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BRIEF COMMUNICATION

Evaluation of genetic relationship in *Typhonium* species through random amplified polymorphic DNA markers

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Abstract

Studies were undertaken to identify genetic relationships in three species of *Typhonium* and to evaluate the genetic variance within populations of *Typhonium trilobatum*, *Typhonium roxburghii* and *Typhonium flagelliforme* by using random amplified polymorphic DNA (RAPD) markers. A total of 193 distinct DNA fragments ranging from 0.2 to 3.2 kb, were amplified using 22 selected random decamer primers. The cluster analysis indicated that the three species of *Typhonium* formed two clusters: the first one consisted of *T. trilobatum* and *T. roxburghii*, the second one was represented by *T. flagelliforme*. A maximum similarity of 63 % was observed in *T. trilobatum* and *T. roxburghii*. *T. flagelliforme* shared up to 43 % similarity with *T. trilobatum* and *T. roxburghii*. The closest genetic distance was obtained within populations of different *Typhonium* species.

Additional key words: *Araceae*, medicinal plant, RAPD.

The genus *Typhonium* (Araceae) is comprises of about 40 species of perennial, tuberous herbs, distributed widely in tropical and subtropical Asia, Australia and Pacific islands of North Africa (Wang and Yang 1996). Four common but confused species namely *T. blumei*, *T. trilobatum*, *T. roxburghii* and *T. flagelliforme* were critically studied by Nicolson and Sivadasan (1981). *T. trilobatum*, *T. roxburghii*, and *T. flagelliforme* are important medicinal plants.

Germplasm characterization is an important link between the conservation and efficient utilization of plant genetic resources. Morphological characters like growth habit, leaf morphology and floral characters have been used to define the taxonomic status. Molecular techniques help researchers not only to identify the authentication of the genotypes, but also in assessing and exploiting the genetic variability (Whitkus *et al*. 1994). Insights into the relative genetic diversity within populations of *Typhonium* species would be useful for the development of strategies for *ex situ* conservation of plant genetic resources. DNA fingerprinting of all the genetic resources of the medicinal plants is a necessity for generating a molecular database as well as to utilize the information in a systematic manner. A better understanding of the genetic relationship among genotypes and molecular markers can be useful for designing strategies for gene introgression and breeding programs to produce desired recombinant hybrid genotypes. The DNA fingerprinting generated by the polymerase chain reaction (PCR), using arbitrary primers, has provided information for the estimating genetic relationships in a number of plant species (Virk *et al*. 1995, Bai *et al*. 1997, Ortiz *et al*. 1997, Pal and Raychaudhuri 2003, Rout *et al*. 2003). This is the first report on genetic relationships and variability within and among *Typhonium* species based on RAPD markers.

The plants were collected from natural forest of Orissa and West Bengal of India and maintained in the greenhouse at Regional Plant Resource Centre, Bhubaneswar, India. The fresh leaf materials were

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Abbreviations: CTAB - cetyldimethyl ethylammonium bromide; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA; TE - Tris-EDTA buffer.

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collected from 10 individuals of *T. trilobatum* (L.) Schott., *T. roxburghii* Schott. and *T. flagelliforme* (Lodd.) and DNA was extracted from by the CTAB method (Doyle and Doyle 1990). DNA quantifications were performed by visualizing under UV radiation, after eletrophoresis on 0.8 % (m/v) agrarose gel. The resuspended DNA was then diluted in Tris-EDTA (TE) buffer to concentration 5 μg cm^{-3} for use in amplification reactions.

Fourty decamer primers, corresponding to kits A, B, C and D from *Operon Technologies* (Alameda, CA, USA) were initially screened using three species to determine the producibility 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 of *Typhonium*. To ensure reproducibility, the primers generating no, weak or complex patterns were discarded.

Fourty arbitrary 10-base primers (*Operon Technologies*) were used for polymerase chain reaction (PCR). Amplification reactions were performed in 0.025 cm^3 [0.002 cm^3 of 1.25 mM each of dNTP's], 15 ng of the primer, 1 × Taq polymerase buffer, 0.5 U of Taq DNA polymerase (*Genei*, Bangalore, India) and 20 ng of genomic DNA. DNA amplification was performed in a PTC-100 DNA Thermal Cycler (*M.J. Research*, Watertown, MA, USA) programmed for 45 cycles: 1st cycle of 3.5 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C; then 44 cycles each of 1 min at 94 °C, 1 min at 37 °C, 2 min at 72 °C followed by one final extension cycle of 7 min at 72 °C. Amplified products were electrophoresed in a 1.2 % (m/v) agarose (*Sigma*, St. Louis, USA) gels with 1 × TAE buffer, stained with ethidium bromide, and photographed under ultraviolet (UV) light. Gel photographs were scanned through a *Gel Doc System* (*Gel Doc. 2000, BioRad*, USA) and the amplification product sizes were evaluated using the *BioRad* software. The sizes of the amplification products were estimated using 100 bp to 3.0 kb ladder (*MBI Fermentas Inc.*, Amherst, USA). All the reactions were repeated in at least two independent experiments.

Data were recorded as presence (1) or absence (0) of band products from the examination of photographic negatives. 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. Each amplification fragment was named by the source of the primer (*Operon Technologies*), 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 indices were estimated using the Dice co-efficient of similarity (Nei and Li 1979). Cluster analysis was carried out on similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) using *NTSYS-PC* version *1.80* (Rohlf 1995).

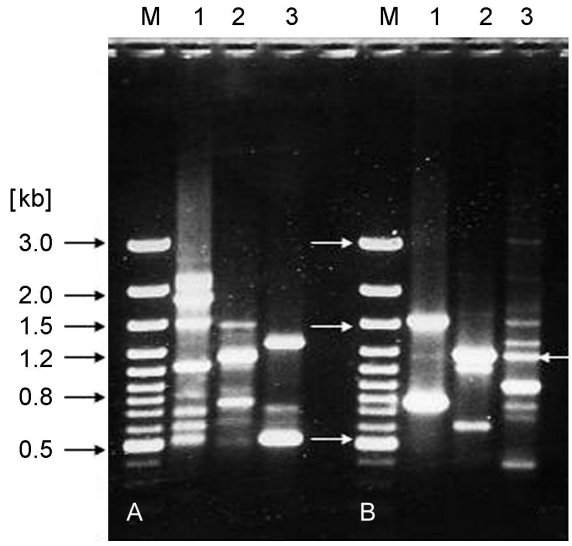

Fig. 1. RAPD patterns of three species of *Typhonium* regenerated by the primer OPA-09 (A) and OPA-14 (B). M - molecular mass ladder, 1 - *T. trilobatum*, 2 - *T. roxburghii*, 3 - *T. flagelliforme*.

The screening of primers resulted in 22 decamer primers which showed polymorphisms within the three species of *Typhonium* used. Eight primers showed polymorphism, but could not distinguish within the species; ten primers showed monomorphic bands within the species. These primers were selected and used to evaluate the degree of polymorphism within all the species of *Typhonium*. The selected primers generated distinctive products in the range of 0.2 to 3.2 kb. Maximum and minimum numbers of bands were produced by the primers OPA-09 (20) and OPD-06 (5), respectively (Table 1). A total of 193 amplified fragments were obtained across three species for the 22 selected primers, and were used to estimate relationships within the species. The patterns of RAPD fragments produced by the decamer primers OPA-09 and OPA-14 are presented in Fig. 1. Further analysis of these RAPD profiles for band similarity indices could clearly differentiate all the species of *Typhonium*. The similarity matrix obtained after multivariate analysis using Nei and Li (1979) coefficient is presented in Table 2. The results of the genetic similarity matrix indicated that *T. trilobatum* had about 63 % similarity with *T. roxburghii* and 43 % similarity with *T. flagelliforme*. The cluster analysis indicates that the three species of *Typhonium* formed two clusters. First cluster consisted of *T. roxburghii* and *T. trilobatum* and second cluster was represented by one specie *i.e. T. flagelliforme* (Fig. 2). The maximum similarity (63 %) obtained in *T. trilobatum* and *T. roxburghii* is in close association with their growth habit and taxonomical classification. *T. flagelliforme*

Table 1. Total number of amplified fragments and number of polymorphic bands generated by PCR using selected random decamers.

Primer	Sequence	Number of amplification fragments	Number of polymorphic bands	Size range [kb]
OPA-02	5`-TGCCGAGCTG-3`	11	6	0.5 - 2.5
OPA-09	5`-GGGTAACGCC-3`	20	8	0.5 - 3.0
OPA-14	5`-TCTGTGCTGG-3`	13	10	0.4 - 3.2
OPA-18	5`-AGGTGACCGT-3`	7	2	0.3 - 2.8
OPB-06	5`-TGCTCTGCCC-3`	6	1	0.5 - 2.4
OPB-09	5`-TGGGGGACTC-3`	8	2	0.3 - 2.0
OPB-12	5`-CCTTGACGCA-3`	7	2	0.4 - 1.8
OPB-13	5`-TTCCCCCGCT-3`	8	2	0.3 - 2.6
OPB-16	5`-TTTGCCCGGA-3`	8	4	0.3 - 3.1
OPB-18	5`-CCACAGCAGT-3`	10	6	0.6 - 2.8
OPB-20	5`-GGACCCTTAC-3`	9	4	0.8 - 2.0
OPC-05	5`-GATGACCGCC-3`	12	6	0.2 - 3.0
OPC-08	5`-TGGACCGGTG-3`	11	6	0.5 - 2.0
OPC-11	5`-AAAGCTGCGG-3`	9	4	0.6 - 2.6
OPC-14	5`-TGCGTGCTTG-3`	6	2	0.4 - 3.0
OPC-18	5`-TGAGTGGGTG-3`	7	1	0.2 - 2.8
OPD-06	5`-ACCTGAACGG-3`	5	1	0.4 - 2.8
OPD-10	5`-GGTCTACACC-3`	8	3	0.5 - 2.0
OPD-12	5`-CACCGTATCC-3`	7	2	0.5 - 3.2
OPD-14	5`-CTTCCCCAAG-3`	6	2	0.6 - 1.8
OPD-15	5`-CATCCGTGCT-3`	7	3	0.5 - 2.4
OPD-18	5`-GAGAGCCAAC-3`	8	2	0.6 - 3.0

Table 2. Similarity matrix (Nei and Li`s coefficient) of three species of *Typhonium*.

	T. trilobatum	*T. flagelliforme*	*T. roxburghii*
T. trilobatum	1.00		
T. flagelliforme	0.63	1.00	
T. roxburghii	0.43	0.46	1.00

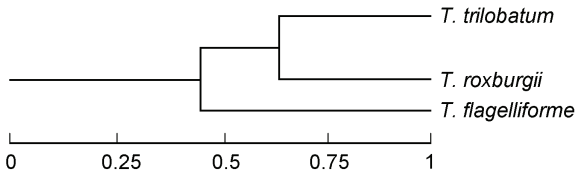

Fig. 2. Dendrogram of cluster analysis of RAPD markers. The scale indicates the fractional similarities among the species.

showed up to 43 % similarity with *T. trilobatum* and *T. roxburghii*. The dendrogram represented the close distances among two species and large distance with one species according to numerical taxonomy (Sneath and Sokal 1973). The DNA profiling in *Typhonium* species clearly showed that it was possible to analyse the RAPD patterns for correlation of their similarity and distance between species, by which one could predict the origin of the species to a great extent. The results indicated that the mean levels of genetic variation were low among the individuals of three *Typhonium* species (data not shown). The close levels of genetic similarity among the populations of *T. trilobatum*, *T. roxburghii* and *T. flagelliforme* were 78.8, 81.6 and 84.2 %, respectively. The differences in number of individuals estimated by RAPD markers in this study are similar to the result obtained by Rajaseger *et al.* (1997) in RAPD studies of the *Ixora coccinea* and *I. javanica*. They also found that the taxa-specific RAPD bands could be utilized to define the identification. It was observed that one species showed a good number of amplification bands in the primers OPA-09 and OPA-14, but another species showed a lesser number of amplified bands on the same primer (Fig. 1). Similarly, unique patterns were observed differentiating all three species from each other by using 22 decamer random primers. Thus RAPD markers can be used for differentiating *Typhonium* species and is helpful for conservation of germplasm and management of genetic resources (Brown and Kresovich 1996).

In conclusion, RAPD markers provide information on genetic relatedness among the *Typhonium* species. This investigation will provide an additional input to researcher for further study on molecular farming in medicinal plants.

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