Identification and Genetic relationship among *Polyscias* and *Schefflera* (Araliaceae) using RAPD and ISSR markers

Gyana Ranjan Rout*, Joyti Kullu, Sunil Kumar Senapati, Subhashree Aparajita, Anuradha Mohapatra

Plant Biotechnology Division, Regional Plant Resource Centre, Bhubaneswar- 751 015, India *E-mail: grrout@rediffmail.com Tel: +91-674-2550274 Fax: +91-674-2550274

Received September 27, 2006; accepted June 21, 2007 (Edited by D. Shibata)

Abstract The species/varieties of *Polyscias* and *Schefflera* are important ornamental foliage pot plants. The germplasm identification and characterization is an important link between the conservation and utilization of plant genetic resources. Investigation were undertaken for identification and determination of genetic variation within 14 species/varieties of *Polyscias* and one species of *Schefflera elegantissima* under family Araliaceae through RAPD (random amplified polymorphic DNA) and ISSR (Inter Simple Sequence Repeats) markers. Genetic analysis was made by using 15 selected decamer primers and 9 selected ISSR markers. A total of 164 and 69 distinct DNA fragments ranging from 300 to 2500 bp were amplified by using selected random RAPD and ISSR primers respectively. The genetic similarity was evaluated on the basis of presence or absence of bands. The cluster analysis was made by using similarity coefficient. The cluster analysis indicated that the 14 varieties/species of *Polyscias* formed one major cluster and *Schefflera elegantissima* forming another major cluster. There were distant variation among the genus or species and close variation was obtained among the varieties of *Polyscias*. The correlation matrix indicates that there was significant correlation between ISSR and RAPD markers. Thus, these markers have the potential for identification of species/varieties and variation within the varieties. This is also helpful in breeding programs as well as a major input into conservation biology of foliage crop.

Key words: Foliage crop, ISSR marker, RAPD markers.

The genus Polyscias and Schefflera belongs to family Araliaceae and about 80 species are distributed in tropical regions of Africa, Asia and the Pacific. The species/varieties of Polyscias and Schefflera are useful foliage plants suitable for growing in semi-shade in ground and in pot. They are propagated by nodal cuttings or separation of rhizomes depending upon the species or varieties. A large number of varieties and mutants are grown in many parts of the world as well as Indian subcontinent. Taxonomically, the species/varieties under family Araliaceae has complicated form with two tribes i.e. Aralieae (characterized by imbricate petals) and Schefflerieae (characterized by valvate petals). Number of varieties with spectacular foliage has been evolved but they are not properly documented. During the past decade, morphological characters, growth habit and floral morphology have been used for classification and identification of plants. Taking into account the utility, the conservation of genetic diversity and building up of nuclear base populations is essential for improvement of foliage plants. The most important role of conservation is to preserve genetic variation and evolutionary process in viable populations of ecologically and commercially

important species in order to prevent potential extinction. The molecular approach for identification of plant species/varieties seems to be more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants (Williams et al. 1990; Paterson et al. 1991). Molecular markers like RAPD and ISSR have been widely used in many plant species for identification, varieties analysis, population studies and genetic linkage mapping (Williams et al. 1990; Rout et al. 2003; Mohapatra and Rout 2005; Barik et al. 2006). In this communication, we report first time the identification and genetic variation among the species/varieties of Polyscias and Schefflera elegantissima under family Araliaceae by using RAPD and ISSR markers.

Fourteen varieties/species of *Polyscias* (*Polyscias* 'Palapala' [*P. balfouriana* Bailey×*P. guilfoylei* (W. Bull.) Bailey], *Polyscias fruticosa* (Linn.) Harms. 'Acc-1', *Polyscias fruticosa* (Linn.) Harms., *Polyscias fruticosa* (Linn.) Harms 'Acc-2', *Polyscias paniculata* Baker, *Polyscias balfouriana* Bailey. 'Albicans', *Polyscias balfourina* Bailey. 'Pennockii', *Polyscias guilfoylei* (W.

This article can be found at http://www.jspcmb.jp/

Bull.) Bailey. 'Victoriae', *Polyscias guilfoylei* (W. Bull.) Bailey. 'Crispa', *Polyscias guilfoylei* (W. Bull.) Bailey. 'Quinquefolia', *Polyscias filicifolia* (Ridley) Bailey. Syn. *Nothopanax filicifolia* (Ridley) Lindl., *Polyscias filicifolia* (Ridley) Bailey. 'Acc-1', *Polyscias guilfoylei* (W. Bull.) Bailey. 'Laciniata', *Polyscias scutellaria* (Burm. f.) Fosberg. and one species of *Schefflera elegantissima* (Veitch. Ex Mast.) Lowey & Frodin Syn. *Dizygotheca elegantissima* (Veitch. Ex Mast.) Vig. & Guill. were collected from the garden of Regional Plant Resource Centre, Bhubaneswar, India. One species *Schefflera elegantissima* was selected to study the tribe under family Araliaceae.

DNA was extracted from fresh leaves collected from nursery garden raised plants of different species/varieties by the CTAB method (Doyle and Doyle 1990). Approx. 200 mg of fresh leaves was ground to powder in liquid nitrogen using a mortar and pestle. The ground powder was transferred to a 50 ml falcon tube with 10 ml of cetyltrimethyl ammonium bromide (CTAB) buffer [2% (w/v) CTAB, 1.4 M Nacl, 20 mM EDTA, 100 mM Tris (tris(hydroxymethyl)aminomethane)-HCl, pH 8.0, and 0.2% (v/v) β -mercaptoethanol]. The homogenate was incubated at 60°C for 2 h, extracted with an equal volume of chloroform/isoamyl alcohol (24:1 v/v) and centrifuged at $10,000 \times q$ for 20 min (Kubota KR-2000 C, Rotor-RA-3R, Tokyo, Japan). DNA was precipitated from the aqueous phase by mixing with an equal volume of isopropanol. After centrifugation at $10,000 \times q$ for 10 min, the DNA pellet was washed with 70% (v/v) ethanol, air-dried and resuspended in TE (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA) buffer. DNA quantifications were performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel. The resuspended DNA was then diluted in TE buffer to $5 \,\mu g/\mu l$ concentration for use in polymerase chain reaction.

Forty 10-mer primers, corresponding to kits A, B, D, and N from Operon Technologies (Alameda, California, USA) and nine synthesized ISSR primers (M/S Bangalore Genei, Bangalore, India) were initially screened using fourteen species/varieties of *Polyscias* and one species of *Schefflera* to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species/varieties. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

Polymerase chain reactions (PCR) with single primer was carried out in a final volume of $25 \,\mu$ l containing 20 ng template DNA, $100 \,\mu$ M of each deoxyribonucleotide triphosphate, 20 ng of decanucleotide primer (M/S Operon Technology, Inc., Alameda, USA), $1.5 \,\text{mM} \,\text{MgCl}_2$, $1 \times \text{Taq}$ buffer (10 mM Tris-HCl [pH- 9.0], 50 mM KCl, 0.001% gelatin), and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, Bangalore, India). Amplification was performed in a PTC-100 thermal cycler (M J Research Inc., Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94°C, followed by 40 cycles of denaturation at 94°C for 20 s., annealing at 38°C for 30 s. and extension at 72°C for 1 min, finally at 72°C for 10 min for RAPD amplification. Amplification products were separated alongside a molecular weight marker by 1.2% agarose gel electrophoresis in 1×TAE (Tris Acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad, California, USA).

For ISSR study, the initial optimization of PCR was conducted including concentration of template DNA primer, MgCl₂, number of PCR cycle and annealing temperature. The PCR reaction had a total volume of 25 µl containing 20 ng templates DNA, 100 mM each dNTPs, 20 ng of oligonucleotides synthesized primer (M/S Bangalore Genei, Bangalore, India), 2.5 mM MgCl₂, $1 \times$ Tag buffer (10 mM Tris-HCl [pH 9.0], 50 mM KCl, 0.01% gelatin) and 0.5 U Taq DNA polymerase. DNA amplification was performed in a PTC -100 thermal cycler (M J Research Inc., Watertown, MA, USA) programmed for a preliminary 5 min denaturation step at 94°C, followed by 40 cycles of denaturation at 94°C for 20 s., annealing temperature depending on the synthesize primer (ranged from 32-56°C) for 30 s. and extension at 72°C for 45 s., finally at 72°C for 5 min. Amplification products were separated alongside a molecular weight marker (1.0 Kb plus ladder, M/S Bangalore Genei, Bangalore, India) by 1.2% agarose gel electrophoresis in 1× TAE (Tris Acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System and evaluated using the software Quantity one (BioRad, California, USA).

Data were recorded as presence (1) or absence (0) of band products from the examination of photographic. Each amplification fragment was named by the source of the primer, the kit letter or number, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity index was estimated using the Dice coefficient of similarity (Nei and Li 1979). The average of similarity matrices was used to generate a tree by UPGMA (Unweighted Pair-Group Method Arithmetic Average) using NTSYS-PC, version 2.0 (Rohlf 1995).

The present investigation offers an optimization of primer screening for evaluation of genetic relationship of

Name of primer	Sequence of the primer	Total number of amplification fragments	Number of polymorphic fragments			
OPA-03	5'-AGTCAGCCAC-3'	12	12			
OPA-05	5'-AGGGGTCTTG-3'	08	08			
OPA-08	5'-GTGACGTAGG-3'	07	06			
OPA-16	5'-AGCCAGCGAA-3'	09	08			
OPC-02	5'-GTGAGGCGTC-3'	11	09			
OPC-04	5'-CCGCATCTAC-3'	08	07			
OPC-08	5'-TGGACCGGTG-3'	12	12			
OPC-11	5'-AAAGCTGCGG-3'	10	10			
OPC-05	5'-GATGACCGCC-3'	13	12			
OPN-02	5'-ACCAGGGGGCA-3'	14	12			
OPN-04	5'-GACCGACCCA-3'	13	12			
OPN-08	5'-ACCTCAGCTC-3'	09	09			
OPD-20	5'-ACCCGGTCAC-3'	15	15			
OPD-03	5'-GTCGCCGTCA-3'	12	11			
OPD-09	5'-CTCTGGAGAC-3'	11	09			

Table 1. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD primers in 14 species/varieties of *Polyscias* and *Schefflera elegantissima*.

14 species/varieties of Polyscias and Schefflera elegantissima through RAPD and ISSR markers. P. guilfoylei 'Crispa' was used for screening primers obtained from different series for amplification by using polymerase chain reactions. The results showed that N- and C-series primers produced relatively more amplification fragments compared to A- and D-series decamer primers. The amplification generated by primers OPN-02, OPN-04 and OPD-20 produced maximum number of DNA fragments; the size of the DNA fragments ranged from 300 to 2500 base pairs. Primer OPD-20 amplified 15 fragments whereas, OPN-02 produced 14 bands in P. guilfoylei 'Crispa'. It was also noted that some primers did not show any amplification by using the variety Crispa. The nineteen decamer primers produced good amplification of RAPD fragments. Among the nineteen primers, fifteen primers were selected to analyze the genetic relationship among the 14 species/varieties of Polyscias and Schefflera RAPD elegantissima through markers. The reproducibility of the amplification product was tested with three independent extraction. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. As a result, fifteen informative primers were selected and used to evaluate the degree of polymorphism within 14 species/varieties of Polyscias and one species of Schefflera elegantissima and also to study the tribe under family Araliaceae. The maximum and minimum number of bands were produced by the primers OPD-20 and OPA-08 respectively (Table 1). A



Figure 1. RAPD pattern of different species/varieties of *Polyscias* (1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14,15) and *Schefflera elegantissima* (5) by using OPC-02 (A) and OPN-02 (B) decamer primers.

total of 164 amplified fragments was scored across the 14 varieties/species of Polyscias and one species of Schefflera for the selected primers, and was used to estimate genetic relationships among themselves. The patterns of RAPD produced by the primers OPC-02 and OPN-02 are shown in the Figures 1A, B. The genetic variation through molecular markers has been highlighted in a number of ornamental foliage crops (Wen et al. 2001; Chen et al. 2004a, b; Phang et al. 1999). Chen et al. (2004b) indicated that the development of different cultivars of Dieffenbachia were originated from either in sports or somaclonal variants. The present findings show the distant variation among the species and close variation among the varieties by using RAPD markers. The similarity matrix was obtained after multivariate analysis using Nei and Li's

Table 2. Similarity Co-efficient of 14 species/varieties of Polyscias and Schefflera elegantissima by using RAPD primers

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
A1	1.00														
A2	0.95	1.00													
A3	0.78	0.81	1.00												
A4	0.60	0.61	0.62	1.00											
A5	0.52	0.51	0.47	0.56	1.00										
A6	0.77	0.76	0.71	0.71	0.48	1.00									
A7	0.76	0.77	0.70	0.62	0.39	0.75	1.00								
A8	0.79	0.80	0.73	0.61	0.42	0.84	0.89	1.00							
A9	0.77	0.80	0.71	0.65	0.42	0.82	0.77	0.78	1.00						
A10	0.80	0.81	0.76	0.66	0.43	0.89	0.82	0.91	0.81	1.00					
A11	0.70	0.71	0.70	0.52	0.43	0.73	0.78	0.83	0.69	0.76	1.00				
A12	0.85	0.88	0.85	0.65	0.53	0.74	0.75	0.78	0.74	0.79	0.73	1.00			
A13	0.81	0.76	0.75	0.63	0.55	0.70	0.69	0.72	0.66	0.71	0.69	0.80	1.00		
A14	0.69	0.74	0.63	0.55	0.38	0.76	0.69	0.68	0.84	0.71	0.67	0.70	0.60	1.00	
A15	0.75	0.72	0.63	0.57	0.51	0.74	0.75	0.84	0.62	0.75	0.75	0.70	0.74	0.60	1.00

P1, Polyscias 'Palapala'; P2, P. fruticosa 'Acc-1'; P3, P. fruticosa; P4, P. fruticosa 'Acc-2'; P5, Schefflera elegantissima; P6, P. paniculata; P7, P. balfouriana 'Albicans'; P8, P. balfouriana 'Pennockii'; P9, P. guilfoylei 'Victoriae'; P10, P. guilfoylei 'Crispa'; P11, P. guilfoylei 'Quinquefolia'; P12, P. filicifolia; P13, P. filicifolia 'Acc-1'; P14, P. guilfoylei 'Laciniata'; P15, P. scuttellaria



Figure 2. Dendrogram of cluster analysis by using RAPD markers. The Scale indicates the fractional similarities among the 14 species/varieties of *Polyscias* and one species of Schefflera *elegantissima*. (Multivariant analysis using Nei and Li's Coefficient)

P1, Polyscias 'Palapala'; P2, *P. fruticosa* 'Acc-1'; P3, *P. fruticosa*; P4, *P. fruticosa* 'Acc-2'; P5, *Schefflera elegantissima*; P6, *P. paniculata*; P7, *P. balfouriana* 'Albicans'; P8, *P. balfouriana* 'Pennockii'; P9, *P. guilfoylei* 'Victoriae'; P10, *P. guilfoylei* 'Crispa'; P11, *P. guilfoylei* 'Quinquefolia'; P12, *P. filicifolia*; P13, *P. filicifolia* 'Acc-1'; P14, *P. guilfoylei* 'Laciniata'; P15, *P. scuttellaria*

coefficient and is presented in Table 2. The similarity matrix was then used to construct a dendrogram with the UPGMA method (Figure 2). The dendrogram shows two major clusters within 14 species/varieties of *Polyscias* and one species of *Schefflera*. Among the two major clusters, one major cluster had only one species (*Schefflera elegantissima*) and other major cluster divided into two minor clusters. First minor cluster had only one species i.e. *P. frutocosa* 'Acc-2' and second minor cluster having 13 varieties/species of *Polyscias*. Second minor cluster again divided into two sub-minor clusters; first sub-minor cluster having two varieties i.e. *P. guifoylei* 'Victoriae' and *P. guifoylei* 'Laciniata' with 85% genetic similarity among each other. Second subminor cluster having 11 varieties/species of *Polyscias* and divided into two groups. First group having six varieties and second group having five varieties/species of *Polyscias*. In the first group, *P. balfouriana* 'Pennockii' and *P. guilfoylei* 'Crispa' having 90% genetic similarity with each other and 86% similarity with *P. paniculata* and also 81% similarity with *P. balfouriana* 'Albicans' *P. guilfoylei* and *P. scuttellaria* having 74% and 76% similarity with *P. balfouriana* 'Albicans'. In the second group, *P. fruticosa* has closely resemblance with *Polyscias* 'Palapala', a hybrid of *P. balfouriana*×*P. guilfoylei* with 96% similarity and also 85% and 81% similarity with *P. filicifolia* and *P. fruticosa* respectively. *P. filicifolia* 'Acc-1' was also 76% similarity

Primer Code Primer sequence	Annealing temperature (°C)	Total no. of amplified products	No. of polymorphic products	Pölymorphic (%)
C1				
AGGGCTGGAGGAGGGC	56	09	09	100
G2				
AGAGGTGGGCAGGTGG	54	08	08	88.8
Т3				
GAGGGTGGAGGATCT	48	09	08	88.8
G4				
ACTGACTGACTGACTG	48	05	05	100
A5				
GACAGACAGACAGACA	48	07	07	100
A6				
GACAGATAGACAGATA	44	03	03	100
G7				
ACAGACAGACAGACAG	48	06	06	100
G8				
CAGCGACAAG	32	11	10	90.9
A9				
TCGTTCCGCA	32	11	08	72.7

Table 3. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected ISSR markers in 14 species/varieties of *Polyscias* and *Schefflera elegantissima*.

with *P. fruticosa*. The similarity matrix showed that the lowest and highest values were 0.39 and 0.95 among the 14 species/varieties of *Polyscias* along with one species of *Schefflera*.

The investigation was also made by using nine ISSR markers. A total of 69 ISSR fragments of which 64 are polymorphic (Table 3). The patterns of ISSR profile produced by the synthesis primers are shown in the Figure 3A, B. The present result indicates that there were distant variation among the genus Polyscias and Schefflera and also species level. However, close variation was occurred among the varieties. The similarity matrix was obtained after multivariate analysis using Nei & Li's coefficient and is presented in Table 4. A dendrogram was constructed using the unweighted pair group method of arithmetic average analysis. The 14 verities/species of Polyscias and one species of Schefflera were divided into two major clusters; first major cluster having only one species i.e. Schefflera elegantissima as a out group and second major cluster comporises 14 varieties/species of Polyscias and with 51% similarity between the two clusters (Figure 4). Second major cluster again sub-divided into two minor clusters; first minor cluster having only one varieties i.e. Pfruticosa 'Acc-2". Second minor cluster comporises 13 varieties/species with 62% similarity among the two minor clusters. Second minor cluster are divided into two sub-minor clusters; first sub-minor cluster having eight varieties/species and second having five varieties/species with 72% similarity among themselves. In second subminor cluster, again divided into two groups; one having two varieties/species with 94% similarity i.e. Polyscias 'Palapala' [a hybrid of *P. balfouriana* 'Albicans' $\times P$. guilfoylei] and P. fruticosa 'Acc-1'. Another group



Figure 3 ISSR pattern of different species of *Polyscias* (1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14,15) and *Schefflera elegantissima* (5) by using synthesis primer (5'-GACAGACAGACAGACAGACA-3') (A) and (B) (5'-CAGCGACAAG-3')

having three varieties [*P. fruticosa*, *P. filicifolia* and *P. filicifolia* 'Acc-1'] with 80% similarity among themselves. In the first sub-minor cluster further divided into two groups; one group having five varieties/species (i.e. *P. paniculata*, *P. guilfoylei* 'Crispa', *P. guilfoylei* 'Laciniata', *P. guilfoylei* 'Quinquefolia' and *P. guilfoylei* 'Victoriae'). *P. guilfoylei* 'Quinquefolia' having 83% similarity with *P. guilfoylei* 'Crispa', 'Laciniata' and 'Victoriae'. In all these cases, the leaves are irregular boundaries. Another group having three species/varieties (i.e. *P. balfouriana* 'Albicans', *P. scuttellaria* and *P. balfouriana* 'State (i.e. *P. balfouriana*, and *P. balfouriana*).

Table 4. Similarity Co-efficient of 14 species/varieties of Polyscias and Schefflera elegantissima by using ISSR primers.

	P1	P2	P3	P4	P5	P6	P7	P8	Р9	P10	P11	P12	P13	P14	P15
P1	1.00														
P2	0.93	1.00													
P3	0.81	0.76	1.00												
P4	0.66	0.70	0.61	1.00											
P5	0.57	0.52	0.49	0.49	1.00										
P6	0.76	0.72	0.78	0.66	0.51	1.00									
P7	0.76	0.69	0.72	0.66	0.51	0.82	1.00								
P8	0.73	0.66	0.72	0.60	0.51	0.82	0.94	1.00							
P9	0.81	0.76	0.73	0.70	0.43	0.90	0.81	0.78	1.00						
P10	0.76	0.75	0.81	0.69	0.45	0.91	0.82	0.82	0.87	1.00					
P11	0.70	0.63	0.72	0.63	0.54	0.85	0.79	0.76	0.81	0.85	1.00				
P12	0.75	0.73	0.79	0.61	0.55	0.72	0.69	0.66	0.64	0.75	0.72	1.00			
P13	0.79	0.72	0.81	0.54	0.48	0.76	0.70	0.70	0.69	0.73	0.67	0.81	1.00		
P14	0.78	0.70	0.82	0.61	0.49	0.90	0.84	0.84	0.85	0.87	0.78	0.70	0.81	1.00	
P15	0.75	0.67	0.73	0.55	0.55	0.75	0.84	0.90	0.67	0.75	0.69	0.67	0.75	0.82	1.0

P1, Polyscias 'Palapala'; P2, P. fruticosa 'Acc-1'; P3, P. fruticosa; P4, P. fruticosa 'Acc-2'; P5, Schefflera elegantissima; P6, P. paniculata; P7, P. balfouriana 'Albicans'; P8, P. balfouriana 'Pennockii'; P9, P. guilfoylei 'Victoriae'; P10, P. guilfoylei 'Crispa'; P11, P. guilfoylei 'Quinquefolia'; P12, P. filicifolia; P13, P. filicifolia 'Acc-1'; P14, P. guilfoylei 'Laciniata'; P15, P. scuttellaria



Figure 4. Dendrogram of cluster analysis by using ISSR markers. The Scale indicates the fractional similarities among the 14 species/varieties of *Polyscias* and one species of *Schefflera elegantissima*. (Multivariant analysis using Nei and Li's Coefficient) P1, *Polyscias* 'Palapala'; P2, *P. fruticosa* 'Acc-1'; P3, *P. fruticosa*; P4, *P. fruticosa* 'Acc-2'; P5, *Schefflera elegantissima*; P6, *P. paniculata*; P7, *P.*



Figure 5. Co-relation between the matrix values of RAPD and ISSR markers generated among 14 species/varieties of *Polyscias* and one species of *Schefflera elegantissima*.

'Pennockii'. *P. balfouriana* 'Albicans' and *P. balfouriana* 'Pennockii' having 96% similarity in both morphologically as well as molecular level. Morphologically, they are busy in nature, leaves are large, leathery, periphery of the leaves is slightly toothed and leaves are provided with more or less creamy white margin. The minimum and maximum similarity coefficients among the 14 varieties/species of *Polyscias* and one species of *Schefflera* are 0.42 and 0.95 respectively. The correlation matrix indicate that there was significant correlation between RAPD and ISSR markers in 14 species/varieties of *Polyscias* and Schefflera elegantissima (Figure 5).

In conclusion, the result of this study indicate that the efficiency and ease of using RAPD and ISSR markers for investigating genetic relationship and identification of ornamental foliage plants. This information is helpful in breeding programs as well as a major input into conservation biology in foliage plant group.

Acknowledgement

The authors wish to acknowledge the help of Department of Forest and Environment, Government of Orissa for providing the laboratory facilities. The authors also thankful to Dr. P. C. Panda, Plant Taxonomy Division, Regional Plant Resource Centre for identification of plant.

References

Barik S, Senapati SK, Aparajita S, Mahapatra A, Rout GR (2006) Identification and genetic variation among *Hibiscus* species (Malavaceae) using RAPD markers. Z. Naturforschung 61C: 123–128

- Chen JJ, Devanand PS, Norman DJ, Henny RJ, Chao CCT (2004a) Genetic relationships of *Aglaonema* species and cultivars inferred from AFLP markers. *Ann Bot* 93: 157–166
- Chen JJ, Henny RJ, Norman DJ, Devanand PS, Chao CCT (2004b) analysis of genetic relatedness of *Dieffenbachia* cultivars using AFLP markers. *J Amer Soc Hort Sci* 129: 81–87
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15
- Mohapatra A, Rout GR (2005) Identification and analysis of genetic variation among rose cultivars using random amplified polymorphic DNA. Z Naturforschung 60C: 611–617
- Nei M, Li WH (1979) Mathematical modes for studying genetic variation in terms of restriction endonuclease. *Proc Natl Acad Sci USA* 76: 5269–5273
- Paterson AH, Tanksley SD, Sorreis ME (1991) DNA markers in plant improvement. *Adv Agron* 46: 39–90
- Phang LJ, Kiew R, Kee A, Huat GL, Yuen GY, Loh JP, Gan LH, Gan YY (1999) Amplified fragment length polymorphism (AFLP) provides molecular markers for the identification of *Caladium* bicolour cultivars. *Ann Bot* 84: 155–161
- Rohlf FJ (1995) NTSYS-PC Numerical taxonomy and multivariate analysis system. Version 1.80, Exeter Software, Setauket, New York
- Rout GR, Bhattacharya D, Nanda RM, Nayak S, Das P (2003) Evaluation of genetic relationships in *Dalbergia* species using RAPD markers. *Biodiversity and Conservation* 12: 197–206
- Wen J, Plunkett GM, Mitchell AD, Wagstaff SJ (2001) The Evolution of Araliaceae: A Phylogenetic analysis based on ITS sequences of nuclear ribosomal DNA. *Systematic Bot* 26: 144–167
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by primers are useful as genetic markers. *Nuclic Acids Res* 18: 6531–6535