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Study of Genetic Diversity among *Philodendron* Varieties by Randomly Amplified Polymorphic DNA Markers

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Abstract

In the present study, Randomly Amplified Polymorphic DNA (RAPD) markers were used to analyze the genetic diversity in 20 varieties of *Philodendron*. The polymerase chain reaction was performed with 60 RAPD primers, out of which 21 primers showed clear amplification as well as more polymorphism. In total, 354 scorable RAPD loci with 348 polymorphic bands (98%) were observed. Percentages of polymorphic bands ranged from 80% to 100%. Amplified DNA fragments were used for statistical analysis. The data was obtained by scoring the bands and analyzed by using STATISTICA software. A dendrogram was obtained by cluster analysis based on the presence or absence of band which indicates similarity and diversities. The cluster analysis grouped the *Philodendron* varieties based on the growth habits, morphological characters and also geographical origin. This indicates that there is an association between the RAPD patterns and the geographic origin of the varieties used. The genetic diversity among the varieties was moderate, which might be due to genetically heterozygous and somaclonal variation.

Keywords: Aroid, Houseplants, Self- heading, Spadix, Tropical forests.

INTRODUCTION

Philodendron is the second largest genus of flowering plants from the Arum family (Araceae), mainly found in many diverse habitats in the humid tropical forests of Americas and the West Indies. None the less, they can also be found in Australia, some Pacific islands, and Asia although they aren't indigenous to these regions. Rather they were introduced or accidentally escaped. According to TROPICOS (a service of the Missouri Botanical Garden), more than 900 species were recognized, however Mayo *et al*. (1997) reported that there are about 400 formally recognized species, whereas according to Croat (1997) and Boyce and Croat (2012) list there are about 700 and in Brazil it has ca. 165 species (Sakuragui *et al.,* 2012) in several biomes. Compared to other genera of the family Araceae, Philodendrons have an extremely diverse array of growth habits. The growth habits were divided into three groups, the first group is veining, and it is grown either in hanging baskets or container where vines are supported by poles. In the second group growing style is upright and third group is erect-arborescent type, which appears self-heading when they are young, but assume more woody and treelike shapes as they mature (McColley and Miller, (1965). However, majority of *Philodendron* species leaf growth gradually go through metamorphosis; there is no immediately distinct difference between juvenile and adult leaves (Bell and Bryan, 2008).

Reproduction in *Philodendron* can occur in two primary ways: through sexual and asexual reproduction. Sexual reproduction is achieved by means of beetles, with many *Philodendron* species requiring the presence of a specific beetle species to achieve pollination. However crosses between *Philodendron* species may perhaps not possible (McColley and Miller, 1965), due to differences in the chromosome number (Henny and Chen, 2003). Chromosome counts in the genus are available for only 10% of the species with a predominance of 2n = 32, 34 and 36, found in *P. scandens, P. wendlandii*, and *P. bipinnatifidum,* respectively (Cotias-de-Oliveira1 *et al.*, 1999). However the genetic relatedness among the *Philodendron* varieties has not been studied thoroughly. Therefore in the present study, RAPD markers were used to investigate genetic divergence among widespread varieties of *Philodendron* and strategies for future hybrid development as well as conservation of genetic resource.

MATERIALS AND METHODS

Plant material

Twenty *Philodendron* varieties were collected from Indo American Hybrid Seeds Company plant tissue culture green house, Bangalore. These varieties were produced through tissue culture

Table 1. Growth habit and origin of 20 *Philodendron* varieties used in the present study.

Sl.No.	Varieties	Growth habit	Origin
1	*Philodendron xanadu*	Self- heading	Brazil
2	*Philodendron panduriforme*	Semi- vining	North & Western parts of Amazon
3	Moonshine	Self-heading	Florida
4	Tropical Sunrise (hybrid)	Self-heading	American tropics
5	Kerala Hybrid (hybrid)	Semi-vining	west coast of Kerala
6	*Philodendron laciniatum*	Semi-vining	South Eastern Brazil
7	*Philodendron ceylon*	Semi-vining	Brazil
8	*P. selloum* var. Gold (hybrid)	Self-heading	Brazil
9	*Philodendron selloum*	Self-heading	Brazil
10	*Philodendron Emerald Duke*	Semi-vining	Thailand
11	Red Emerald (hybrid)	Semi-vining	Florida
12	*Philodendron wendlandii*	Semi-vining	Florida
13	Royal Queen (hybrid)	Semi-vining	Florida
14	Pluto (Hybrid)	Self-heading	unknown
15	Black Cardinal (hybrid)	Self-heading	Florida
16	*Philodendron deflexum*	Semi-vining	Brazil (South America)
17	Green Emerald (hybrid)	Semi-vining	Florida
18	Painted lady (hybrid)	Semi-vining	Brazil
19	Pink princes (hybrid)	Semi-vining	Florida
20	Golden Xanadu (hybrid)	Self-heading	Brazil

Fig. 1. Photograph of 20 *Philodendron* varieties used in the present study.

in company laboratory. The growth habit and country of origin is being given in Table 1 and Fig. 1. The genotypic study was conducted during January 2013.

Genomic DNA extraction and primer selection

Total genomic DNA was also extracted from fresh leaves according to the CTAB method described by Porebski *et al*. (1997). After isolation of DNA, its quantity was determined spectrophotometrically at a wavelength of 260 and 280 nm using Spectrophotometer. The ratio between absorbance at 260 and 280 nm (260/280) was used to estimate DNA purity. DNA of each cultivar was diluted to a working concentration of 20 ng/µl for PCR/RAPD analysis.

Randomly five varieties of DNA were used to optimize the RAPD protocols and selected the suitable primers which exhibit clear amplification. Overall, 60 arbitrary decamer oligonucleotides, from Operon Technologies Inc. (Alameda, California, USA), were tested as single primers to identify the most promising ones for detecting polymorphism. Eventually 21 primers were chosen on the basis of their ability to detect the polymorphism and produce the reliable and easily scorable banding patterns (Table 2).

PCR-amplification and gel electrophoresis

A modified RAPD method based on Williams *et al*. (1990) was used with a model T-100Tm thermal cycler (BIO-RAD, USA). The PCR reaction mix included the following: 20 µl reaction mixture containing 1 × PCR buffer [10 mM Tris HCl (pH 8.3), 50 mM KCl], 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide triphosphate (dNTPs), 0.4 µM of 10-mer primer (Operon Technologies Inc., Alameda, CA), 1 unit Taq DNA polymerase and 20 ng of template DNA for the amplification of genomic DNA. The PCR profile started with 94°C for 5 min followed by 45 cycles of denatu-

Table 2. List of 21 random primers used for generating RAPDs in varieties of *Philodendron.*

Primers	Sequence (5' to 3')	No. of Bands	No. Polymorphic bands	% polymorphism
OPA01	CAGGCCCTTC	16	16	100
OPA03	AGTCAGCCAC	16	15	94
OPA04	AATCGGGCTG	15	15	100
OPA05	AGGGGTCTTG	19	19	100
OPA09	GGGTAACGCC	14	14	100
OPA12	TCGGCGATAG	18	18	100
OPA13	CAGCACCCAC	13	13	100
OPA15	TTCCGAACCC	21	21	100
OPA16	AGCCAGCGAA	21	21	100
OPA17	GACCGCTTGT	18	18	100
OPA18	AGGTGACCGT	25	25	100
OPA19	CAAACGTCGG	21	21	100
OPC02	GTGAGGCGTC	15	15	100
OPC05	GATGACCGCC	14	14	100
OPC06	GAACGGACTC	11	11	100
OPC07	GTCCCGACGA	15	15	100
OPC08	TGGACCGGTG	15	12	80
OPD18	GAGAGCCAAC	14	14	100
OPF03	CCTGATCACC	12	11	92
OPF13	GGCTGCAGAA	18	17	94
OPN12	CACAGACACC	23	23	100
	Total	354	348	98

ration at 94°C for 1 min, annealing at 37°C for 1 min and extension at 72°C for 2 min. A final extension, 72°C for 8 min was included, followed by soaking at 20°C.

Electrophoresis

The RAPD-PCR products were analyzed directly on 1.5% agarose gels in 0.5X TBE buffer, visualized by staining with ethidium bromide and transillumination under short-wave UV light.

Data analysis

The clear and unambiguous RAPD bands were scored to the presence (1) and absence (0) of the corresponding band among the varieties. The marker data subjected to cluster analysis using the software STATISTICA" (Statsoft, 1996). A dendrogram was constructed for genotypic data using UPGMA (unweighted pair-group method using arithmetic averages) method. The dissimilarity matrix was developed using Squared Euclidean Distance (SED) that estimated all pairwise differences in the amplification product (Sokal and Sneath, 1973).

RESULTS AND DISCUSSION

RAPD profile and analysis

The 10-mer 60 RAPD primers were tested for genetic analysis of 20 *Philodendron* varieties. However, the best 21 primers that rendered polymorphic and reproducible banding pattern were chosen. The selected few gel profiles of 20 *Philodendron* varieties are furnished in Fig. 2.

Using twenty one selected primer combinations, a total of 354 scorable RAPD fragments were generated, out of which 348 (98%) were polymorphic (Table 2). Out of 21 primers, 16 primers showed 100% polymorphism and four other primers also showed 94 to 98% polymorphism except one primer OPC08, which showed 80% polymorphism. One of the reasons for this high level of polymorphism could be the extensive inter species variation in *Philodendron*. The other explanation could be that primers with 60 to 70% GC content were used. Fukuoka et al. (1992) observed an increase in the number of bands with increasing GC content of the primers. They got an average of 0.8 bands primer-1 with 40%, 6.1 bands with 50% and 8.6 bands with 60% GC content.

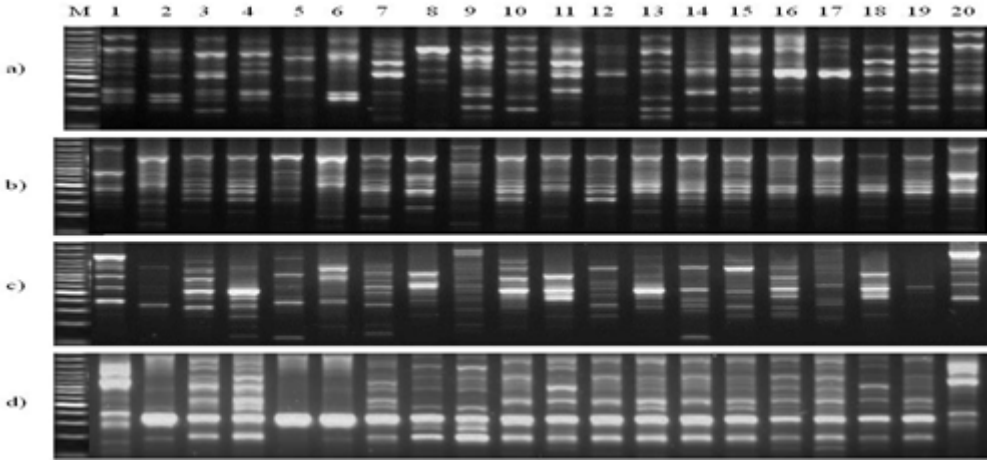

Fig. 2. RAPD profiles of twenty different varieties of *Philodendron* using primers OPA12 (a), OPA13 (b), OPA15 (c), OPD18 (d). M: Molecular weight marker (1000 bp DNA ladder), Lane 1: *P. xanadu*, 2: *P. panduriforme*, 3: Moonshine, 4: Tropical Sunrise (hybrid), 5: Kerala Hybrid (hybrid), 6: *P. laciniatum*, 7: *P. Ceylon*, 8: *P. selloum* var. Gold (hybrid), 9: *P.selloum*, 10: *P. Emerald Duke*, 11: Red Emerald (hybrid), 12: *P. wendlandii*, 13: Royal Queen (hybrid), 14: Pluto (Hybrid), 15: Black Cardinal (hybrid), 16: *P. deflexum*, 17: Green Emerald (hybrid), 18: Painted lady (hybrid), 19: Pink princes (hybrid), 20: Golden Xanadu (hybrid).

The DNA fragments ranged from 100 to 1500 bp with a majority of polymorphism. The level of polymorphism from this study is corroborated to that of 43 *Philodendron* varieties and five aroid genera, where AFLP technique was used to measure the genetic diversity (63% polymorphism). Furthermore Chen *et al*. (2004a, 2004b) reported 69% polymorphism in *Aglaonema* spp. and 71% polymorphism in *Dieffenbachia* spp., the two other aroid genera.

As expected, genetic distances between the species varieties were comparatively low. Eu-

Table 3. Euclidean distance metric based on RAPD marker data showing the relationships between *Philodendron* species.

	Green xanadu	*p.panduriform*	Moonshine	Tropical Sunrise	Kerala Hybrid	Lacinatum	Ceylon	slm var. Gold	*p. selloum*	Emerald Duke	Red Emerald	wendlandii	Royal Queen	Pluto	Black Cardinal	Deflexum	Green Emerald	Painted lady	Pink princes	Golden Xanadu
Green xanadu	0																			
p.panduriform	10	0																		
Moonshine	10	9.7	0																	
Tropical Sunrise	11	9.9	7.3	0																
Kerala Hybrid	10	9.2	9.9	9.9	0															
Lacinatum	11	9.4	9.6	9.8	9.7	0														
Ceylon	11	9.8	9.7	9.9	10	9.7	0													
slm var. Gold	10	9.7	9.8	11	9.7	10	10	0												
p. selloum	9.9	11	9.4	10	11	**10**	11	9.7	0											
Emerald Duke	11	9.8	8.8	9.3	10	10	9.8	10	10	0										
Red Emerald	11	9.6	9.2	9.5	11	10	10	10	11	8	0									
wendlandii	10	9.6	8.9	9.2	10	10	9.9	10	10	8.1	7.9	0								
Royal Queen	11	9.9	8.9	9.3	10	10	10	9.7	11	7.9	7.9	7.8	0							
Pluto	11	9.8	9.5	9.6	9.9	9.5	10	10	11	8.3	8.4	8.3	8.1	0						
Black Cardinal	11	9.9	8.8	9.1	10	10	11	9.8	11	8.1	8.2	8.5	7.1	7.9	0					
Deflexum	11	11	9.6	9.3	11	10	10	10	11	8.9	8.4	7.8	8.9	9.6	8.8	0				
Green Emerald	11	10	10	9.9	11	10	10	10	11	9.4	9.7	8.8	9.7	9.5	9.5	8.7	0			
Painted lady	11	9.5	90	9.2	10	11	10	9.8	11	8.4	6.2	8.1	8.1	8.4	8.1	8.1	9.8	0		
Pink princes	11	9.9	9	9.2	10	10	9.8	10	11	7.9	8	7.7	6.9	8.4	7.6	8.4	9	7.6	0	
Golden Xanadu	8.9	10	11	11	9.7	11	11	10	11	10	10	10	10	10	10	11	11	9.9	10	0

clidean distance metrics estimates of distance for 21 varieties ranged from 7.6 to 11 (Table 3). In fact most of them are assorted 10 to 11.

Genetic relationship among the varieties

A dendrogram was constructed for 20 *Philodendron* varieties based on UPGMA using squared euclidean distance matrix (Fig. 3). The generated three major clusters and revealed maximum linkage distance of 108 units. Cluster I, II, and III covered a linkage distance of 108, 102 and 90 units, respectively. The first cluster contains *Philodendron xanadu* varieties viz. green xanadu, golden xanadu and P. *bipinnatifidum* varieties viz. selloum and selloum gold. They are native to Brazil, multi-lobed and self heading. However *P. xanadu* was referred as miniature of *P. selloum* (Devanand *et al.,* 2004), but there is an entirely different plant also called miniature *P. selloum,* which is in fact a unique natural variation of P. *bipinnatifidum*, however, that plant is rare (Croat *et al*., 2002). These two species were differentiated with spathe and spadix. The spathe of *P. xanadu* is dark violet-purple bordering on red and the spathe of *P. bipinnatifidum* is basically green with a white interior. In fact P. bipinnatifidum (syn. *P. selloum*), is one of the world's most ubiquitous houseplants which has provided a rich source of variation for breeding different foliage forms.

However in our study grouping of these species in first cluster infers there is close genetic similarity between species and they may be rather genetically heterozygous.

3 The cluster II contains four varieties and all are scandent (climbing) vine. 'Kerala hybrid with pale green color, ovate leaves, *P. ceylon* with yellow color, ovate leaves, *P. laciniatum* and *P. panduriforme* with light green and dark green lobed leaves respectively. The genetic distance among them ranges 9.7 to 10 (Table 3). This indicates that they are not genetically distant, even though they are genetically heterozygous.

Cluster III contains twelve varieties and most of them are climbers except four varieties which are self headers. As estimated in the distance matrix, cluster analysis placed most of the varieties close to each other showing a high level of genetic relatedness. Among climbers and self heading varieties, cluster generally corresponds to color and shape of the leaf as well as plants growth habit. For example tropical sunrise and moonshine are grouped together with a genetic distance of 0.73 and both are self header, stout or almost no stems with bright red new growth. Black Cardinal is a hybrid developed from a series of crosses involving *P. wendlandii, P. domesticum, P. erubescens, P. imbe*, P. *fragrantissimum* (Devanand *et al.*, 2004) and is grouped with 'Pink prices

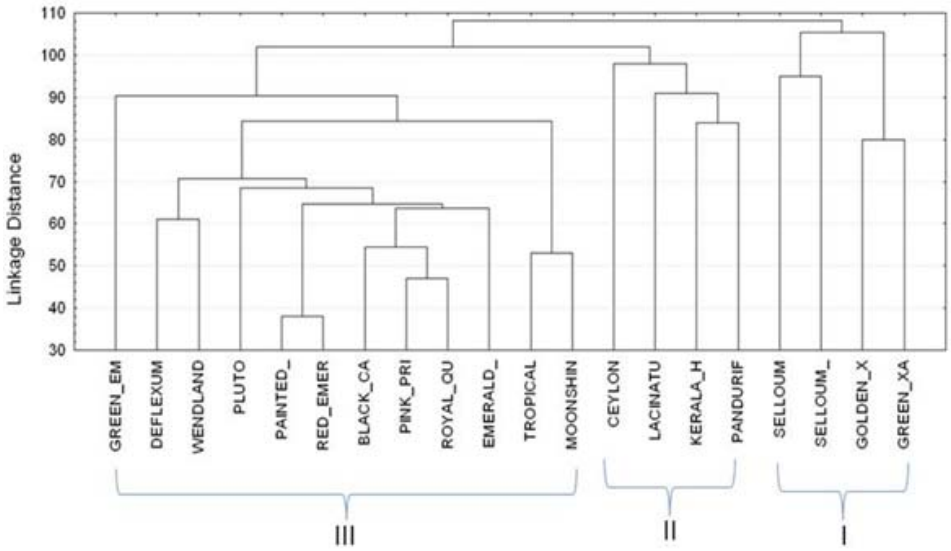

Fig. 3. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram showing the genetic relationships among the 20 *Philodendron* varieties using RAPD markers.

(dark leaves and light pink variegated blotches)', 'Emarld Duke (deep green leaves)' and 'Royal Queen' (deep maroon foliage)'. The grouping of these hybrids could be due to similarity of morphological characteristics as well as narrow genetic base, furthermore it is supported with Euclidean distance range 7.1 to 8.1(Table 3).

In general agreement, our RAPD-based analysis categorize the three clusters based on varietal growth habit and origin, indicating that, there is an association between the RAPD patterns and the geographic origin of the varieties used, despite more polymorphism (Fig. 2, Table 2). However, more genetic polymorphism would infer most of the *Philodendrons* are open pollinated and propagated by vegetative means and hence species might be extremely heterozygous. Moreover, somaclonal variation is another additional factor for the addition of new varieties, since plants have been cloned heavily through tissue culture. Furthermore, *Philodendron* species are known to be highly variable and not every leaf of every species will always appear the same and size is also not a determining factor when it comes to determining the species. For example the size of any morphological structure in *Anthurium* is highly variable because plants often change remarkably in size over the course of their lives and also in response to the general climatic conditions where they occur, particularly in geographically widespread species (Croat *et al*., 2002).

This study reveals the ability of RAPD markers to reliably differentiate between different *Philodendron* varieties and their use as a tool for the estimation of genetic diversity. The information about genetic similarity will be helpful to avoid the chances of using genetically similar landrace/genotypes and will also be helpful in future breeding programs to select genetically diverse parents for *Philodendrons.*

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