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DNA FINGER PRINTING IN *ACORUS CALAMUS:* THE MEDICINAL HERB

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ABSTRACT:

Germplasm is a vital resource and if properly described, characterized and evaluated once, can be utilized for long term methodical crop improvement. Thus, the present investigation "DNA finger printing in *Acorus calamus*" was carried out with the objective to document information on plant morphology and assess the extent of genetic diversity in *Acorus calamus* pool by using RAPD and SSR marker.

The DNA finger printing revealed that Accession-9, the clone collected from Chinthapalli of Vizag district, A.P showed diversity at molecular level. The morphological characterization also revealed that Accession – 9 produced high yields compared to other accessions.

KEY WORD: *DNA, Acorus calamus, Medicinal herb, Molecular, Morphological.*

INTRODUCTION:

Acorus calamus of Araceae family is an important medicinal herb that is widely used. B asarone, primary constituents of *Acorus calamus* are mainly believed to be responsible for its wide therapeutic actions. The fragrant oils obtained by alcoholic extraction of the rhizome are mainly used in the pharmaceutical industries (Balakumbahan *et al.*, 2010). The improvement of any crop lies in exploring the rich gene pool available in it. Germplasm is a

vital resource and if properly described, characterized and evaluated once, can be utilized for long term methodical crop improvement. At AICRP on Medicinal and Aromatic Plants & Betelvine, eleven accessions of *Acorus calamus* were added as part of germplasm enrichment programme. Interestingly, most of these accessions in gene bank look akin to one another with respect to majority of the phenotypic characters except for few characters. Hence, detailed characterization and evaluation of *Acorus calamus* genepool is necessary for identification and exploitation of agronomic and qualitatively desirable traits in crop improvement programmes and also for better conservation and management of genetic resources. The assessment of diversity based on morphological parameters has been often constrained by lack of adequate data on distinguishable morphological characters. However, DNA marker technologies largely overcome these limitations and are being extensively used in several crop plants not only for genetic diversity analysis but also for a wide range of molecular genetic studies. As detailed information on genetic diversity *Acorus calamus* is lacking, the present investigation "DNA finger printing in *Acorus calamus*" was carried out with the objective to document information on plant morphology and assess the extent of genetic diversity in *Acorus calamus* pool by using RAPD and SSR markers.

MATERIALS AND METHODS:

The experimental plant material for the present study comprised of 11 *Acorus calamus* genotypes collected from various places of India and maintained at All India coordinated project on Medicinal and Aromatic Plants & Betelvine, Venkataramannagudem. The list of *Acorus calamus* genotypes selected for the study is presented in Table 1. Morphological characterization of *Acorus calamus* genotypes with respect to their plant, leaf and rhizome characters was done using three plants in each accession. Detection of molecular polymorphism among the genotypes was performed by RAPD and SSR analysis. DNA was extracted as per modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson, 1980 and Ginwal and Mittal, 2010). The concentration and quality of DNA was estimated using Nano Drop spectrophotometer at 260/280 nm and verified by running sample on 1.0 per cent agarose along with λ DNA marker. For RAPD analysis, the primer OPAG-19 (AGC CTC GGT T) was used. For SSR analysis, the primer AC-06 (F: ttacaaatgcgatgctctaa and R: ggaatcctgctctgctataa) was used. Series of polymerase chain reaction (PCR) products were generated employing long range PCR techniques. The banding pattern was visualized in Gel Doc.

RESULTS AND DISCUSSION:

The eleven *Acorus calamus* accessions maintained in germplasm block at AICRP on Medicinal and Aromatic plants & Betelvine, Venkataramannagudem were studied for their morphological and molecular diversity.

Morphological characterization

Accession-3, the clone collected from Gadipalli of Nalgonda district in Andhra |Pradesh recorded highest number of leaves with less disease reaction percentage and leaf area index (Table 2 & 3). However, the leaf length (> 45 cm) and width (> 3.20 cm) recorded higher with the clones collected from other states Kalyani (W. Bengal) and Thrissur (Kerala). Difference in leaf emergence was higher in clone collected from Chinthapalli of Vizag district in Andhra Pradesh (Table 3). With respect to rhizome characters, Accession 11, the clone collected from Thrissur (Kerala) recorded higher number of scales (17) in rhizome while higher rhizome length (45 cm) and width (7.1 cm) recorded with Accession – 10, clone collected from Kalyani and Accession-2, clone collected from Betegudem of Nalgonda districts respectively. Higher yield characters viz. rhizome weight (60 g/pl.) and rhizome yield (16.80 q/ha) recorded with the Accession-3 (Gadipalli of Nalgonda district, A.P.), Accession-6 (Munipalle of Guntur district, A. P.) and Accession-9 (Chinthapalli of Vizag district, A.P.) (Table 4). The morphological characterization consists of calculating the genetic distances between *Acorus calamus* accessions using morphological characters and this analysis type is simpler and less cost, although present limitations related to the characters that were influenced by the environment and to cultivars with great phenotypic similarity (Oliveira *et al*., 2000). However, it is the predominant method of characterization, were easily differentiated based on different leaf, flower, fruit and seed characters.

Molecular characterization

Molecular analysis with RAPD and SSR marker revealed that the presence of polymorphism among the accessions collected. In RAPD analysis, Accession-9, the clone collected from Chinthapalli of Vizag district, A.P., had single band (Fig. 1). The fingerprint profile indicated that a unique band of 800 bp were absent in Accession-9 when the DNA was amplified with primer OPAG-19 showing its polymorphism with other cultivars. In the present study, eleven *Acorus calamus* accessions were studied for their genetic diversity at molecular level using simple sequence repeat (SSR) markers. SSR primer used in the present study produced discrete, scorable and unambiguous bands (Fig. 2). All studied accessions were found to have unique allelic profiles with SSR loci. The information obtained using molecular markers like RAPD and SSR offer many benefits for identifying variation and establishing diversity among the genotypes. Molecular markers enable the assessment of genetic similarity between genotypes. It was observed that clones collected from the Munipalle, Guntur dist. and Chinthapalli, Vizag dist. of Andhra Pradesh were found to be similar and varied with all other accessions in all aspects. However, the clones collected from different geographical origins are found to be genetically similar and the phenotypic variation expressed in those clones may be due to environment. The findings were in line with the reports of Golani et al (2007) and Nandan Mehta and

Mayuri Sahu (2009), who did not observe association between geographical distribution of accessions and genetic divergence.

CONCLUSION:

Molecular investigations carried out in this study allowed an evaluation of the degree of genetic differentiation among accessions. The analysis of *Acorus Calamus* genetic variability and the set-up of a fine molecular characterization system can form the basis for future genetic breeding programme aiming at development of new and improved cultivars.

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Table 1: List of Acorus calamus genotypes

Sl..	Accesión number	Place	District
		Andhra Pradesh:	
1	Acc - 1	Nagireddigudem	Nalgonda
2	Acc - 2	Betegudem	Nalgonda
3	Acc - 3	Gadipalli	Nalgonda
4	Acc - 4	Kantipudi	Guntur
5	Acc - 5	Bandivaripalem	Guntur
6	Acc - 6	Munipalle	Guntur
7	Acc - 7	Aiahagaripalli	Warangal
8	Acc - 8	Damaramadagu	Nellore
9	Acc - 9	Chintapalli	Vizag
		Other States:	
10	Acc - 10	Kalyani	W. B.
11	Acc - 11	Thrissur	Kerala

Table 2: Evaluation of *Acorus calamus* germplasm for Plant characters

Accesión number	Plant growth habit	No. of leaves /plant	Disease Reaction (%)	Insect Reaction (%)
Acc - 1	Marshy herb	11	14	-
Acc - 2	-do-	10	22	-
Acc - 3	-do-	11	17	-
Acc - 4	-do-	13	12	-
Acc - 5	-do-	12	18	-
Acc - 6	-do-	11	21	-
Acc - 7	-do-	12	18	-
Acc - 8	-do-	13	16	-
Acc - 9	-do-	11	14	-
Acc - 10	-do-	12	18	-
Acc - 11	-do-	11	17	-

Table 3: Evaluation of *Acorus calamus* germplasm for Leaf characters

Accesión number	Leaf length (cm)	Leaf breadth (cm)	Diff. in leaf em. (cm)	LAI	Leaf color	Leaf veins
Acc - 1	33.83	1.73	1.9	0.25	Green	Parallel
Acc - 2	37.67	1.87	1.8	0.20	-do-	-do-
Acc - 3	42.67	2.90	2.1	0.26	-do-	-do-
Acc - 4	42.00	3.00	2.0	0.24	-do-	-do-
Acc - 5	37.00	3.13	2.1	0.24	-do-	-do-
Acc - 6	43.00	3.20	2.3	0.28	-do-	-do-
Acc - 7	41.00	3.03	2.2	0.23	-do-	-do-
Acc - 8	42.00	3.10	2.4	0.24	-do-	-do-
Acc - 9	45.00	3.10	2.5	0.21	-do-	-do-
Acc - 10	50.00	3.23	2.2	0.20	-do-	-do-
Acc - 11	47.00	3.37	2.1	0.23	-do-	-do-

Table 4: Evaluation of *Acorus calamus* germplasm for Rhizome characters

Accesión number	Rhiz. Clr.	No. of Scale	Rhiz. Length (cm)	Rhiz. width (cm)	Rhiz. Wt. (g)	Rhiz. Yld. (q/ha)
Acc - 1	Brown	9	30	6.5	35	9.80
Acc - 2	Red	11	39	7.1	40	11.20
Acc - 3	Red	14	40	6.8	60	16.80
Acc - 4	Brown	16	35	6.5	45	12.60
Acc - 5	-do-	13	39	6.4	55	15.40
Acc - 6	-do-	15	42	7.0	60	16.80
Acc - 7	-do-	14	35	6.5	45	12.60
Acc - 8	-do-	15	30	6.3	50	14.00
Acc - 9	-do-	16	40	6.4	60	16.80
Acc - 10	Brown	16	45	6.2	50	14.00
Acc - 11	-do-	17	30	6.1	55	15.40

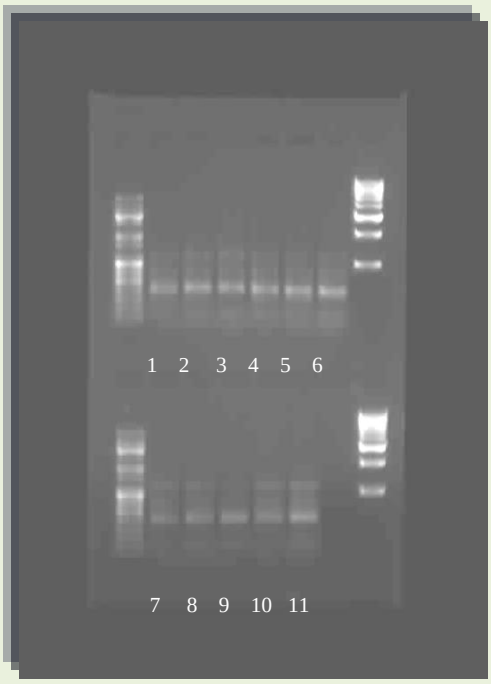

Fig. 1: Molecular analysis with RAPD marker

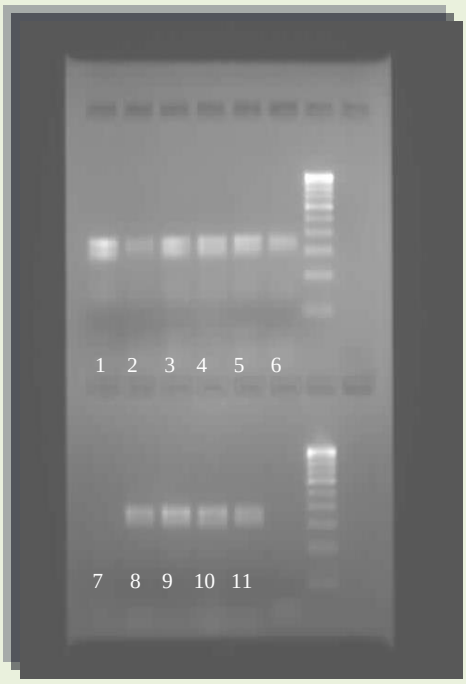

Fig. 2: Molecular analysis with SSR marker