



# Genetic Diversity Analysis in Taro Using Molecular Markers – An Overview

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## Abstract

Taro (*Colocasia esculenta* L. (Schott.)) is an important root crop especially in the humid tropics and sub-tropics. It is one of the few crops that can adapt well to different agro-climatic conditions. Taro is thought to have been originated in the Indo-Malayan region probably in North Eastern India and Bangladesh. However, some studies suggest that there exists a parallel Pacific gene pool for taro, quite distinct from the Asian one. Due to the vegetative nature of the crop and as a result of fixing of somatic mutations, morphotypes are quite distinct even when they share the same genetic material. Hence, for breeding purposes, selection of the most divergent parents becomes difficult if one goes by morphological characterization alone. It is in this regard that molecular characterization attains its relevance. Here, the actual genetic diversity exhibited by the crop can be measured and utilized for breeding and conservation of genetic resources. Though a good amount of molecular work has been carried out in the Pacific and South-east Asian genepools, no systematic work has been undertaken in Indian taro germplasm collections, except for a few scattered reports. In this review, an attempt has been made to document thoroughly the major molecular markers used for the analysis of genetic diversity in taro and its application in the management of genetic resources.

**Key words:** *Colocasia esculenta*, molecular marker, genetic diversity, germplasm characterization, conservation, genetic resources

## Introduction

Taro (*Colocasia esculenta* L. (Schott.)), is an ancient and important root crop belonging to the monocotyledonous family, Araceae. The family consists of about 110 genera with over 2500 species. About 400 million people include taro in their diets and it forms a staple food, mainly in the humid tropics (Bown, 1988). Taro is mainly grown as a root crop or as a leafy vegetable. The plants have peltate, cordate leaves and corms, which differ in sizes and shapes.

## History

Because of a long history of vegetative propagation, there is considerable confusion in the taxonomy of the genus *Colocasia*. Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be

polymorphic. There are at least two botanical varieties (Purseglove, 1972) viz., *Colocasia esculenta* (L.) Schott. var. *esculenta* (dasheen type) and *Colocasia esculenta* (L.) Schott. var. *antiquorum* (Schott.) Hubbard and Rehder (eddoe type), which is synonymous with *C. esculenta* var. *globulifera* Engl. and Krause. *C. esculenta* var. *esculenta* is characterized by the presence of a large cylindrical central corm and very few side cormels, whereas. *C. esculenta* var. *antiquorum* has small globular central corm, with several relatively large side cormels arising from the corm. Most of the taro grown in the Asia/Pacific region is of the dasheen type. However in India, eddoe type is more common, especially in South India. It is reported that *C. esculenta* var. *esculenta* is diploid and *C. esculenta* var. *antiquorum* is triploid (Kuruvilla and Singh, 1981; Irwin et al., 1998).

This classification is, however, controversial and it has not yet been demonstrated that all diploids belong to the var. *esculenta* and all triploids belong to var. *antiquorum*. This view is supported by reports that both diploids as well as triploids are present in eddoe type (Sreekumari, 1992; Kuruvilla and Singh, 1981; Nusaifa Beevi, 2009). It is generally accepted that the majority of triploids are of Asian origin (Matthews, 1990) and that they are abundant in areas of high altitudes and latitudes and less abundant in other areas (Kuruvilla and Singh, 1981).

### Origin

The problems associated with the origin, domestication and spread of taro have been reported earlier (Spier, 1951; Yen and Wheeler, 1968; Matthews, 1990; Lebot and Aradhya, 1991; Lebot, 1992). It is possible that the centre of origin will never be found because much genetic evidence has already been lost. Many cultivars have disappeared or were transferred to different places and many wild populations have been destroyed by intense agricultural practices (Ivancic and Lebot, 2000). However, various lines of ethno-botanical evidence suggest that taro originated in South Central Asia, probably in India or the Malay Peninsula. Wild forms occur in various parts of South Eastern Asia (Purseglove, 1972). From its centre of origin, taro spread eastward to the rest of South-east Asia and to China, Japan and the Pacific Islands. Some authors have suggested that the island of New Guinea may have been another centre of origin for taro, quite distinct from the Asian centre (Lebot, 1999, Ivancic and Lebot, 2000). From Asia, taro spread westward to Arabia and the Mediterranean region. By 100 B.C., it was being grown in China and in Egypt. It arrived on the east coast of Africa over 2,000 years ago, through voyagers, first across the continent to West Africa and later on by slave ships to the Caribbean. Today, taro is pan-tropical in its distribution and cultivation.

### Propagation and flowering

Taro is a vegetatively propagated crop and propagation is effected through planting of corms/cormels. For a long time it was believed that taro plants did not flower and therefore failed to produce seeds (Plucknett, 1970; Shaw, 1975). However, later studies from several countries indicated that many clones flowered and produced viable seeds (Abraham and Ramachandran, 1960; Jackson et

al., 1977; Jos and Vijaya Bai, 1977; Strauss et al., 1979; Ghani, 1979). It is reported that many Melanesian cultivars flower naturally and set seed (Lebot, 1999). Flowering and seed setting under natural conditions were also reported in the Philippines (Pardales Jr., 1981), Solomon Islands (Strauss et al., 1979; 1980) and Hawaii (Kikuta et al., 1938). Under Indian conditions, flowering was seasonal mostly starting by mid June (2-3 months after planting) and lasted till mid September (Sreekumari et al., 2003). It was observed that few clones flowered in February too, four months after planting (personal observation).

### Cytology

The species *Colocasia* exists in two cytotypes – as diploids with  $2n = 28$  and triploids with  $2n = 42$  chromosomes (Yen and Wheeler, 1968; Ramachandran, 1978; Coates et al., 1988). The basic chromosome number is considered to be  $x = 14$  (Yen and Wheeler, 1968; Kuruvilla and Singh, 1981; Matthews, 1990). However, meiotic and karyomorphological data according to Krishnan and Magoon (1977) and Sreekumari (1997) favour the contention of  $x = 7$  as the original basic chromosome number. However, recent investigations using fluorescent *in situ* hybridization with ribosomal DNA probe (Kokubugata and Konishi, 1999) showed evidence for basic chromosome number  $x = 14$ . Research work done in Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, and other reports showed that Indian taro consisted of diploids as well as triploids (Sreekumari, 1992, Nusaifa Beevi, 2009; Kuruvilla and Singh, 1981). Kuruvilla and Singh (1981) reported that clones collected from the North Eastern hill state of Meghalaya were diploids and triploids, whereas those from the plains of South India were diploids.

### Genetic diversity

The existing genetic variation within a population is called as genetic diversity. The importance of genetic diversity is evident in terms of survival and adaptability of a species. For instance, a species with high genetic diversity will tend to produce a wider range of offspring, where some of them may become the most fit variants in a population. In contrast, a species that has little or no genetic diversity will produce offspring that are genetically alike and therefore will likely be susceptible

to diseases or problems affecting their parents. Hence, little or lack of genetic diversity reduces biological fitness and increases the chances of species extinction. Genetic diversity within and between populations can be assessed routinely using morphological, biochemical and molecular characterization and evaluation.

Taro is an old cultivated species and a great portion of its genetic diversity has probably been lost due to diseases and pests, natural catastrophes and changes in climate, culture and population structure. The remaining genetic variation in the genus *Colocasia* may not be sufficient to answer all the important questions associated with the several thousand years of history of the crop (Ivancic and Lebot, 2000). Though the origin of taro in Asia is well documented, confusion prevails over the domestication of taro in the Pacific islands, which is suggested by many as another centre of origin for taro. Various studies support two Centres of origin for taro – one in South-east Asia and another in Melanesia (Lebot and Aradhya, 1991; Lebot et al., 2004; Kreike et al., 2004; Sardos et al., 2011). Molecular tools can help improve our knowledge in studying these issues related to origin, phylogenetic relationship, geographical distribution, hidden genetic diversity and predictions on susceptibility of the crop to any particular disease, amongst other applications, in this crop.

### Markers for genetic diversity analysis

The diversity among varieties could be assessed based on morphological (growth habit, stolon formation, plant height, shape, colour and orientation of lamina, maturity, shape and weight of corms and cormels, corm and cormel yield, flesh colour and edibility of tubers, resistance against leaf blight etc.), biochemical (protein expression profiles and isozymes) and molecular markers (DNA markers *viz.*, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), Simple Sequence Repeats (SSRs) etc.). Many reports are available on the morphological characterization in taro (Lebot et al., 2004; Quero-Garcia et al., 2004; Trimanto et al., 2010; Singh et al., 2011). However, diversity analysis based on morphology alone has a major disadvantage in the fact that it is highly influenced by the environment. Another drawback is that, being a vegetatively propagated crop for thousands

of years, morphotypes can be quite distinct as a result of fixation of somatic mutations (Kuruville and Singh, 1981), even when they share the same genetic background. Somatic mutations are changes occurring in the somatic cells and hence will not be carried forward to the next generation. Hence for breeding purpose, if one goes by morphological characterization alone to identify diverse parents, the result would be undesirable. To overcome this problem, molecular characterization comes in handy. Molecular techniques have had critical roles in studies of phylogeny and species evolution and have been applied to increase our understanding of the distribution and extent of genetic variation within and between species. Some of the advanced marker techniques utilize newer classes of DNA elements, such as retrotransposons, mitochondrial and chloroplast based microsatellites, thereby revealing genetic variation through increased genome coverage (Mondini et al., 2009). Among the several methods available, one of the earlier used ones was isozyme analysis followed by other DNA based markers such as RAPD, ISSR, AFLP and SSRs. Whereas isozymes represent allelic expression of the same locus, other DNA based markers like RAPD are independent genetic markers (Ochiai et al., 2001) with a lower proportion of non-neutral markers than the former (Bartish et al., 2000) and hence more superior. Germplasm characterization and evolutionary process in viable populations are important links between the conservation and utilization of plant genetic resources. The development of molecular and biochemical techniques help researchers not only to identify genotypes, but also in assessing and exploiting the genetic variability (Whitkus et al., 1994). Insights into the relative genetic diversity among taro cultivars would be useful in plant breeding and *ex situ* conservation of plant genetic resources. However, compared to other crops such reports are few in this crop and hence an attempt is made to review the major molecular studies done towards studying the genetic diversity in taro.

### Isozyme markers

The use of biochemical and molecular markers for taro germplasm characterization is quite recent and expensive, when thousands of accessions have to be analyzed. The first isozyme studies (Lebot and Aradhya, 1991) covering a wide geographical region included 1417 cultivars and

wild forms from South-east Asia and Oceania. Malate dehydrogenase, isocitrate dehydrogenase, phosphoglucose isomerase, 6-phosphoglucose dehydrogenase, malic enzyme, shikimic dehydrogenase and alcohol dehydrogenase (Table 1) were used by them and results showed a great variation in Asia than in the Pacific, with Indonesia being the area of the greatest diversity. Multivariate analysis of the isozyme data indicated that the majority of the Indonesian cultivars were different from the Philippines and the Pacific cultivars. This study supported the existence of two independent gene pools, one in South-east Asia and the second in Melanesia, indicating the possibility of two independent domestication processes.

Prana et al. (2000) studied genetic variation of 328 taro samples collected from various parts of West Java, Indonesia, using isozyme markers and identified 100 zymotypes which reflected the wide diversity present in the collection. Six enzyme systems were studied by them, namely phosphoglucose isomerase, 6-phosphoglucose dehydrogenase, shikimic dehydrogenase, malic enzyme, malate dehydrogenase and isocitrate dehydrogenase (Table 1).

In the work of Manzano et al. (2001), 42 accessions of taro (*Colocasia esculenta* L. Schott.), from the genebank of the Research Institute on Tropical Roots and Tubers (INIVIT), Cuba, were studied using morphological as well as isozyme pattern (esterase and peroxidase) (Table 1) for establishing a list of minimum descriptors enabling its characterization, genotype identification and formation of a core collection and to verify the absence of duplicates in the germplasm collection of *Colocasia esculenta* in Cuba. Isozymic studies indicated that clones with striped petioles, white cormel flesh, pink buds and white roots were grouped together in the dendrogram derived from the cluster analysis of the esterase and peroxidase isozymes and possibly originated from introductions from Asia. Twelve polymorphic loci with 27 alleles, 20 of which were rare alleles, were found for the esterase isozyme system.

Trimanto et al. (2010) characterized 18 samples of taro plants growing in Karanganyar district, Central Java, which included high, medium and low altitude, based on morphological and isozyme pattern to find if any variation existed between plants growing in the different

altitudes and to study whether the isozymic data supported the morphological data. Their study was conducted using three enzyme systems namely esterase, peroxidase and shikimate dehydrogenase (Table 1) and results revealed that morphological characteristics were not affected by altitude and isozymic banding pattern data supported the morphological character data.

### DNA markers

A few molecular approaches were attempted by earlier workers based on nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) for studying the genetic diversity in taro. Matthews et al. (1992) examined the restriction fragment length polymorphism (RFLP) in the rDNA and mitochondrial DNA (mtDNA) in Japanese taro cultivars. It was observed that the rDNA variation was consistent with previous classifications based on morphological and biochemical data.

Irwin et al. (1998) evaluated genetic diversity of taro from Hawaii and other regions using random amplified polymorphic DNA (RAPD) markers (Table 1). Forty four *Colocasia esculenta*, two *Xanthosoma* spp. and one *C. gigantea* accessions were subjected to molecular characterization. Their study showed that high genetic diversity was observed in taro from Indonesia, which confirmed the earlier studies of Lebot and Aradhya (1991) using isozymes. The RAPD markers were also able to distinguish among Hawaiian taros and could separate diploid and triploid accessions, which were found to be monomorphic with isozymes. An attempt was made by Lakhanpaul et al. (2003) to assess the genetic diversity in Indian taro by RAPDs (Table 1). Thirty two taro accessions collected from different parts of India belonging to 28 morphotypes were subjected to RAPD. This study showed the presence of high genetic diversity and distinctness in Indian taro germplasm as revealed by the absence of even two accessions showing a similarity coefficient value of one. RAPD analysis was found to be better suited for detecting genetic differences within *Colocasia esculenta* and amongst its related species (Ochiai et al., 2001). Similar observations were also made by Shen et al. (2003) for the use of RAPD markers in genetic diversity analysis of taro, where 88% polymorphism was observed. Genetic diversity of 28 taro accessions collected from 14 counties and cities in Yunnan province of China was done by them using

Table 1. Details of some major marker systems used for the genetic diversity analysis of taro reflecting the material studied

Marker system	Primer / Isozyme details	Material(s) used for study	References
Isozyme	Malate dehydrogenase, isocitrate dehydrogenase, phosphoglucoc isomerase, 6-phosphogluconate dehydrogenase, malic enzyme, shikimic dehydrogenase, and alcohol dehydrogenase	1417 cultivars and wild forms of taro collected in Asia and Oceania	Lebot and Aradhya, 1991
RAPD	AM8, AN1, B11, B20, B13, AN20, AN7, AN2, B3, B9, AM17, AM18, B13, AL4, AM3, AN2, B3, A17, D15, AM17, B9, AN15, B11, B5, C5, C8, D2, D5, AM19, B1, B4, B8, C9, D9, D16, AM12, B5, B6, C5, AM3, B1, D10 (Operon Technologies)	Forty four taro ( <i>Colocasia esculenta</i> ), two tannia ( <i>Xanthosoma</i> species) and one <i>Colocasia gigantea</i> accession	Irwin et al., 1998
Isozyme	Phosphoglucoc-isomerase, 6-phosphogluco dehydrogenase, shikimic dehydrogenase, malate enzyme, malate dehydrogenase and isocitrate dehydrogenase	328 taro samples collected from various parts of West Java, Indonesia	Prana et al., 2000
Isozyme	Esterase and peroxidase	42 accessions of taro from the genebank of the Research Institute on Tropical Roots and Tubers (INIVIT), Cuba	Manzano et al., 2001
RAPD	OPW – 01, OPW – 02, OPW – 04, OPW – 08, OPW – 09, OPW – 10, OPW – 12, OPW – 13, OPW – 15, OPW – 16, OPW – 17, OPW – 18, OPW – 19 (Operon Technologies, Alameda, USA)	32 Indian taro accessions belonging to 28 morphotypes collected from different parts of India	Lakhanpaul et al., 2003
SSR	Ces-1A06 - (CT)31; Ces-1A08 - (CT)21(CA)20; Ces-1B02 - (GA)9, (GA)16; Ces-1B03 - (TA)3(GATA)3(GA)12; Ces-1B09 - (AG)27; Ces-1C03 - (CT)14 and Ces-1C06 - (CT)16	105 accessions of taro collected from Indonesia, Malaysia, Thailand, Vietnam, the Philippines, Papua New Guinea and Vanuatu	Noyer et al., 2003
Isozyme, RAPD and AFLP	Isozyme systems: Peroxidase, cytochrome oxidase, superoxide dismutase, polyphenol oxidase and esterase. RAPD analysis: OPN 07; OPN 09; OPN 10; OPN 14; OPO 01; OPO 03; OPO 07; OPO 18; OPO 19; OPP 02; OPP 03; OPP 14; OPP 15; OPP 16; OPP 20; OPQ 04; OPQ 05; OPQ 06 and OPQ 20 AFLP analysis: “3 + 2” primer combination was adopted. 7 primer combinations were screened out from the 32 combinations between E-AA, E-AC, E-TT, E-TG and M47, M48, M49, M50, M59, M60, M61, M62.	28 taro accessions collected from 14 counties and cities of Yunnan province, China	Shen et al., 2003
SSR	uq84-207 – (CT)18; uq110-283 – (TGA)6(TGGA)4; uq73-164 – (CT)15; uq55-112 – (CAC)5; uq88B-94 – (CAT)9; uq97-256 – (CA)8 and uq91-262 – (TG)6(GA)4	511 taro accessions collected as part of the Pacific Island Country TaroGen network	Godwin et al., 2003

AFLP	Three AFLP primer combinations tried. The first amplification was carried out using Pst+0 and Mse +A primers. The second amplification was carried out using different primer combinations: – P+/M+ : 5'-GAC TGC GTA CAT GCA GAA-3' / 5' -GAT GAG TCC TGA GTA AAA C-3' – P+/M+ : 5' -GAC TGA GTA CAT GCA GGG-3' / 5' -GAT GAG TCC TGA GTA AAC C-3' – P+/M+ : 5' -GAC TGC GTA CAT GCA GGG-3' / 5' -GAT GAG TCC TGA AAG G-3'.	255 taro accessions from Vietnam, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea and Vanuatu	Kreike et al., 2004
Isozyme and AFLP	Isozyme systems: Malate dehydrogenase, phosphogluco isomerase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, shikimic dehydrogenase. AFLP analysis: The first amplification was carried out using Pst + 0 and Mse + A primers. The second amplification was carried out using different primer combinations: P + 2/M + 3: 5-GAC TGC GTA CAT GCA GAA-3 / 5-GAT GAG TCC TGA GTA AAA C-3 P + 2/M + 3: 5-GAC TGA GTA CAT GCA GGG-3 / 5-GAT GAG TCC TGA GTA AAC C-3 P + 2/M + 3: 5-GAC TGC GTA CAT GCA GGG-3 / 5-GAT GAG TCC TGA AAG G-3	2298 accessions of taro collected from Indonesia, Malaysia, Thailand, Vietnam, the Philippines, Papua New Guinea and Vanuatu	Lebot et al., 2004
RAPD and ISSR	RAPD primers: OPA 10, OPC 01, OPC 11, OPC 12, OPC14, OPC 19, OPC 20, OPD 02, OPD 10, OPW 15, OPW 16, OPW 17, R 10 (Operon Technologies, USA and M/s Genetix, India) ISSR primers: ISSR 01 – (GA) <sub>9</sub> T; ISSR 02 – (GA) <sub>9</sub> AC; ISSR 03 – (GA) <sub>9</sub> AT; ISSR 04 – (ACC) <sub>6</sub> G; ISSR 05 – (GACA) <sub>4</sub> ; ISSR 06 – (GATA) <sub>4</sub>	Taro germplasm - regenerated plants from <i>in vitro</i> formed corms after <i>in vitro</i> conservation	Hussain and Jyagi, 2006
Isozyme and RAPD	RAPD primers: S122, S123, S124, S126, S127, S128, S129, S131, S132 and S133 (Integrated DNA technologies – Coralville, USA) Isozyme systems: Esterase, malate dehydrogenase, glutamate dehydrogenase, glucosyl transferase, isocitrate dehydrogenase, hexokinase, alcohol dehydrogenase	14 Indian taro accessions based on tolerance to taro leaf blight (TLB), corm/cormel yield, tolerance to drought and salinity	Sharma et al., 2008
SSR	Xuqtem84 - (CT) <sub>18</sub> ; Xuqtem110 - (TGA) <sub>6</sub> (TGGA) <sub>4</sub> ; Xuqtem73 - (CT) <sub>15</sub> ; Xuqtem55 - (CAC) <sub>5</sub> ; Xuqtem88 - (CAT) <sub>9</sub> ; Xuqtem97 - (CA) <sub>8</sub> and Xuqtem91 - (TG) <sub>6</sub> (GA) <sub>4</sub>	Taro accessions collected from 15 provinces of Papua New Guinea	Singh et al., 2008
Isozyme	Esterase, peroxidase, shikimate dehydrogenase	Taro from Karanganyar district, Indonesia	Trimanto et al., 2010
RAPD	OPA 03, OPA 04, OPA 08, OPA 09, OPA 12, OPA 13, OPA 15, OPA 16 and OPA 17 (Operon Technologies – Bioenzyme)	60 accessions of taro collected from three South Indian States of Kerala, Tamil Nadu and Andhra Pradesh	Nusaifa Beevi et al., 2011

RAPD and ISSR	RAPD primers: OPH – 01, OPH – 02, OPH – 03, OPH – 04, OPH – 05, OPH – 06, OPH – 07, OPH – 08, OPH – 09, OPH – 10, OPH – 11, OPH – 13, OPH – 14, OPH – 15, OPH – 16, OPH – 20 (OP series - Gene1, Bangalore, India) ISSR primers: UBC -11 - (AT <sub>8</sub> T); UBC -14 - (CT <sub>8</sub> A); UBC -15 - (CT <sub>8</sub> G); UBC-16 (CA <sub>8</sub> ); UBC-19 - (GT <sub>8</sub> A); UBC - 20 (GT <sub>8</sub> C); UBC - 21 (GT <sub>8</sub> T); UBC - 22 (AG <sub>8</sub> YT); UBC - 23 (AG <sub>8</sub> YC); UBC - 25 (AG <sub>8</sub> YA); UBC - 26 (CT <sub>8</sub> YA); UBC - 36 (AG <sub>8</sub> YG); UBC - 50 (CA <sub>8</sub> ART); UBC - 53 (CA <sub>8</sub> RC); UBC - 54 (TC <sub>8</sub> RG); UBC - 56 (AC <sub>8</sub> YA); UBC - 64 (AC <sub>8</sub> CYT); UBC - 65 (CCG <sub>6</sub> ); UBC - 66 (CTC <sub>6</sub> ); UBC - 67 (GGC <sub>6</sub> ); UBC - 68 (AC <sub>8</sub> YG); UBC - 70 (AT <sub>8</sub> G); UBC - 86 (AT <sub>8</sub> C); UBC - 92 (CA <sub>8</sub> YC); UBC - 95 (GT <sub>8</sub> GA) (R = Purines; Y = Pyrimidine).	21 taro lines from Andaman Islands along with 3 commercial varieties from Indian mainland	Singh et al., 2011
SSR	Ce1A06 – (CT)31, Ce1B02 – (GA)9(GA)16, Ce1B03 – (GA)3(GATA)3 (TA)12, Ce1B09 – (AG)27, Ce1B12 – (TA)8(AG)14CG(AG)12, Ce1D12 – (CT)24, Ce1F04 – (CT)29, Ce1F12 – (TC)16TT(TC)10 and Ce1H12 – (GA)18	344 landraces of taro collected from ten villages, each located on different islands of Vanuatu, an archipelago	Sardos et al., 2011
SSR	Xuqtem55, Xuqtem73, Xuqtem84, Xuqtem88, Xuqtem91, Xuqtem97 and Xuqtem110	Seven taro cultivars from the State of Espirito Santo, Brazil	Nunes et al., 2012

isozymes, RAPD and AFLP (Table 1). Nineteen RAPD primers showed polymorphism with high polymorphic percentage (88.0%). Seven AFLP primers also showed high polymorphic percentage of 91.8%. All the three marker systems showed significant genetic diversity. Sharma et al. (2008) carried out RAPD and isozyme studies in 14 Indian taro lines (Table 1) to evaluate the levels of genetic variation present amongst them. They observed 97% polymorphism in those lines using RAPD. Cultivars collected from the northern part of India formed a separate group and clustered together, whereas the cultivar collected from the southern part of India formed a separate solitary group. The RAPD and ISSR markers were used by Hussain and Tyagi (2006) (Table 1) to assess the genetic stability in *in vitro* regenerants of taro. A total of 13 RAPD primers and six ISSR primers gave 111 (RAPD) and 43 (ISSR) distinct bands, which exhibited uniform banding patterns in R<sub>0</sub> plants. These results showed the usefulness of both the marker systems in identifying the genetic stability of regenerants. Pillai and Lekha (2008) observed high variability within 45 Indian taro accessions using RAPD. Nusaifa Beevi et al. (2011) used RAPDs to reveal genetic diversity of 60 lines of South Indian taro (Table 1). The RAPD primers were able to differentiate between wild and cultivated forms as well as distinguish between diploids and triploids. Diversity of 21 taro accessions from different parts of the Andaman Islands in addition to three commercial varieties as reference genotypes was assessed by Singh et al. (2011) using both RAPD and ISSR (Table 1). Both the marker systems divided the population into two sub clusters, one having the reference genotypes and the other having the island population and showed correlation with morphological parameters.

The AFLP was used for genetic diversity studies in taro by few workers (Kreike et al., 2004; Quero-Garcia et al., 2004; Lebot et al., 2004). In the work of Kreike et al. (2004), 255 taro accessions belonging to Vietnam, Thailand, Malaysia, Indonesia, the Phillipines, Papua New Guinea and Vanuatu were studied for genetic diversity. The

highest gene diversity was observed for the wild group from Thailand and the lowest for the diploid cultivars from Thailand. Two major groups of clusters were identified, one assembling accessions from Asian countries and the other consisting of accessions from the Pacific, thus supporting the presence of a separate Asian and Pacific gene pool. Another important observation was that the AFLP markers could not discriminate between diploid and triploid accessions. This was in contrast to the observations made by Irwin et al. (1998), who observed unique RAPD bands in the two triploids, which were absent in the 42 diploids screened. From the dendrogram it was observed that the accessions formed clusters that reflected their geographical origin. Contrary to this, accessions of the same geographical origin did not group together in the study of Quero-Garcia et al. (2004), where an attempt was made to validate the germplasm stratification of more than 450 accessions of taro collected from Vanuatu based on agro-morphological descriptors, using AFLP. These studies further confirmed the narrow genetic base of the Vanuatu taro germplasm and also have been useful in detecting duplicates and to fingerprint the accessions. Lebot et al. (2004) made a detailed molecular characterization of 2298 accessions of taro collected from Indonesia, Malaysia, Thailand, Vietnam, the Philippines, Papua New Guinea and Vanuatu using AFLP and isozymes (Table 1). Electrophoresis was done on more than 2000 accessions, whereas AFLP characterization was carried out in all cultivars included in the core samples, which consisted of approximately 10% of the total number of elite cultivars from each country. The results of these studies also showed that genetic base of cultivars was narrow.

Of the molecular techniques available, SSRs are fast emerging as markers of choice mostly due to their co-dominant nature, transferability, reproducibility and amenability to high throughput. Mace and Godwin (2002) identified around 16 polymorphic microsatellite markers (SSRs), which gave good polymorphism when screened against a restricted set of taro genotypes from South-east Asia and Oceania, showing an average of 3.2 alleles detected on each locus. Seven polymorphic primers from this set were utilized by Godwin et al. (2003) (Table 1) to evaluate genetic diversity of 511 taro genotypes and rationalize ten national collections from

the Pacific Island Countries. In total, 38 alleles were amplified from the seven SSR loci. The SSRs were informative in revealing the genetic differences within and among the different countries. Solomon Islands showed the highest proportion of polymorphic loci (1.0) and the highest average number of alleles per locus (5.3). Another microsatellite enriched library was constructed by Bastide (2000) and using these, Noyer et al. (2003) (Table 1) analyzed a subset of samples earlier characterized by Kreike et al. (2004). A differentiation between South-east Asian and Melanesian taros was observed in this study, confirming AFLP and isozyme results of Kreike et al. (2004). Mace et al. (2006) developed a regional core collection for taro germplasm based on phenotypic and molecular characterization from germplasm collected by *TaroGen* (Taro Genetic Resources: Conservation and Utilization) from 10 countries in Oceania *viz.*, Papua New Guinea, Solomon Islands, Vanuatu, New Caledonia, Fiji, Palau, Niue, Tonga, Cook Islands and Samoa. A total of 515 accessions were genotyped (23.4% overall) using taro specific simple sequence repeat (SSR) markers. DNA fingerprint data showed that great allelic diversity existed in Papua New Guinea and the Solomon Islands. Interestingly, rare alleles were identified in taros from the Solomon Islands province of Choiseul which were not observed in any of the other collections. The first national core collection of any species established in Papua New Guinea (PNG) based on molecular markers was that of taro through SSR markers by Singh et al. (2008) (Table 1). Around 859 accessions of taro collected from 15 provinces of Papua New Guinea were morphologically characterized using 30 agro-morphological descriptors initially and a core of 20% was selected, which was further reduced to 10% based on SSR data using seven SSR markers. Recently, Sardos et al. (2011) analyzed the genetic diversity of the National Sample of taro using a set of nine SSR markers (Table 1). The samples were collected from ten villages of Vanuatu, an archipelago located in the South-west Pacific, each of which was located on a different island. The nine SSR markers used to study 344 landraces revealed a total of 89 alleles, ranging from 6 to 17 alleles per locus with an average of 9.89. Rare alleles could also be detected (57.3%). Microsatellite marker and molecular dataset revealed that these were effective tools to monitor the diversity and evolution of taro in the future. Nunes et al. (2012) investigated the



genetic diversity of the seven regional core collections of Brazilian taro from the State of Espirito Santo using seven microsatellite loci (Table 1). The study revealed that the Xuqtem 110 loci showed 100% polymorphism and the polymorphism observed in this study could differentiate between the taro cultivars. Molecular data was useful in demonstrating the primitiveness of the clones, which were cultivated in Brazil. It also showed that SSR markers could be used for allelic identification.

## Conclusion

In short, insights into the relative genetic diversity using morphological as well as molecular data among taro cultivars would be useful in making a core collection, which includes maximum variability available in a crop thus enhancing its use in plant breeding and *ex situ* conservation of plant genetic resources. Markers were able to separate between diploid and triploid lines, identify duplicates within the germplasm as well as reflect their geographic origin. Taro germplasm characterization using molecular and biochemical markers would contribute to the knowledge of genetic relationships between accessions of wild and cultivated gene pool, thereby facilitating breeding of taro cultivars to suit the various requirements of end users. Markers were also found to be effective in understanding the domestication and evolution of taro.

## References

- Abraham, A. and Ramachandran, K. 1960. Growing *Colocasia* embryos in culture. *Curr. Sci.*, 29: 342 – 343.
- Bartish, I. V., Garkava, L. P., Rumpunen, K. and Nybom, H. 2000. Phylogenetic relationship and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes. *Theor. Appl. Genet.*, 101: 554 – 563.
- Bastide, C. 2000. Création de banques microsatellites pour l'igname (*Dioscorea alata*, *Dioscorea praehensilis* et *Dioscorea abyssinica*) et le taro (*Colocasia esculenta*). Mémoire de DUT, Dépt Génie Biologique Option Agronomie. IUT de Perpignan, France. 13 p.
- Bown, D. 1988. *Aroids. Plants of the Arum Family*. Century Hutchinson Ltd., London, United Kingdom.
- Coates, D. J., Yen, D. E. and Gaffey, P. M. 1988. Chromosome variation in taro, *Colocasia esculenta*: Implications for origin in the Pacific. *Cytologia*, 53: 551 – 560.
- Ghani, F. D. 1979. The status of Keladi china (*Colocasia esculenta*) cultivation in peninsular Malaysia. *IFS Provisional Rep.*, 5: 35 – 54.
- Godwin, I. D., Mace, E. S., Mathur, P. N. and Izquierdo, L. 2003. Application of DNA markers to management of taro (*Colocasia esculenta* (L.) Schott.) genetic resources in the Pacific Island region. In: *Proceedings of Third Taro Symposium*. Guarino, L., Taylor, M. and Osborn, T. (Eds.). Secretariat of the Pacific Community, Nadi, Fiji Islands. pp. 64 – 68.
- Hussain, Z. and Tyagi, R. K. 2006. *In vitro* corm induction and genetic stability of regenerated plants in taro (*Colocasia esculenta* (L.) Schott.). *Indian J. Biotech.*, 5: 535 – 542.
- Irwin, S. V., Kaufusi, P., Banks, K., de la Peña, R. and Cho, J. J. 1998. Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica*, 99: 183 – 189.
- Ivancic, A. and Lebot, V. 2000. *The Genetics and Breeding of Taro*. CIRAD Publication Series, France.
- Jackson, G. V. H., Ball, E. A. and Arditti, J. 1977. Seed germination and seedling proliferation of taro, *Colocasia esculenta* (L.) Schott. *in vitro*. *J. Hort. Sci.*, 52: 169 – 171.
- Jos, J. S. and Vijaya Bai, K. 1977. Stigma receptivity in *Colocasia*. *J. Root Crops*, 2(3): 25 – 28.
- Kikuta, K., Whitney, L. D. and Parris. 1938. Seeds and seedlings of the taro, *Colocasia esculenta*. *Am. J. Bot.*, 25: 186 – 188.
- Kokubugata, G. and Konishi, T. 1999. Implication of a basic chromosome number of  $x = 14$  in seven cultivars of two varieties of *Colocasia esculenta* by fluorescent *in situ* hybridization using rDNA probe. *Cytologia*, 64: 77 – 83.
- Kreike, C. M., Van Eck, H. J. and Lebot, V. 2004. Genetic diversity of taro, *Colocasia esculenta* (L.) Schott., in South-east Asia and the Pacific. *Theor. Appl. Genet.*, 109: 761 – 768.
- Krishnan, R. and Magoon, M. L. 1977. Edible aroids – new insights into phylogeny. In: *Proceedings of the Third International Symposium on Tropical Tuber Crops*. Leakey, C. L. A. (Ed.). Ibadan, Nigeria, International Institute of Tropical Agriculture. pp. 58 – 60.
- Kuruvilla, K. M. and Singh, A. 1981. Karyotypic and electrophoretic studies on taro and its origin. *Euphytica*, 30: 405 – 413.
- Lakhanpaul, S., Velayudhan, K. C. and Bhat, K. V. 2003. Analysis of genetic diversity in Indian taro (*Colocasia esculenta* (L.) Schott.) using random amplified polymorphic DNA (RAPD) markers. *Genet. Resour. Crop Evol.*, 50: 603 – 609.
- Lebot, V. 1992. Genetic vulnerability of Oceanias traditional crops. *Exp. Agric.*, 28: 309 – 323.
- Lebot, V. 1999. Biomolecular evidence for plant domestication in Sahul. *Genet. Res. Crop Evol.*, 46: 619 – 628.
- Lebot, V. and Aradhya, M. 1991. Isozyme variation in taro (*Colocasia esculenta* (L.) Schott.) from Asia and the Pacific. *Euphytica*, 56: 55-66.
- Lebot, V., Prana, M. S., Kreike, N., van Heck, H., Pardales, J., Okpul, T., Gendua, T., Thongjiem, M., Hue, H., Viet, N. and Yap, T. C. 2004. Characterisation of taro (*Colocasia esculenta* (L.) Schott.) genetic resources in South-east Asia and Oceania. *Genet. Resour. Crop Evol.*, 51: 381 – 392.

- Mace, E. S. and Godwin, I. D. 2002. Development and characterization of polymorphic microsatellite markers in taro, *Colocasia esculenta* (L.) Schott. *Genome*, 45: 823 – 832.
- Mace, E.S., Mathur, P.N., Izquierdo, L., Hunter, D., Taylor, M. B., Singh, D., DeLacy, I. H., Jackson, G. V. H. and Godwin, I. D. 2006. Rationalization of taro germplasm collections in the Pacific Island region using simple sequence repeat (SSR) markers. *Plant Genet. Resour.: Characterization Util.*, 4: 210 – 220.
- Manzano, A. R., Nodals, A. A. R., Gutierrez, M. I. R., Mayor, Z. F. and Alfonso, L. C. 2001. Morphological and isozyme variability of taro (*Colocasia esculenta* L. Schott.) germplasm in Cuba. *Plant Genet. Resour. Newsl.*, 126: 31 – 40.
- Matthews, P. J. 1990. The origin, dispersal and domestication of taro. *Ph.D. Thesis*, Australian National University, Australia.
- Matthews, P., Matsushita, Y., Sato, T. and Hirai, M. 1992. Ribosomal and mitochondrial DNA variation in Japanese taro (*Colocasia esculenta* L. Schott.). *Japan J. Breed.*, 42: 825 – 833.
- Mondini, L., Noorani, A. and Pagnotta, M. A. 2009. Assessing plant genetic diversity using molecular tools. *Diversity*, 1(1): 19 – 35.
- Noyer, J. L., Billot, C., Weber, A., Brottier, P., Quero-Garcia, J. and Lebot, V. 2003. Genetic diversity of taro (*Colocasia esculenta* (L.) Schott.) assessed by SSR markers. In: *Proceedings of Third Taro Symposium*. Guarino, L., Taylor, M. and Osborn, T. (Eds.). Secretariat of the Pacific Community, Nadi, Fiji Islands. pp. 174 – 180.
- Nunes, R. S. C., Pinhati, F. R., Golinelli, L. P., Reboucas, T. N. H., Paschoalin, V. M. F. and da Silva, J. T. 2012. Polymorphic microsatellites of analysis in cultivars of taro. *Horticultura Brasileira*, 30: 106 – 111.
- Nusaifa Beevi, P. 2009. Biosystematic studies in taro (*Colocasia esculenta* (L.) Schott.). *Ph.D. Thesis*. University of Kerala, Thiruvananthapuram, India.
- Nusaifa Beevi, P., Sreekumari, M. T. and Kumar, V. 2011. Study of genetic diversity in South Indian taro (*Colocasia esculenta* (L.) Schott.) using random amplified polymorphic DNA markers. *J. Root Crops*, 37(2): 162 – 167.
- Ochiai, T., Nguyen, V. X., Tahara, M. and Yoshino, H. 2001. Geographical differentiation of Asian taro, *Colocasia esculenta* (L.) Schott., detected by RAPD and isozyme analyses. *Euphytica*, 122: 219 – 234.
- Pardales Jr., J. R. 1981. Floral morphology and biology, fruit and seed germination and seedling development in taro. *Ann. Trop. Res.*, 3: 169 – 176.
- Pillai, S. V. and Lekha, S. L. 2008. Molecular variability in 45 Indian *C. esculenta* cultivars. *Asian Australas J. Plant Sci. Biotech.*, 2(1&2): 102 – 106.
- Plucknett, D. L. 1970. *Colocasia*, *Xanthosoma*, *Alocasia*, *Cyrtosperma* and *Amorphophallus*. In: *Proceedings of the Second International Symposium on Tropical Root and Tuber Crops*. Honolulu, Hawaii, College of Tropical Agriculture, University of Hawaii. pp. 127 – 135.
- Prana, M. S., Hartati, N. S. and Kuswara, T. 2000. Evaluation on genetic variation in taro (*C. esculenta* (L.) Schott.) collected from West Java using isozyme markers. *Annales Bogorienses*, 6(2): 80 – 87.
- Purseglove, J.W. 1972. *Tropical Crops. Monocotyledons*. Longman, London.
- Quero-Garcia, J., Noyer, J. L., Perrier, X., Marchand, J. L. and Lebot, V. 2004. A germplasm stratification of taro (*Colocasia esculenta*) based on agro-morphological descriptors, validation by AFLP markers. *Euphytica*, 137: 387 – 395.
- Ramachandran, K. 1978. Cytological studies in South Indian Aracea. *Cytologia*, 43: 289 – 303.
- Sardos, J., Noyer J. L., Malapa, R., Bouchet, S. and Lebot, V. 2011. Genetic diversity of taro (*Colocasia esculenta* (L.) Schott.) in Vanuatu (Oceania): an appraisal of the distribution of allelic diversity (DAD) with SSR markers. *Genet. Resour. Crop Evol.*, (Online ISSN 1573-5109)
- Sharma, K., Mishra, A. K. and Mishra, R. S. 2008. The genetic structure of *C. esculenta*: a comparison of RAPD and isozyme markers. *Pl. Biotechnol. Rep.*, 2: 191 – 198.
- Shaw, D. E. 1975. Illustrated notes on flowering, flowers, seed and germination in taro *Colocasia esculenta*. *Papua New Guinea Department of Agriculture Stock and Fisheries Res. Bull.*, 13: 39 – 59.
- Shen, D., Zhu, D. W., Li, X. X. and Song, J. P. 2003. Analysis of genetic diversity in taro in China. In: *Proceedings of Third Taro Symposium*. Guarino, L., Taylor, M. and Osborn, T. (Eds.). Secretariat of the Pacific Community, Nadi, Fiji Islands. pp. 89 – 94.
- Singh, D., Mace, E. S., Godwin, I. D., Mathur, P. N., Okpul, T., Taylor, M., Hunter, D., Kambuou, R., Rao, R. V. and Jackson, G. 2008. Assessment and rationalization of genetic diversity of Papua New Guinea taro (*Colocasia esculenta*) using SSR DNA fingerprinting. *Genet. Resour. Crop Evol.*, 55: 811 – 822.
- Singh, S., Singh, D. R., Faseela, F., Kumar, N., Damodaran, V. and Srivastava, R. C. 2011. Diversity of 21 taro (*Colocasia esculenta* (L.) Schott.) accessions of Andaman islands. *Genet. Resour. Crop Evol.* (Online)
- Spier, R. F. G. 1951. Some notes on the origin of taro. *South-western J. Anthropology*, 7: 69 – 76.
- Sreekumari, M. T. 1992. Cytomorphological and cytogenetic studies in edible aroids. *Ph.D. Thesis*. University of Kerala, Thiruvananthapuram, India.
- Sreekumari, M. T. 1997. Cytological studies in taro – a review. *J. Root Crops*, 23(1): 1 – 7.
- Sreekumari, M. T., Abraham, K., Edison, S. and Unnikrishnan, M. 2003. Taro breeding in India. In: *Proceedings of Third Taro Symposium*. Guarino, L., Taylor, M. and Osborn, T. (Eds.). Secretariat of the Pacific Community, Nadi, Fiji Islands. pp. 202 – 206.

- Strauss, M. S., Michaud, J. D. and Arditti, J. 1979. Seed storage and germination and seedling proliferation in taro, *Colocasia esculenta* (L.) Schott. *Ann. Bot.*, 43: 603 – 612.
- Strauss, M. S., Stephens, G. C., Gonzales, C. J. and Arditti, J. 1980. Genetic variability in taro, *Colocasia esculenta* (L.) Schott. (Aracea). *Ann. Bot.*, 45: 429 - 437.
- Trimanto, Sajidan and Sugiyarto. 2010. Characterization of taro (*Colocasia esculenta*) based on morphological and isozymic patterns markers. *Nusantara Bioscience*, 2(1): 7 – 14.
- Whitkus, R., Doebley, J. and Wendel, J. F. 1994. DNA-based markers in plants. In: *Nuclear DNA Markers in Systematics and Evolution*. Phillips, L. and Vasil, I. K. (Eds.). Kluwer, Amsterdam. pp. 116 – 141.
- Yen, D. E. and Wheeler, J. M. 1968. Introduction of taro into the Pacific: the indications of the chromosome numbers. *Ethnology*, 7: 259 – 267.