was air dried completely and dissolved in 100µl TE buffer. The DNA samples were stored at -20 °C for further analysis. The final DNA quantity was determined by loading the sample on 0.8% agarose gel and using Qubit Fluorometer 2.0 (Table 1).

Primer screening

About thirty primers were initially screened using the selected varieties to determine the suitability of each primer to the study. From these screened primers only 10 (OPA-1, OPA-2, OPA-4, OPA-9, OPC-7, OPD-19, OPG-03, OPG-10, OPN-05, OPM-16) showed high polymorphism and were used for the characterization of the six genotypes .

PCR Amplification

The DNA amplification was performed by Agilent surecycler 8800 using ten arbitrary 10-base RAPD primers (Table: 2) following the protocol where the reaction mix comprised with 25µl volume of 2.5µl of 1x buffer, 0.175µl of 1.5µM MgCl2, 1µl of 200µM dNTPs, 1.25µl of 1 unit Taq polymerase, 2µl of 10pmole Primer and 4µl of 25ng/µl of template DNA. The reaction was performed using the PCR profile with 1 cycle initial denaturation of 3min at 94°C, 39 cycles of 30 sec at 94°C, 1min at 37°C, 1 min at 72°C and a final elongation of 15min. After amplification the products were separated on 2% agarose gel, stained with ethidium bromide. The gels were documented with Biorad Geldoc EZ imager.

Data Analysis

The amplification products for each DNA sample with primers were considered as polymorphic when they were present in some individual and not in others. The prominent DNA bands were scored visually on the basis of their presence (1) or absence (0) for all of the samples studied. The sizes of fragments (molecular weight in base pairs) were estimated by using 100-bp ladder marker, which was run along with the amplified products. The scores obtained using all primers in the RAPD analysis were then used for constructing a single matrix. The total number of bands, polymorphic bands and the percent of polymorphism were calculated. The Polymorphism information content (PIC) values for each RAPD primer was calculated using the formula PIC = 2fi(1-2fi) (Powell et al 1996). Jaccard's similarity coefficient was used to generate pairwise similarity matrices (Jaccard 1908)using the SIMQUAL format of NTSYS-pc version 2.02i (Rohlf 2002).Based on the similarity matrix a dendrogram was constructed using UPGMA with the SAHN module of NTSYS-pc (Sneath and Sokal 1973). A two dimensional plot of six accessions of the Jackfruit was obtained using EIGEN procedure in the NTSYSpc version 2.02i.

III. RESULTS AND DISCUSSION

Tender leaf samples were used for the isolation of DNA because mature leaves were not useful as they were rich in phenols and polysaccharides. The protocol used resulted in dull white translucent DNA pellets, which were easily dissolved in TE buffer, if the purification process was not proper the pellet resulted in slight yellowish color. The DNA purified using the protocol was homogenous and did not degrade. It was also amplifiable using Taq DNA polymerase. This pellet when ran on 0.8% agarose for checking the presence of DNA, bands were not seen as the polyphenols might have suppressed the precipitation of DNA. The components for the amplification was varied so as to get a reproducible profile. The amount of DNA was found to be optimum at 4µl (25ng/µl) as the fragments formed were clear. A higher and a lower volume of DNA resulted in a smear effect or no amplification respectively. The concentration of the DNA isolated from all the samples were checked for their suitability for amplification using Qubit fluorometer 2.0. The concentration of the isolated DNA samples are given in the table 1 . Good DNA concentration was obtained for all the samples.

Preliminary screening facilitated the selection of primers producing a higher level of polymorphism and to reject primers with informations not enough for the analysis. The present study proceeds through the random analysis of 30 Operon random ten-base long, single stranded primers. Among these 10 primers generated amplification products for all the 6 jack genotypes with maximum numbers of clear-cut polymorphic bands with minimum smearing. The remaining 20 primers couldn't amplify or gave monomorphic bands for our selected genotypes and were omitted for further analysis. The primers selected, no. of scorable bands and no. of polymorphic bands is given in table no.2. Among the selected 10 primers, the number of bands for each population for a single primer range from about 1-9 bands. The band size ranged approximately from 100bp – 1000bp. The largest band was in the size range of 900- 1000bp and the smallest was approximately 100bp. In total the 10 primers amplified 166 different reproducible bands.

Out of 166 bands scored, 110 bands (66.27%) were found polymorphic and the rest of the bands (33.73%) were monomorphic in nature (Table 2). Saleh (2013) reported moderate polymorphism of 71.59% in Ficus sps (Moraceae).

One probable reason for the polymorphism exhibited by the jack genotypes would be because the primers used in this study had 60-70% GC content. The maximum number of bands per lane (9) was produced by primer OPA-1(86.66% polymorphism) and minimum (1) was for OPN-05.The average number of bands per primer was 16.6 and average numbers of polymorphic bands per primer was found to be 11.0. All such polymorphic bands were considered to be potential source of markers for estimation of genetic diversity of *A.heterophyllus* . The agarose gel profiles (2%) of amplified products obtained by all the 10 primers used for the amplification of 6 jack genotypes was done. The primer OPA1 gave 4-9 bands per lane while OPA2 gave 3-7 bands per lane. OPA-4 gave a maximum of 6 bands and a minimum of 3 per lane. OPA9 also gave 3-7 bands per lane. OPC7 had a maximum of 7 bands and a minimum of 3. OPD19 gave a 5-7 bands while OPG03 ,OPG10 and OPN05 gave 2-3 bands and OPM16 with 4-6 bands. Prasad et al (2014) has reported the primers OPA 9 (25 bands) and OPA 8(32 bands) gave the highest polymorphic bands in Jackfruit. The PIC value was significantly good ranging from 0.222(OPA9 and OPM16) to 0.250 (OPA-1) with an average value of 0.239 proving the usefulness of RAPD primers in detecting polymorphism across the selected accessions. A study conducted in the analysis of apricot genotypes using RAPD primers showed that the PIC value ranges from 0.08 to 0.5 with an average of 0.33 (Mir et al 2012).

A dendrogram was constructed using the binary data of RAPD primers based on UPGMA clustering and showed four major clusters, which followed geographical separation (Fig: 1). Kumarakom-1 (SP1) and Kumarakom-2 (SP6) represented the 1st cluster, Pathamuttom (SP2) and Vaikkom-1 (SP3) represents two separate clusters and the last cluster was represented with Veloor-1 (SP4) and Veloor-2(SP5) .The pairwise similarity matrix values using Jaccard's similarity coefficient (Table 3) between genotypes ranged from 0.48 to 1.00. The highest level of genetic similarity(0.91) was noticed between Kumarakom-1 and Kumarakom- 2 accessions and the least (0.53) was observed between Veloor-1 and Veloor-2. The accessions Vaikkom-1 and Pathamuttom showed a genetic similarity of 0.67.The results of PCA analysis were comparable to the cluster analysis (Fig:2). This clearly shows that significant genetic exists among these Jack selections. The high genetic diversity present among these cultivars clearly suggests that they must have originated from genetically divergent parents.

IV.CONCLUSION

The present study has revealed the genetic diversity in the six local Jackfruit genotypes using RAPD which were found superior on the basis of organoleptic and other physico-chemical characters. The powerful capability of molecular technique to distinguish closely related cultivars based on their RAPD patterns has been brought out by this study. A similar study on the genetic diversity of six populations of Colombian mango was done by Marcella Diaz et al (2009).Characterization of diversity is a necessary requirement for the improvement, use and conservation of plant genetic resources (Krishna and Singh, 2007).Being horticulturally superior and genetically distinct, the Jackfruit cultivars are conserved in the RARS farm and are used for the multiplication and commercial exploitation.

V. REFERENCES

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APPENDIX

Table 1: Concentration of the DNA samples isolated from the 6 Jackfruit genotypes (Qubit fluorometer 2.0)

No.	Sample ID	Concentration of DNA at 260nm (ng/ml)
1	Kumarakom 1	60
2	Kumarakom 2	58.1
3	Pathamuttom	60.2
4	Vaikkom 1	63.4
5	Veloor 1	59.8
6	Veloor 2	68.1

Table 2: Details of bands by 10 random primers in 6 Jack genotypes

Primers	Sequence(5'-3')	Total Number of fragments scored	Number of monomorphic loci	Number of polymorphic loci	% of polymorphism	PIC
OPA1	CAGGCCCTTC	15	2	13	86.66	0.250
OPA2	TGCCGAGCTG	16	5	11	68.75	0.248
OPA4	AATCGGGCTG	20	6	14	70.00	0.222
OPA9	GGGTAAACGCC	13	4	9	69.23	0.245
OPC7	GTCCCGACGA	16	6	10	62.50	0.248
OPD19	CTGGGGACTT	13	4	9	69.23	0.245
OPG03	GAGCCCTCCA	16	6	10	62.50	0.248
OPG10	AGGGCCGTCT	19	7	12	63.16	0.231
OPN05	ACTGAACGCC	18	7	11	61.11	0.240
OPM16	GTAACCAGCC	20	9	11	55.00	0.222
TOTAL		166	56	110		
Average		16.6	33.73	11.0		0.239
% of polymorphic loci				66.27		

Fig:1 Dendrogram obtained by UPGMA cluster analysis based on Jaccard's similarity coefficient of six Jackfruit accessions using RAPD data

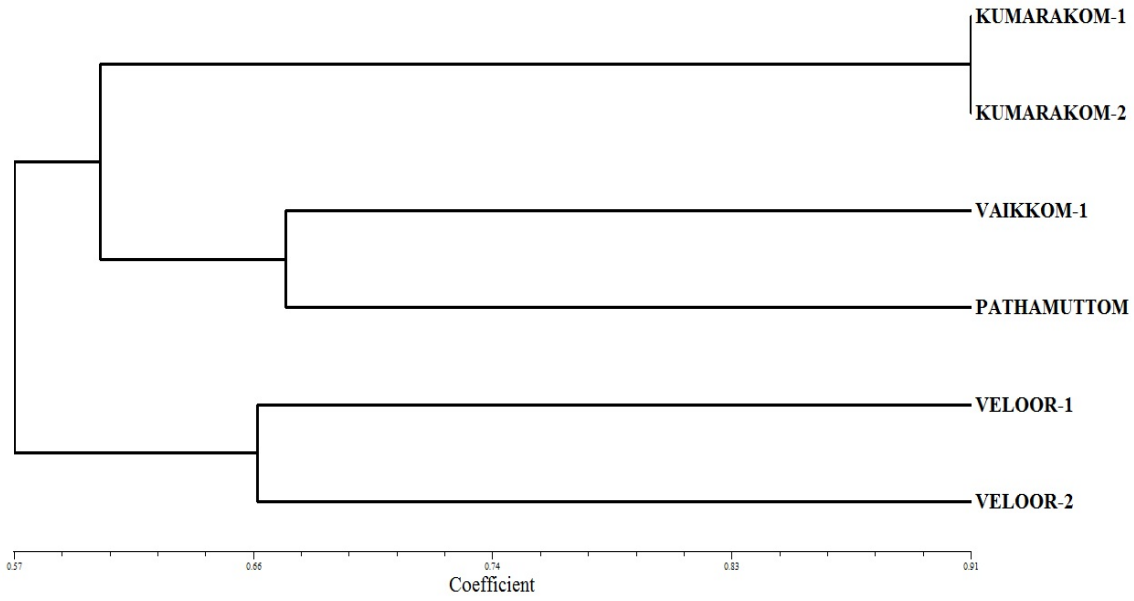

Fig :2 Two dimensional plot of six Jackfruit accessions based on PCA (Principal component analysis) using RAPD data

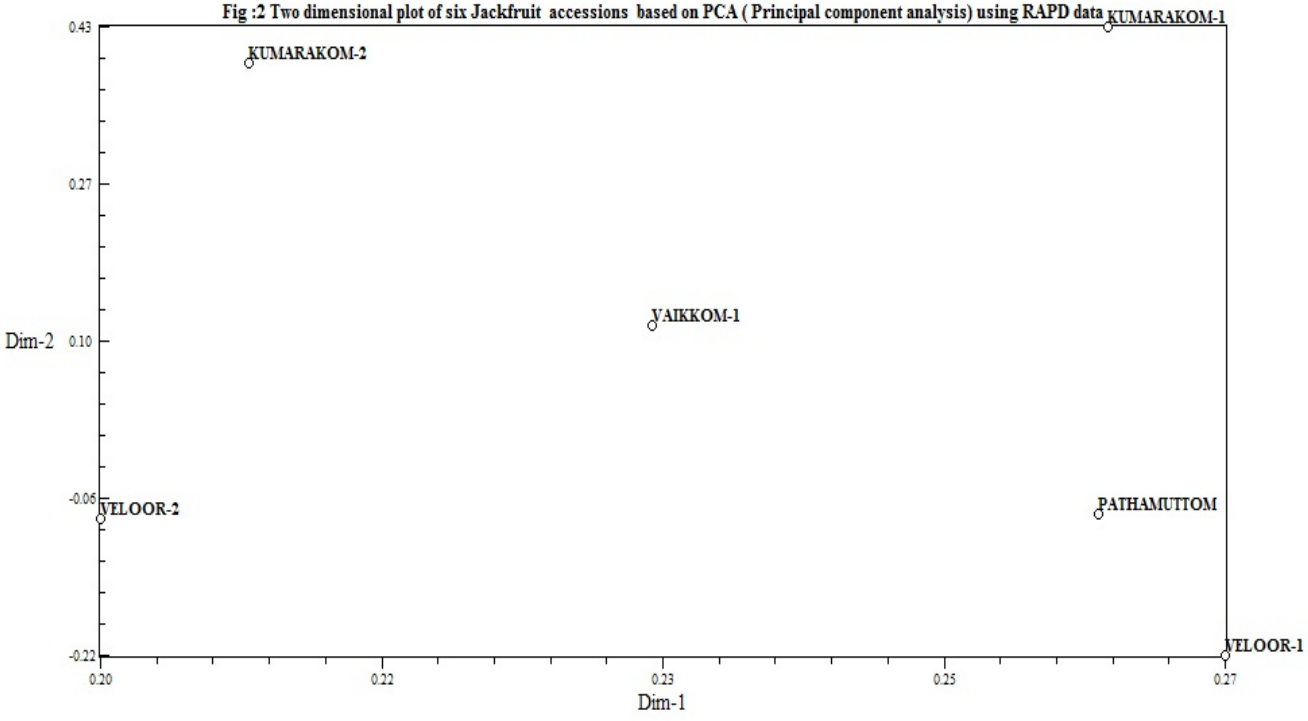

Table 3: Jaccard's matrix of six Jackfruit accessions using RAPD

KUMARAKOM-1	VELOOR-1	VAIKKOM-1	VELOOR-2	PATHAMUTTOM	KUMARAKOM-2
1.0000000					
0.4800000	1.0000000				
0.6200000	0.5400000	1.0000000			
0.5800000	0.6600000	0.5800000	1.0000000		
0.5500000	0.6500000	0.6700000	0.6300000	1.0000000	
0.9100000	0.5300000	0.6700000	0.6100000	0.5800000	1.0000000