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Short communication

Genetic diversity of *Kaempferia siphonantha* King. ex Baker: An indigenous medicinal plant of Andaman and Nicobar Islands

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Kaempferia siphonantha King. ex Baker belongs to the family Zingiberaceae. In Andaman and Nicobar Islands Zingiberaceae is the largest family, comprising nearly 50 genera and 1,000 species. In Asia, the family comprises at least 52 genera and more than 1,500 species (Sirirugsa, 7), which are scattered throughout the tropical and subtropical regions. *K. siphonantha* is the only representative of the genus in this Island territory (Karthikeyan *et al*., 3). *K. siphonantha* as a shade-loving plant, having beautifully zoned patterns of silver, blue, black and shades of green makes this plant very attractive even without flowers. The plant possess horizontal rhizomatous rootstock grows luxuriantly on clay loam soil. It is stemless, perennial herb growing up to 20 cm tall with slender root fibres. White flowers attract attention as the lip is tined with purple patches at the distal end.

Kaempferia siphonantha plant samples were collected from various locations of Middle and North Andaman and South Andaman, districts *viz.,* Manjeri (A1), Chidiyatapu, (A2), Chouldari (A3), Little Andaman (A4), Betapur (A5), Mayabunder (A6), Togapur (A7), Wandoor (A8), Guptapara (A9) and Manpur (A10) of Bay Islands. The collected plants were identified morphologically with the help of Botanical Survey of India, Port Blair. The plants are maintained in the glasshouse at Central Agricultural Research Institute, Port Blair for further studies. Genetic polymorphism in plants has been documented by use of various fingerprinting systems including the analysis of isozymes, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single sequence repeats (SSR), and random amplified polymorphic DNA (RAPD). PCR based RAPD markers were widely used to study genetic diversity in many species (Elanchezhian *et al*., 1).

Genomic DNA was isolated from young leaves using CTAB method (Mandal *et al*., 4). The random amplification was performed using twenty five primers procured from Operon Technologies (Table 1). PCR amplification mix contained 10X reaction buffer with 1.5 mM $MgCl_2$ (2.0 µl), 10 mM dNTPs (1.0 µl), 1 unit *Taq* polymerase enzyme (Finnzymes, Finland), 1 µl each of forward and reverse primers (250 ng/µl) and 1 µl of template DNA (200 ng/µl) and the volume was made to 20 µl with sterile distilled water. The reactions were subjected to 94ºC for 1 min., followed 45 cycles of 94ºC for 1 min., 36ºC for 2 min. and 72ºC for 3 min., with a final extension at 72ºC for 5 min. The amplified products were separated on 1.2% agarose in 1X TAE buffer visualized using ethidium bromide staining and photographed in a gel documentation system (Vilber Lourmat, France). Only the primers which displayed reproducible, scorable and clear bands were considered for analysis. Similarity index (SI) was calculated based on Rf values (Molecular Analyst version 1.5) for individual primer. Similarity index of bands which were common between two plants was estimated using Dice's coefficient, a similarity matrix involving ten samples were generated with NTSYS-pc (Numerical Taxonomy System, Applied Biostatistics, Inc, New York, USA). A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetical Average (UPGMA).

The dendrogram was constructed based on similarity index showed distinct variability of the collected samples though morphologically they were similar and undividable (Fig. 1; Table 2). The dendrogram separated the accessions collected from ten different locations into two major groups having 50% similarity. Among the two major groups, the plant belongings to the upper group were collected from Manjeri (A1), Chidiyatapu, (A2), Chouldari (A3), Little Andaman (A4), Betapur (A5), Mayabunder (A6), Togapur (A7), Guptapara (A9), and Manpur (A10), while accessions belongs to the lower group consisted of only one accession Wandoor (A8), which was found distinct from other samples. Accessions of the upper group were grouped into two major sub-clusters, plants A1 and A3 in the upper cluster showed maximum 98% similar to each other. The lower sub-cluster consisting with sample A2 showed 92% similarity with plants A1 and A3, plant A9 had 87% similarity with A2, plant A5 showed 83% similarity with A9, plant A4 shows 75%

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Table 1. Primer sequences used for RAPD-PCR and number of amplified products/primer.

Sl. No.	Primer	Sequence (5´ - 3´)	Accession(s) showing amplification	Total No. of accession(s)
1	OPA - 01	CAGGCCCTTC	4	1
2	OPA - 02	TGCCGAGCTG	6,7,8	3
3	OPA - 03	AGTCAGCCAC	7,10	2
4	OPA - 05	AGGGGTCTTG	8	1
5	OPA - 06	GGTCCCTGAC	8,9	2
6	OPA - 09	GGGTAACGCC	8	1
7	OPA - 10	GTGATCGCAG	9	1
8	OPA - 11	CAATCGCCGT	7	1
9	OPA - 12	TCGGCGATAG	9	1
10	OPA - 13	CAGCACCCAC	8	1
11	OPA - 14	TCTGTGCTGG	8	1
12	OPF - 02	GAGGATCCCT	7	1
13	OPF - 03	CCTGATCACC	3,6	2
14	OPF - 04	GGTGATCAGG	10	1
15	OPF - 05	CCGAATTCCC	8	1
16	OPF - 06	GGGAATTCGG	5,8,9,10	4
17	OPF - 07	CCGATATCCC	4,6	2
18	OPF - 09	CCAAGCTTCC	5,6,8,10	4
19	OPF - 18	TTCCCGGGTT	8	1
20	OPF - 20	GGTCTAGAGG	5,8	2
21	OPE - 01	CCCAAGGTCC	6,7,8	3
22	OPE - 02	GGTGCGGGAA	8	1
23	OPE - 13	CCCGATTCGG	8	1
24	OPE - 14	TGCGGCTGAG	6,8	2
25	OPE - 18	GGACTGGAGA	3,6,7,8,10	5

similarity with A5, plant A7 shows 74% similarity with A4, plant A10 shows 71% similarity with A7, plant A6 shows 68% similarity with A10' respectively. Finally all the groups in the dendogram were identified bearing least similarities among them.

The RAPD method used in this study projected appreciable intra-population variation, which pre-existed in different collection. In spite of their morphological identity, substantial polymorphism was observed among the plants under this study (Welsh and McClelland, 8) The RAPD profiles in individual lines were found to be different from each other and ample polymorphism was apparent in respect of different oligonucleotide primers used in this study. The genome amplified by different primers remains extremely less, the probability extent of polymorphism to be high. The dendrogram also displayed genetic variation among different accessions and considerable changes that occurred in the genome in the processes of evolution (Padmesh *et al.*, 5; Sangwan *et al.*, 6) The study confirmed that the suitability of RAPD is a reliable tool and easy to perform molecular diagnose of different medicinal

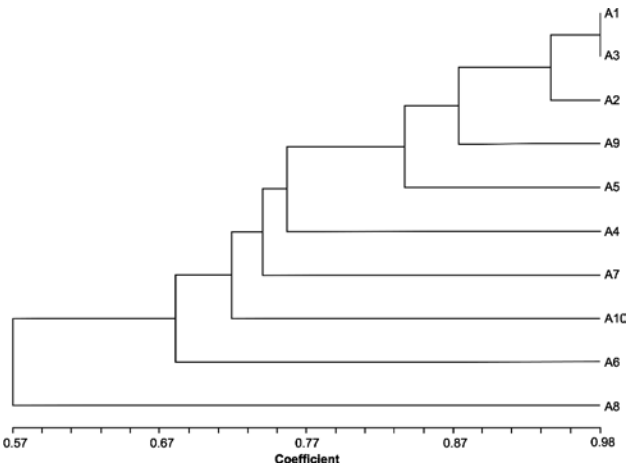

Fig. 1. Dendrogram showing the genetic relationship among the collections of *K. siphonantha*.

Table 2. Similarity matrix among the *K. siphonantha* collections.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
A1	1.000									
A2	0.931	1.000								
A3	0.977	0.954	1.000							
A4	0.795	0.772	0.818	1.000						
A5	0.863	0.840	0.886	0.704	1.000					
A6	0.727	0.750	0.750	0.659	0.681	1.000				
A7	0.795	0.772	0.818	0.659	0.704	0.568	1.000			
A8	0.590	0.568	0.613	0.545	0.545	0.545	0.522	1.000		
A9	0.863	0.886	0.886	0.704	0.772	0.727	0.704	0.636	1.000	
A10	0.772	0.750	0.795	0.659	0.681	0.590	0.590	0.545	0.727	1.000

plant species of Bay Islands. RAPD proved that it is a useful tool in molecular profiling of different plants of *K. siphonanthsa* collected from various places of South, Middle and North Andaman (Jarret and Austin, 2). This study also proved that the entries which were found to be similar in taxonomical classification based on morphological characters; but have divergence at DNA level.

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