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Full Length Research Paper

Molecular characterization of six populations of *Acorus calamus* L. using randomly amplified polymorphic DNA (RAPD) markers

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Studies were undertaken to identify genetic relationship in six different populations of *Acorus calamus* L. through randomly amplified polymorphic DNA (RAPD) markers. A total of 574 DNA fragments ranging from 281 to 1353 bp were amplified using 10 selected primers. The number of amplification products produced by a primer ranged from as low as six to a maximum of 13, with an average of nine bands per primer. The cluster analysis revealed three major clusters; the first cluster contained sample collected from Lucknow and Paonta Uttaralkand, India. The second cluster had single sample from Bangalore India and the third cluster contained sample collected from Solan, Nauni and Hissar, India. The similarity coefficient value ranged from 0.97 to 0.88. The highest similarity coefficient (0.97) was detected between samples collected from Nauni and Hissar as well as between Solan and Hissar and the lowest (0.88) was detected between the pairs Lucknow and Bangalore, Lucknow and Solan, Bangalore and Solan. The level of polymorphism in our study was very low which showed that samples used for analysis would have close relationship.

Key words: Randomly amplified polymorphic DNA (RAPD), similarity coefficient, polymorphism, *Acorus calamus*, primer.

INTRODUCTION

Acorus calamus L. (commonly known as sweet flag) is one of the highly valued medicinal and important aromatic herbs. It belongs to the family Acoraceae. The genus consists of two species, *A. calamus* and *Acorus gramineus* (Liao and Hsiao, 1998). It is a semi aquatic perennial herb with profuse underground stem and exhaustive root system. The plant flourishes on marshy land or near the perennial source of water in North America, Europe except Spain and Asia. In India, it naturally occurs at higher altitudes of Himachal Pradesh, Uttarakhand, Jammu and Kashmir and North Eastern States (Kapoor, 2001; Chauhan, 1999). The rhizome, roots and leaves have been used from ancient times in India for the treatment of bronchial diseases, indigestion, chronic diarrhea, epilepsy and as a repellent, toxicant and tranquillizer (Dev, 2006). The rhizome contains active ingredient, asarone which is found in three isomeric forms viz. alpha, beta and gamma asarone and possesses insecticidal, antifungal, antibacterial and allelopathic properties (Ravindran et al., 2004; Bertea et al., 2005).

Germplasm characterization is an important link between the conservation and efficient utilization of plant genetic resources. Morphological characters have been used extensively to study diversity of different forms in the past. In recent years, attempts to study biodiversity at molecular level have gained importance. 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). Randomly amplified polymorphic DNA (RAPD) has been extensively used to study diversity in different crops (Williams et al., 1990) Insights into the relative genetic diversity within populations of *A. calamus* would be useful for the development of strategies for *ex situ*

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Table 1. List of RAPD primers sequences used for the study.

S/N	Name of primer	Base sequence (5'-3')
1	OPA-4	AATCGGGCTG
2	OPA-7	GAAACGGGTG
3	OPA-10	GTGATCGCAG
4	OPA-11	CAATCGCCGT
5	OPA-12	TCGGCGATAG
6	Mosseler-31	CCGGCCTTCC
7	Mosseler-147	GTGCGTCCTC
8	Mosseler-156	GCCTGGTTGC
9	Mosseler-119	ATTGGGCGAT
10	Mosseler-191	CGATGGCTTT

conservation of plant genetic resources. DNA finger-printing 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 systemic 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 estimating the genetic relationships in a number of plant species (Bai et al., 1997; Ortiz et al., 1997; Pal and Raychaudhri, 2003; Rout et al., 2003; Virk et al., 1995). The present study reports the extent of diversity between different populations of *A.* calamus.

MATERIALS AND METHODS

The populations of *A. calamus* were collected from Lucknow (A-3), Hissar (A-4), Bangalore (A-6), Paonta (A-8), Solan (A-9) and Nauni (A-12) of India and maintained in the greenhouse at the Genetics and Tree Propagation Division, Forest Research Institute, Dehradun, Uttarakhand, India. Their average mean sea level is 869, 211, 898, 350, 350 and 3000 m, respectively. The fresh leaf materials were collected from five individuals of each population of six month old and DNA was extracted using the cetyltrimethylammonium bromide (CTAB) modified method of Stange et al. (1998). In this method, we used lithium chloride in the prewarmed extraction buffer with some additional steps like extraction which was done with chloroform and octanol (Ginwal and Mittal, 2010). The quality of DNA was checked on 1.0% (w/v) agarose gel containing ethidium bromide (0.5 µg/ml) at 90 V for 1 to 2 h and quantification was done by using Biophotometer (Eppendrof, Germany) and all the samples were brought down to a uniform concentration of 10 ng/µl to be used as template DNA for PCR. For PCR DNA and Mg^{2+}, concentration was standardized (Ginwal and Mittal, 2010). The isolated DNA from five individuals of each population was bulked uniformly and a composite sample representing the population was made. Ten decamer primers from Operon Company (QIAGEN Operon, 1000 Atlantic Avenue, Almeda, CA 94501, USA) and from Mosseler et al. (1992) were used as primers (Table 1). Tests were performed for standardizing PCR conditions and finally PCR amplification was carried out at 94°C for 3.5 min for initial denaturation, followed by 44 cycles of denaturation at 94°C for 1 min, primer annealing for 1 min at 37°C, extension at 72°C for 2 min and final extension at 72°C for 7 min. Reaction mixture (25 µl) contained 10 ng genomic DNA, 1X reaction buffer [100 mM Tris pH 9.0; 500 mM KCl; 0.1% gelatin, 2.0 mM $MgCl_2$, 200 µM (0.2mM) of each dNTPs (Bangalore Genei, Bangalore, India), 0.4 µM of each primer, 1 unit of Taq DNA polymerase (Bangalore Genei, Bangalore, India)]. The amplified products was separated by electrophoresis in 1.5% (w/v) agarose (Genei, Bangalore) gel with 1X TBE buffer, stained with ethidium bromide (0.5 µg /ml) at 90 V for 3.0 to 3.5 h and photographed using Gel Documentation system (UVP, USA). The sizes of the amplification product were estimated using 100 bp to 3.0 kb ladder (Φ × 174 DNA/ BsuRI (Hae III), fermentas. All the reactions were repeated in at least two independent experiments.

All the amplified bands were scored as present or absent for each DNA sample and further, the RAPD reaction results were analyzed using software Gene Profiler. In order to analyze the relatedness among the species, a dendrogram based on unweighted pair group method with arithmetic average (UPGMA) and NEI and Li genetic distance matrix (Nei and Li, 1979) value was obtained.

RESULTS AND DISCUSSION

Out of the twenty RAPD primers screened, ten gave reproducible and consistent amplification and out of these ten decamer primers, five decamer primers (OPA-7, OPA-12, OPA-11, M-119 and M-191) (Figure 1a and b) showed polymorphism, while the rest of the five primers showed monomorphism within the six populations of *A. calamus*. The details and nucleotide sequence of the RAPD primers is given in Table 1. The number of amplification products produced by a primer ranged from as low as six to a maximum of 13, with an average of nine bands per primer. The size of amplification products varied from 281 to 1353 bp. The maximum and minimum numbers of bands were produced by the primers OPA-10 (13) and M-31 (6), respectively. A total of 574 amplified fragments were obtained across six populations for the ten selected primers and were used to estimate relationship among them. The patterns of RAPD fragments produced by the decamer primer M-191 showed 2 distinct band in A-6, primer OPA-7 showed 1 extra band in A-6, primer OPA-12 showed 1 extra band in

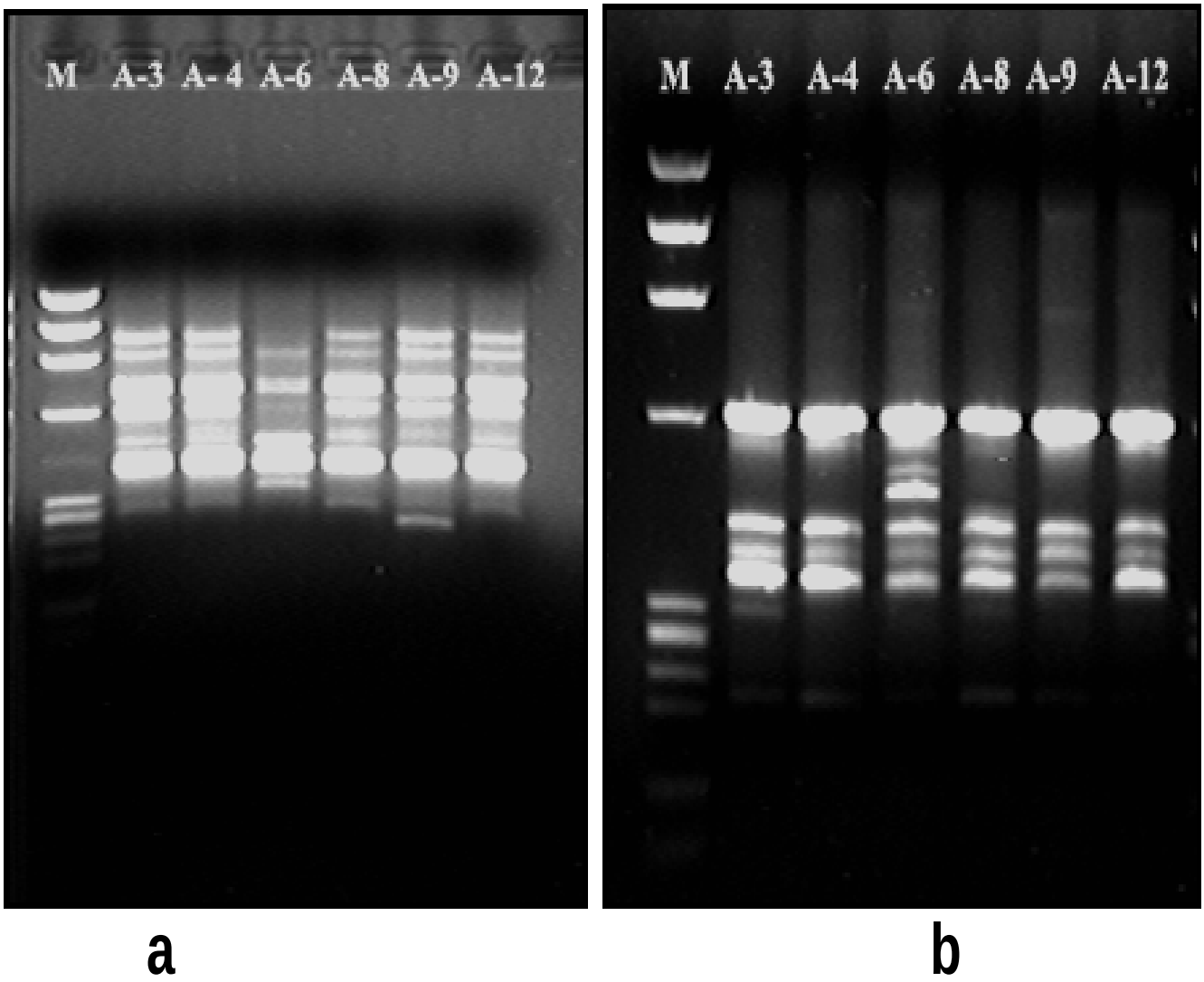

Figure 1. Gel image showing the RAPD amplified products using a) primer M-119 and b) primer M-191, in 1.5% agarose gel.

A-6, primer band showed that 1 band was missing in A-9 and primer M-119 showed 1 extra band in A-9 as well as 1 band missing in A-6. The UPGMA based dendrogram (Figure 2) revealed three major clusters; the first cluster contained sample collected from Lucknow and Paonta, the second cluster had single sample from Bangalore and the third cluster contained sample collected from Solan, Nauni and Hissar. The similarity coefficient value varied from 0.97 to 0.88. The highest similarity coefficient 0.97 were detected between samples collected from Nauni and Hissar as well as between Solan and Hissar and the lowest 0.88 was detected between the pairs Lucknow and Bangalore, Lucknow and Solan and Bangalore and Solan (Table 2).

The second groupings relate closely to their geographical distribution; accession A-9 (Solan), A-12 (Nauni) both belongs to Himachal Pradesh. The most geographically distant accession A-6 is also among the most distant genetically according to UPGMA. However, accession A-8 (Paonta) was grouped with the first group on the UPGMA analysis; geographically it was closed to populations A-9 (Solan) and A-12 (Nauni).

Several studies have been made and molecular polymorphism has been studied by using RAPD in plants. A RAPD analysis was done in three species of *Typhonium* to identify genetic relationship and to evaluate the genetic variance within population of three different species (Rout et al., 2006). Similarly, polymorphic studies were done among six *Piper* species by RAPD using hundreds of decamer primer of arbitrary sequence. Genetic divergence have also been assessed, employing RAPD among 29 neem accessions collected from two agro-ecological regions of India, which cover three states: Punjab, Haryana and Rajasthan (Deshwal et al., 2005).

The level of polymorphism in our study was very low which showed that samples used for analysis would have

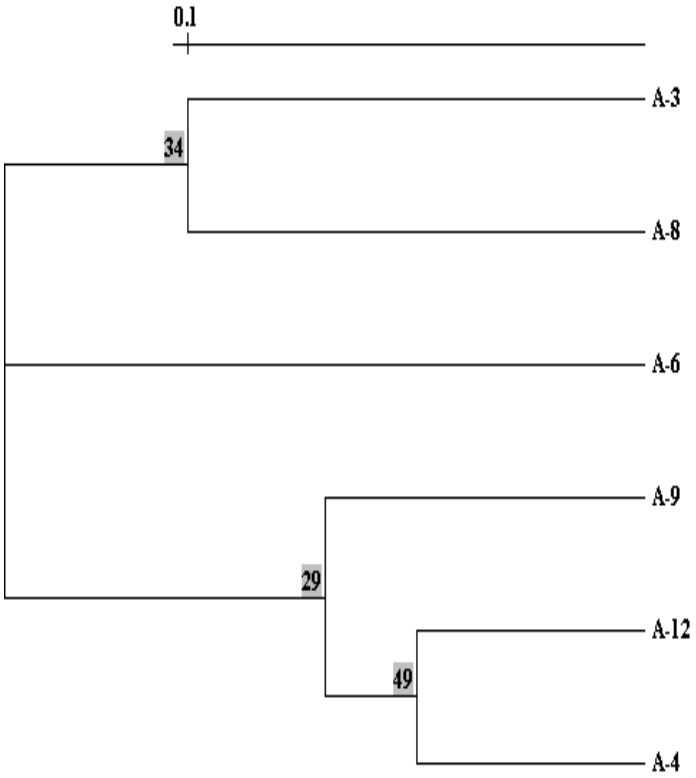

Figure 2. Genetic divergence between the six populations of *A. calamus* based on UPGMA cluster analysis.

Table 2. Similarity index for simple matching coefficient of the six populations of *A. calamus* L.

	A-12	A-3	A-4	A-6	A-8	A-9
A-12	1					
A-3	0.94	1				
A-4	0.97	0.91	1			
A-6	0. 94	0.88	0.91	1		
A-8	0.94	0.94	0.91	0.94	1	
A-9	0.94	0.88	0.97	0.88	0.88	1

close relationship. Similar kind of studies have been conducted in *Acorus* where low genetic variability was observed among populations collected from Southeast Ohio, USA as reported earlier (Pai et al., 2005).

Although there were some levels of polymorphism among the given population, an elaborated work is needed by using more number of primers and large number of populations from different regions to draw a suitable conclusion.

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