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GENETIC DIVERSITY STUDIES IN OPEN POLLINATED SEEDLING PROGENIES OF ELEPHANT FOOT YAM *AMORPHOPHALLUS PAEONIIFOLIUS* (DENNST.) USING SSR MARKERS

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ABSTRACT

A study was undertaken at Horticultural research station, Kovvur, Dr. Y.S.R.H.U, Venkataramannagudem, West Godavari district, Andhra Pradesh to assess the genetic diversity in 24 accessions of open pollinated seedling progenies of elephant foot yam along with two checks Gajendra and Sree Padma at molecular level using SSR markers. High quality DNA was isolated by using CTAB protocol and used for polymerase chain reaction (PCR). Nineteen sets of SSR primers based on elephant foot yam sequences were used to amplify the fragments. Out of 19 SSR primers, 18 resulted in band amplification. Polymorphic Information Content (PIC) was computed based on banding pattern produced. PIC ranged from 0.14 to 0.81 which indicated the presence of high genetic diversity. A dendrogram generated using NTSYS- p.c 2.0 version formed two main clusters. The average genetic similarity calculated by Jaccard's similarity coefficient revealed a similarity value of 0.14 to 0.97 thus revealing the considerable level of genetic diversity among elephant foot yam accessions.

***Key words*: SSR markers, UPGMA dendrogram, Jaccard's similarity coefficient, elephant foot yam**

Introduction

Amorphophallus paeoniifolius (Dennst.) is a herbaceous, perennial, monoecious C_3 crop. It is basically a crop of South Eastern Asian origin. It serves as a source of protein as well as starch. It has been used as a local staple food in many countries like Philippines, Java, Indonesia, Sumatra, Malaysia, Bangladesh, India, China and South Eastern Asian countries. Owing to its production potential and popularity as a vegetable in various delicious Indian cuisines, it is commercially cultivated in India in the states of Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh and Jharkhand. In the Northern and Eastern states of India local cultivars grown in wild form are generally being used for making vegetable pickles and indigenous for various ailments. The corms are usually eaten as vegetable after boiling or baking and are rich in calcium, (50 mg g^{-1}), phosphorus (34 mg g^{-1}) and vitamin A (260 IU g^{-1}). The leaves of elephant foot yam are used as a vegetable by local tribes in India because they contain high concentration of vitamin A (Raghu *et al.*, 1999). Elephant foot yam has got great potentiality to provide nutritional security to the developing countries along with its medicinal aspects. It is a vegetatively propagated crop and hence genetic variability among the accessions is very limited.

Research exploring molecular genetics of elephant foot yam is limited till date. However, few genomic molecular markers have been developed for this crop. SSR markers have been shown to be an effective tool to carry out germplasm characterization and genetic diversity studies in this crop. Hence in-depth studies based on molecular markers like SSRs will help definitely in understanding the genetic diversity of germplasm as well as identification, conservation and utilization of this crop. This study was initiated to access the utility of SSR

Table 1: Similarity Index of 26 elephant foot yam genotypes based on Jaccard's similarity coefficient

	1	**2**	**3**	**6**	**11**	**12**	**17**	**20**	**21**	**22**	**24**	**26**	**27**	**28**	**31**	**34**	**42**	**44**	**47**	**57**	**59**	**61**	**63**	**65**	**GJ**	**SP**
OP/14-1	1.00																									
OP/14-2	0.78	1.00																								
OP/14-3	0.85	0.68	1.00																							
OP/14-6	0.78	0.61	0.78	1.00																						
OP/14-11	0.73	0.80	0.83	0.71	1.00																					
OP/14-12	0.75	0.73	0.76	0.68	0.83	1.00																				
OP/14-17	0.78	0.76	0.78	0.66	0.80	0.78	1.00																			
OP/14-20	0.36	0.14	0.46	0.49	0.34	0.41	0.39	1.00																		
OP/14-21	0.73	0.66	0.83	0.80	0.80	0.78	0.71	0.49	1.00																	
OP/14-22	0.76	0.73	0.85	0.73	0.83	0.71	0.73	0.36	0.73	1.00																
OP/14-24	0.78	0.80	0.88	0.71	0.90	0.78	0.80	0.34	0.76	0.83	1.00															
OP/14-26	0.68	0.66	0.83	0.76	0.80	0.68	0.66	0.44	0.85	0.78	0.76	1.00														
OP/14-27	0.80	0.73	0.80	0.78	0.73	0.71	0.68	0.41	0.68	0.66	0.83	0.73	1.00													
OP/14-28	0.78	0.66	0.78	0.76	0.71	0.63	0.71	0.49	0.66	0.63	0.76	0.66	0.83	1.00												
OP/14-31	0.80	0.78	0.85	0.78	0.88	0.80	0.78	0.36	0.82	0.90	0.83	0.78	0.66	0.68	1.00											
OP/14-34	0.76	0.83	0.71	0.73	0.73	0.76	0.73	0.31	0.63	0.76	0.78	0.63	0.85	0.73	0.76	1.00										
OP/14-42	0.75	0.78	0.76	0.78	0.78	0.76	0.68	0.36	0.68	0.80	0.83	0.73	0.85	0.68	0.80	0.90	1.00									
OP/14-44	0.83	0.80	0.83	0.80	0.85	0.78	0.76	0.34	0.80	0.88	0.80	0.76	0.68	0.71	0.97	0.78	0.83	1.00								
OP/14-47	0.88	0.76	0.83	0.80	0.76	0.83	0.80	0.39	0.76	0.78	0.80	0.71	0.83	0.76	0.83	0.88	0.88	0.85	1.00							
OP/14-57	0.88	0.85	0.83	0.71	0.85	0.83	0.85	0.29	0.76	0.78	0.85	0.71	0.78	0.76	0.88	0.83	0.78	0.90	0.90	1.00						
OP/14-59	0.78	0.80	0.88	0.71	0.95	0.78	0.80	0.34	0.80	0.88	0.90	0.80	0.73	0.71	0.93	0.73	0.78	0.90	0.80	0.90	1.00					
OP/14-61	0.80	0.83	0.85	0.73	0.93	0.80	0.83	0.32	0.83	0.85	0.88	0.78	0.71	0.73	0.95	0.76	0.76	0.93	0.81	0.91	0.97	1.00				
OP/14-63	0.83	0.71	0.88	0.76	0.80	0.78	0.71	0.44	0.76	0.78	0.80	0.71	0.83	0.76	0.78	0.78	0.83	0.80	0.85	0.80	0.80	0.78	1.00			
OP/14-65	0.80	0.83	0.75	0.73	0.83	0.85	0.73	0.32	0.78	0.76	0.83	0.68	0.76	0.68	0.85	0.80	0.85	0.88	0.88	0.88	0.83	0.85	0.88	1.00		
GJ	0.73	0.61	0.78	0.71	0.66	0.63	0.61	0.49	0.66	0.68	0.71	0.61	0.68	0.71	0.73	0.68	0.68	0.76	0.71	0.71	0.66	0.68	0.80	0.73	1.00	
SP	0.88	0.80	0.83	0.80	0.80	0.78	0.80	0.34	0.76	0.88	0.85	0.71	0.73	0.71	0.93	0.83	0.88	0.95	0.90	0.90	0.85	0.88	0.80	0.88	0.76	1.00

markers in differentiating some elephant foot yam accessions to identify markers that could be useful for future genetic mapping and improvement programs.

Materials and methods

Plant material

Twenty-four accessions of open pollinated seedling progenies of elephant foot yam along with the two checks Gajendra and Sree padma which were being maintained at Horticultural research station, Kovvur were taken to study the SSR allele diversity.

DNA extraction

The DNA was isolated from young leaves of 26 accessions of elephant foot yam by using CTAB protocol. The DNA isolated was homogenous and not degraded. The quality of DNA was verified by nanodrop and 1% agarose gel electrophoresis. The DNA yields ranged from 420ng/µl to 2340ng/µl and determined by A_{260}: A_{280} ratio which varied from 1.6 to 1.93 indicating that DNA was relatively of high purity and suitable for PCR amplification.

PCR assay and gel analysis

A set of nineteen SSR primers developed by Santosa *et al.*, (2010) were used for amplification. The reaction mixture (25µl) consisted of DNA 5ng (4µl), forward primer (1pmoles) (2.0µl), reverse primer (1pmoles) (2.0 µl), dNTP mix (2.5mM) (2.0 µl), 10X Taq buffer (2.5 µl), Taq DNA polymerase (0.2 µl) and sterile water (12.3 µl). PCR was carried out in a thermal cycler under the following conditions: an initial denaturation at 95^0c for 5 minutes followed by 35 cycles of 94^0c for 1 minute each, annealing at 58^0c for 1 minute and 72^0c for 1 minute and final extension at 72^0c for 7 minutes. Approximately 10µl of the amplified SSR products were loaded onto a 2% agarose gel and separated by electrophoresis in TBE buffer at 80V. Amplified products were resolved on a 1.5% agarose gel containing 4µl of ethidium bromide and visualised under UV light. Gel photographs were scanned through Gel Doc system.

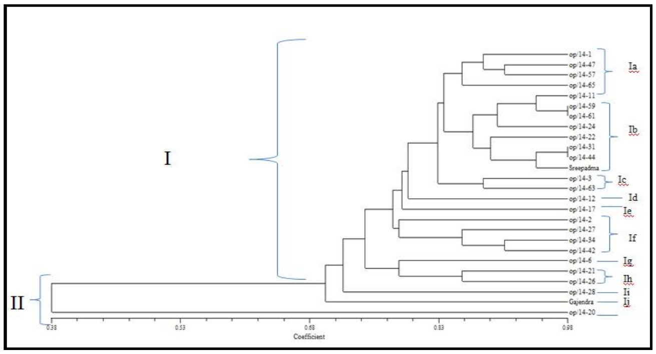

Fig. 1: Dendrogram of elephant foot yam accessions showing clustering pattern based on SSR analysis.

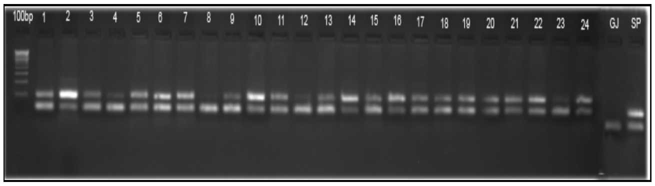

Fig. 2: Gel profile of 26 elephant foot yam accessions with SSR primer Ampa-1.

Genetic data analysis

Allelic frequencies for SSR markers were used to estimate the percentage of polymorphic loci. Loci were considered polymorphic if more than one allele was detected. PIC was calculated by the formula PIC = 1- “Pi^2 - “ “Pi^2 Pj^2. Cluster analysis of the SSR data was performed with the software version NYSYS-p.c 2.0. An unweighted pair grouping by mathematical averaging (UPGMA) cluster analysis was produced from similarity matrices constructed for SSR data and resulting dendrograms were compared.

Results and Discussion

Nineteen primers were used to perform the amplification reactions out of which eighteen primers have generated distinct bands. Polymorphic DNA bands were scored for the presence (1), absence (0) for each accession by visual inspection. SSR primers were used in DNA amplifications resulted in scorable PCR bands or loci and SSR primers used in this study were highly informative. The SSR fragments observed in the 26 accessions showed a high degree of polymorphism. Each band produced by the primers was distinct and reproducible. The polymorphic information content (PIC) demonstrates the informativeness of the SSR loci and their potential to detect differences among the genotypes based on their genetic relationships. The highest PIC was scored by the SSR marker Ampa-18 (0.81), while it was found to be lowest with the SSR marker Ampa-4 (0.14). The highest allelic frequency was recorded by the SSR marker Ampa-2, Ampa-3, Ampa-4 and Ampa-19 (0.84), where as lowest allele frequency was recorded by Ampa-18 (0.18). All the SSR primers has produced polymorphic bands with all the elephant foot yam accessions, except the primers Ampa-2, Ampa-3 and Ampa-14 which were found to be monomorphic. The expected heterozygosity for SSR markers ranged from 0.08 to 0.53. Lowest allele heterozygosity was observed with the primer Ampa-19 (0.08) and highest allele heterozygosity was noticed with the primer Ampa-17 (0.70). The results are in accordance with the findings of Santosa *et al.*, (2010), Santosa *et al.*, (2017), Yong *et al.*, (2017), Rupsanatan *et al.*, (2016), Zheng *et al.*, (2013), Pan *et al.*, (2012), Santosa *et al.*, (2007).

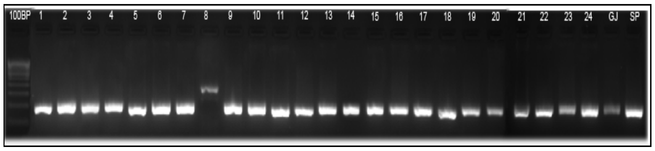

Fig. 3: Gel profile of 26 elephant foot yam accessions with SSR primer Ampa-19.

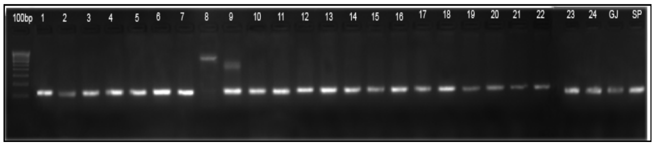

Fig. 4: Gel profile of 26 elephant foot yam accessions with SSR primer Ampa-15.

Cluster analysis of the genetic similarity values was performed to generate a dendrogram illustrating the overall genetic relationships between the accessions studied. Based on SSR bands amplified by 18 primers, genetic distances among 26 elephant foot yam accessions were calculated and a dendrogram was constructed by UPGMA method which separated the accessions into two main clusters. The UPGMA dendrogram based on genetic distance indicated that the genetic similarity values ranged from 0.14 to 0.97. Among all the accessions, the accession OP/14 -20 was found to be distinct with low similarity coefficient values and it is considered to be more diverse from all the other accessions. The accessions OP/14-31 with OP/14-44 has shown 97% of similarity and considered to be less diverse.

Conclusions

The present study of use of microsatellite markers in characterisation of elephant foot yam accessions revealed that 18 polymorphic microsatellite markers were found to be efficient to distinguish some important accessions and certainly will be useful for purposes such as certification of varieties and development of high yielding coupled with low oxalate content varieties. Identification and elimination of duplicates in the germplasm collection can save time and resources. Further there may be also some accessions derived from somatic mutations that were not detected and identified by the molecular markers used in this study.

Our results signify the presence of great genetic variability among open pollinated elephant foot yam progenies. SSR markers are useful in the assessment of diversity enhancing the efficiency of elephant foot yam genotypes for use in breeding and germplasm conservation. Since, elephant foot yam is a vegetatively propagated and highly heterozygous crop, hybridisation between divergent genotypes could be made which broaden the genetic base for the development of elite genotypes with desirable and required qualitative and quantitative characters. The notable wealth of genetic variability evident in the present study is of much important from breeding point of view as it is essential for undertaking any planned breeding programme.

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