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Study of Genetic Diversity in South Indian Taro (*Colocasia esculenta* (L.) Schott.) Using Random Amplified Polymorphic DNA Markers

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Abstract

Sixty accessions of taro (*Colocasia esculenta* (L.) Schott.), collected from the three South Indian states were analyzed using nine Random Amplified Polymorphic DNA (RAPD) primers at Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. The results obtained were utilized for assessing the extent and pattern of intraspecific variation within the species complex. Out of the nine primers, four proved to be highly informative as they could detect high levels of polymorphic fragments and were able to differentiate the accessions. The phylogenetic tree generated using UPGMA cluster analysis revealed that genetic diversity in taro was correlated with the wild and the cultivated forms. The accessions were separated into five major clusters. Out of the five clusters obtained in the dendrogram at 40% level of similarity coefficient, cluster I and cluster II included mainly cultivars. Cluster IV included almost exclusively wild accessions, majority of them being true wild ones with frequent flowering and significant stolon production. The primers were able to distinguish between diploids/triploids accessions. The diploid (clusters I, II, III and IV) and triploid (cluster V) group of accessions tended to form independent clusters and this may be suggestive of agro-ecological and sexual separation between them. These primers will be useful for future genetic analysis and provide taro breeders with a genetic basis for selection of parents for crop improvement programmes.

Key words: Taro, *Colocasia esculenta*, genetic diversity, molecular marker, RAPD

Introduction

Taro (*Colocasia esculenta* (L.) Schott.) is a polymorphic species belonging to the monocot family Araceae. The plant is believed to have originated in South East Asia, probably India or Malaysia. The rapidity and efficiency of its vegetative propagation have brought about its establishment and worldwide distribution, especially in the humid tropical and subtropical regions. The plant is cultivated extensively as a tuber crop for its edible corms and cormels that are very rich in easily digestible starch. Cytological literature on taro, especially on materials from India indicates some confusion regarding the basic chromosome number within the species complex. But, the overall situation to date, strongly suggests that x = 14 is the basic line in *C. esculenta*, occurring either as a diploid with 2n = 2x = 28 or triploids with 2n = 3x = 42 chromosomes (Kuruvilla and Singh, 1981; Sreekumari and Mathew, 1991).

Assessment of genetic variation in a species is a pre-requisite for initiating efficient breeding programmes, as it provides the basis for tailoring desirable genotypes. In recent years, several molecular techniques have been

used for germplasm characterization, identification of varieties, molecular diagnostics, phylogenetic studies and diversity analysis. Among the different molecular markers, Randam Amplified Polymorphic DNA (RAPD) approach offers the advantage of being technically undemanding, without the use of radioactivity or polyacrylamide and they are relatively cost effective as compared to other procedures. A few molecular approaches have been attempted to analyze the genetic diversity of taro germplasm all over the world. Screening of Indonesian germplasm using RAPD primers revealed that it is a powerful tool in molecular systematics with numerous successful applications in crop species including taro (Irwin et al., 1998). The geographical differentiation and phylogenetic relationship of Asian taros were analyzed using RAPD and isozymes and established that the genetic distance estimates among these differentiated taro groups were similar in both RAPD and isozyme analysis (Ochiai et al., 2001). A high genetic diversity of Indian taro was also reported using RAPD (Lakhanpaul et al., 2003).

The present study aimed at analyzing the diversity in the genetic base of South Indian taro accessions using RAPD markers.

Materials and Methods

Plant material

Sixty accessions of taro from three South Indian states were subjected to molecular study using RAPD primers at Central Tuber Crops Research Institute (CTCRI) Thiruvananthapuram, Kerala, India. The collection comprised of 43 cultivars and 17 wild types and was denoted by accession numbers Co-1 to Co-60. List of accessions used to assess molecular diversity along with place of collection (state-wise) is given in Table 1.

Isolation of genomic DNA

One gram of fresh leaf samples of each collection were used for DNA isolation. DNA extraction was

Table 1. Collection sites and germplasm types of the taro accessions

Ploidy	Locality	Germplasm type	Accession Number	Phenotypic characters
2n	Kerala	Cultivars (20)	Co-11,12, 20	Tall, non-stoloniferous
			Co- 25	Medium, stoloniferous, flowering
			Co- 6, 13, 44	Medium, non-stoloniferous, flowering
			Co- 4, 21,33, 36,38, 48,49, 57,58	Medium, non-stoloniferous, non- flowering
			Co- 27	Dwarf, stoloniferous
			Co- 26, 35, 54	Dwarf, non-stoloniferous
		Wild (9)	Co- 28, 39, 41	Medium, stoloniferous, flowering
			Co- 30, 42	Medium, stoloniferous, non- flowering
			Co- 29, 59	Dwarf, stoloniferous
			Co- 37, 53	Dwarf, non-stoloniferous
	Tamil Nadu	Cultivars (15)	Co- 8	Tall, stoloniferous, flowering
			Co- 10	Tall, stoloniferous and non-flowering
			Co- 19, 22	Tall, non-stoloniferous and flowering
			Co- 9,16,18	Tall, non-stoloniferous, non-flowering
			Co- 7	Medium, stoloniferous
			Co- 1,2,3,5,17,23,50	Medium, non-stoloniferous
		Wild (8)	Co-31	Medium, stoloniferous,flowering
			Co-40,55	Medium, stoloniferous, non- flowering
			Co-32, 51, 56	Medium, non-stoloniferous
			Co-60	Dwarf, stoloniferous
			Co-46	Dwarf, non-stoloniferous
	Andhra Pradesh	Cultivars (3)	Co-14,15,24	Medium, non-stoloniferous
3n	Kerala	Cultivars (5)	Co-34	Tall, stoloniferous
			Co- 52	Medium, non-stoloniferous, flowering
			Co-43	Medium, non-stoloniferous, non- flowering
			Co-45, 47	Dwarf, stoloniferous

done using a modified CTAB (Cetyltrimethylammonium bromide) method (Gawel and Jarret, 1991). The extracted DNA was re-suspended in 100μl of 1x TE buffer (Tris (10mM), EDTA (1mM) and stored at -20°C. The concentration of extracted DNA was determined using spectrophotometer at 260nm and diluted to 10 ng μl^{-1}. DNA concentrations thus estimated were rechecked by ethidium bromide staining of the gels after electrophoresis in 1% agarose gel along with a λ-Hind III ladder.

PCR amplification and electrophoretic analysis

PCR amplification was performed according to Williams et al. (1990) using primers synthesized by Operon Technologies (Bioenzyme). The reaction mixture (25μl) consisted of 2.5μl of 10X buffer with Mg, 200μM each of dATP, dTTP, dGTP and dCTP (Bioenzyme), 15pM of each primer, 0.5-1 unit of Taq polymerase (Accura Taq, Bioenzyme) and 50ng template DNA. PCR was carried out in a thermal cycler (Corbett Research), under the following conditions: an initial denaturation for 2 min at 94°C, followed by 40 cycles of 30 second at 94°C (denaturation), 1 min at 35°C (annealing) and 1 min at 72°C (extension), with a final extension step at 72°C for 7 min. The amplification products were electrophoresed on 1.5% agarose gel at 100 volts followed by staining with ethidium bromide, DNA bands were visualized under the UV transilluminator and images were captured with a Gel documentation system (Gene Genius). A 100bp ladder (Pharmacia) was loaded in all gels as a molecular weight standard.

Scoring and data analysis

Each amplification product was considered as an RAPD marker. These were scored across all samples. Bands were recorded as present (1) or absent (0). Molecular weights of the bands were estimated by using 100 bp DNA ladder (Pharmacia) as standards. The scores were entered into a database programme and compiled in a binary matrix for phenetic analysis using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis for Personal Computers System Version 2.02) (Rohlf, 1998) and analysed using the method SIMQUAL (similarity for qualitative data) with Jaccard's similarity coefficients (Jaccard, 1908) and a similarity coefficient matrix was constructed. This matrix was subjected to unweighted pair-group method for arithmetic averages analysis (UPGMA) to generate a dendrogram using average linkage procedure.

Results and Discussion

RAPD polymorphism among accessions

The amplification patterns obtained from nine primers out of the 20 primers of OPA series were used in the statistical analysis. Eleven primers amplified few or no PCR bands (or loci). A total of 44 bands, ranging in size from 100bp to 1000bp were scored with nine primers, of which 31 bands (63.6%) were polymorphic. The number of scorable bands ranged from 1-9 with an average of 4.9 bands per primer. The extent of polymorphism was higher with five of the primers such as OPA-03, OPA-04, OPA-09, OPA-12 and OPA-13 with 67- 83% of polymorphism (Table 2). The maximum number of bands were produced by OPA-03, OPA-04, OPA-12 and OPA-13. With OPA-03 and OPA-13, seven of the total nine bands were polymorphic (78% each). OPA-12 produced six polymorphic bands out of nine (67%) and OPA-04 produced five polymorphic bands out of six (83%). These four primers were highly informative because they amplified more than five bands. List of primers selected along with their sequences

Table 2. List of primers along with their sequences and some characteristics of the amplification products in 60 taro accessions

Primer	DNA sequence (5'-3')	Total number of bands	Number of polymorphic bands	Polymorphism (%)
OPA-03	AATCGGGCTG	9	7	78
OPA-04	AGGGGTCTTG	6	5	83
OPA-08	GTGACGTAGG	2	1	50
OPA-09	GGGTAACGCC	3	2	67
OPA-12	TCGGCGATAG	9	6	67
OPA-13	CAGCACCCAC	9	7	78
OPA-15	TTCCGAACCC	2	1	50
OPA-16	AGCCAGCGAA	2	1	50
OPA-17	GACCGCTTGT	2	1	50
	Total	44	31	70.4

and some characteristics of the amplification products in the 60 accessions of taro are provided in Table 2. The amplification products obtained by the primer OPA-03 is presented in Fig. 1 (a-d), which exemplify the typical RAPD banding pattern obtained in the present study.

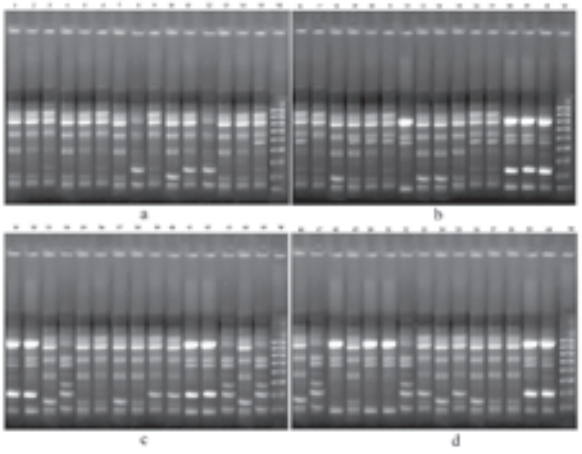

Fig. 1 (a-d). RAPD banding pattern produced from DNA amplification of 60 taro accessions using Operon Primer A03

Band sizes across the primers

Analysis of 60 accessions of taro with nine RAPD primers revealed that the band size ranged from 100bp - 1000bp. The largest fragment amplified was in the range of 800 - 1000bp, while the smallest, but easily recognizable fragment was approximately of 100bp. The highest fragment size of 1000bp was observed with the primer OPA-12, where the band size ranged between 100-1000bp. Most of the bands were concentrated between 400-800bp.

Similarity coefficient

A fairly wide range (0.46-1.00) of Jaccard's similarity coefficient was observed among the 60 accessions. The values were suggestive of wide range of genetic diversity. The frequency distribution of Jaccard's similarity coefficient values for RAPD markers are given in Fig. 2 which revealed that most of the values were concentrated within the range of 0.65- 0.70 and between 0.70- 0.75.

Phylogenetic tree

A dendrogram generated using UPGMA cluster analysis separated the sixty accessions into five major clusters at

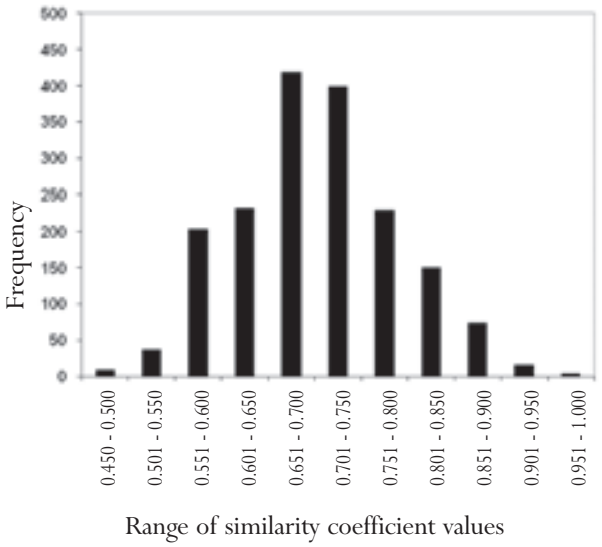

Fig.2. Frequency distribution of similarity coefficient values in 60 taro accessions

about 40% Jaccard's similarity coefficient level. The first cluster (cluster I) was the smallest among the five and included only three diploid accessions. The second cluster (cluster II), which was the largest one, consisted of 33 accessions, all diploid cultivars. The third cluster (cluster III) consisted of nine accessions which included diploids, seven cultivated and two wild ones. cluster IV included 10 accessions, all diploids and wild and the last cluster (cluster V) with only five accessions, which were all triploid cultivars. As the similarity coefficient level increases, these clusters were subdivided into smaller clusters and produced eleven clusters, when the similarity coefficient level increased to 50%.

The RAPD markers proved to be more useful in detecting variations between varieties than any other method. The method is also important as a reliable, simple and easy tool in the diagnosis of different varieties. It has been employed for cultivar identification (Ravi et al., 2003) and also for plant genomic analysis including population genetics, systematics and phylogeny (Demeke et al., 1992; Adams and Demeke, 1993). A great asset of RAPD is the large number of available random primers and the large data sets that can be generated from them, which is important for the prediction of bio-wealth. In the present study, the 60 accessions of *C. esculenta* were analyzed using nine RAPD primers and a high level of polymorphism has been observed with these markers indicating a diverse genetic base of the South Indian taro

accessions.

Jaccard's similarity coefficient value is suggestive of high genetic diversity of the present taro accessions. This agrees with the result of Lakhanpaul et al. (2003), who previously reported a similarity coefficient value of 0.50 to 0.98 and a high genetic diversity among Indian taro based on RAPD markers. Within the highest range of 0.95 -1.00, four values were obtained between four pairs of accessions, which suggested close similarity among them. Within the lowest range of 0.450-0.455, there were nine pairs of accessions which were suggestive of highest dissimilarity between these accessions. Among them, the lowest values were observed for the pairs Co-47/Co-2, Co-47/Co-1, Co-47/Co-10 and Co-47/Co-23. Co-47 is a non flowering triploid cultivated accession commonly called as 'adakka chempu' collected from Kannur district. This triploid accession was found to be the most divergent one among the present collection.

The phylogenetic tree generated using UPGMA cluster analysis revealed that genetic diversity in taro was correlated with the wild and the cultivated forms to some extent. Cluster analysis also showed that they were divided into both diploid and triploid types with 2n= 2x= 28 and 2n= 3x= 42 forms. Irwin et al. (1998) have investigated genetic diversity of taro using RAPD markers (OPD-10, OPD-15) and they could distinguish between Hawaian morphotypes into diploids and triploids, which were considered as monomorphic zymotypes by Lebot and Aradhya (1991) in their isoyme studies. Similarly, a high level of amplified fragment length polymorphic (AFLP) variation was observed between wild and traditional cultivars (Kreike et al., 2003) and also using simple sequence repeats (SSR) and inter-simple sequence repeats (ISSR) in taro (Mace and Godwin, 2002; Okpul et al., 2005).

The overall result showed a high level of genetic variability among the clones tested, particularly among the cultivated ones. Some of the cultivars flowered naturally. Insect pollinators were very active and natural hybridization occurred very regularly. As a result, natural seed set was common in the field, especially in Kerala and Tamil Nadu and it may be that natural cross-pollination was readily occurring. This resulted in a greater genetic diversity. In cluster III of the present dendrogram holding mostly wild accessions, the high genetic diversity might reflect lack of improvement made in this crop in these areas (majority from Tamil Nadu). Cluster IV of the five major clusters, included mainly wild accessions, majority of them being true wild ones with frequent flowering and significant stolon production. Genetic diversity of these accessions was found to be low when compared to those under group II and III. This might be due to lack of breeding among the wild taros in the South Indian region, especially Kerala (majority of accessions under this cluster were from Kerala). The diploid (clusters I, II, III and IV) and triploid (cluster V) groups of the present accessions tended to form independent clusters. This supports Tahara et al. (1999), who have precisely compared the accessions and successfully differentiated them into diploids and triploids by isozyme analysis. Clear separation of diploids and triploids into different clusters may be suggestive of the agro- ecological and sexual separation between them.

The fact that the triploid cluster remained intact in the dendrogram demonstrated that the high genetic similarity within the triploid accessions was genuine and not artificial despite differences in ploidy. Triploid taros were sterile and were propagated clonally. Once a triploid was formed and established as an adapted clone after natural selection, somatic mutation was the only source of genetic diversification in its descendents. Genetic changes by mutation are by far slower and much restricted than those by genetic recombination through sexual propagation. This might be the reason for their homogeneity.

The overall result suggests that South Indian taros in particular gave region wise greater difference, especially in South West areas (Kerala). Genetic variability was very high, particularly among the cultivars. Triploids were found to be the least divergent group. The clustering of accessions into diploids and triploids was well established, while the differences were not as much as between the wild and cultivated. The result, however, did not reveal any specific bias in the clustering of the accessions with reference to their geographical origin except in cluster III and IV.

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