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RAPD AND ISSR BANDING PATTERN OF *ALPINIA GALANGA* POPULATIONS FROM EASTERN INDIA

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ABSTRACT: India is very well known for presence of medicinal aromatic plants for treatment of various diseases which are day by day diminishing from the nature. This requires an urgent need for conservation of germplasm of those high yielding varieties which arises to help in various ways to the pharmaceutical, cosmetic as well as other industries. So also their genetic study is very much essential in order to explore their molecular structure. The present study was undertaken to characterize *Alpinia galanga* collected from four different populations of Odisha using two molecular markers as Random Amplified Polymorphic DNA and Inter Simple Sequence Repeats. Further a dendrogram was constructed through sequential agglomerative hierarchical and nested clustering, unweighted pair group method with arithmetic mean analysis and Jaccard's similarity coefficient of combined markers which segregated these genotypes into two main clusters moreover showing the genetic similarity between the germplasm. Hence, the molecular analysis could be further used for identification of important novel gene present in *Alpinia galanga* which can be utilized for future crop improvement.
Key words: *Alpinia galanga,* Polymerase Chain Reaction, Random Amplified Polymorphic DNA, Inter Simple Sequence Repeats.

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INTRODUCTION

Alpinia galanga an aromatic rhizomatous herb belonging to Zingiberaceae is cultivated in India and China [1]. The plant can grow upto 10 feet high, rhizomes in clumps, with abundant leaves and is commonly known as blue ginger or greater galangal. The plant has leaves that are lance-shaped, 30-60cm long and 10-15cm broad with fringed borders and reedy stems along with thick fragrant rootstocks. The raw rhizome is a popular ingredient used in many Indonesian and Malaysian dishes for its ginger like flavour. The rhizomes of *A.galanga* smell as cardamom so mainly used for flavouring food as well as condiment with medicinal properties. The rhizome is used to treat arthritis, ulcer, whooping colds in children, fever, vomiting etc. The rhizomes have antimicrobial, antioxidant, antiulcer, anti-inflammatory, antitumour, cytotoxic, anti-spasmodic, anti-hepatotoxic, anti depressant and mosquito repellant activities [2-8]. Besides its vast importance there are very few reports available on its genetic study for which the present work has been reported from the eastern ghats.

MATERIALS AND METHODS

Plant material and molecular analysis

For the present study, *Alpinia galanga* were collected from the wild habitats of Koraput, Phulabani, Raikia and G.Udaigiri populations and were maintained in the medicinal garden of Centre for Biotechnology, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha. DNA was isolated by the standard protocol followed by polymerase chain reaction (PCR) reaction as Random Amplified Polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) following the reported standard protocol [9-11].

For RAPD random decamer Operon Primers (Operon Tech., Alameda, USA) were dissolved in double sterilized $T_{10}E_1$ buffer, pH 8.0 to the working concentration of 15 ng/µl. RAPD primers as per the amplified pattern from A, C, D, N and AF series namely OPA04, OPA07, OPA09, OPA18, OPC02, OPC05, OPC011, OPD03, OPD07, OPD08, OPD12, OPD18, OPD20, OPN04, OPN16, OPN18, AF5, AF14 and AF15 were used. Similarly ISSR primers namely $(GAC)_5$, $(GTGC)_4$, $(GACA)_4$, $(AGG)_6$, $(GA)_9T$, $T(GA)_9$, $(GTG)_5$, $(GGA)_4$ and $(CAA)_5$ (Bangalore Genei Pvt. Ltd, Bangalore, India) were used. After PCR reaction, 2.5 µl of 6X loading dye (MBI Fermentas, Lithuania) was added to the amplified products and separated in 1.5-2% agarose gel with ethidium bromide. The electrophoresis was performed for 3 hours at 60 volt and visualized under UV-transilluminator (BioRad, USA) and photographed using Gel Documenting System (Bio-Rad, USA) for scoring the bands [12]. The sizes of the amplicons were determined by comparing them with that of the DNA ladder and were repeated at twice to confirm the reproducibility patterns.

Statistical analysis

Resolving power (Rp) of the primers were calculated [13]. Resolving power is: Rp=ΣIB (IB (Band informativeness) = 1-[2×(0.5-P)], P is the proportion of the species containing the band. The Primer Index (PI) was calculated from the polymorphic index. A polymorphic index (PIC) was calculated as PIC = $1-\sum P^2_i$, P_i is the band frequency of the i^{th} allele [14]. In the case of RAPDs and ISSRs, the PIC was considered to be $1-p^2-q^2$, where p is band frequency and q is no band frequency [15]. The PIC value was then used to calculate the primer index (PI). PI is the sum of the PIC of all the markers amplified by the same primer.

Jaccard's coefficient of similarity was measured and a phylogram based on similarity coefficients was generated by unweighted pair group method using arithmetic averages (UPGMA) and the SAHN (Sequential Agglomerative Hierarchical and nested) clustering was obtained [16, 17]. The entire analysis was performed using the statistical package NTSYS-pc 2.02e [18]. In addition to the classical resampling methods the statistical testing of robustness of the obtained trees, such as bootstrapping was implemented. This test has been created to give a possibility of having more distances with the same values. In such case, the order of taxa influences the result of the tree building. Rearrangement of taxa can reveal this situation.

RESULTS

RAPD and ISSR analysis

The DNA profiles as obtained by RAPD markers were represented in Table-1 (Fig. 1 A, B). A total of 79 bands were amplified, 78 bands were found to be monomorphic and only 1 was polymorphic in nature. The highest number of bands (11) were amplified with primer OPD18 (230-1400bp) and lowest number of band (3) was amplified with primer OPC5 (750-2000bp). No unique bands were found with all the primers. Average number of bands per primer was found to be 6.1. The resolving power of the primers were varied from 6-22 where the primer with maximum resolution power was OPD18 (22) and the primer with minimum resolution power was OPC5 (6).

The DNA profiles as obtained by ISSR markers were represented in Table-1 (Fig. 1 C, D). A total of 73 bands produced, 66 were monomorphic, 5 were polymorphic and 2 were found to be unique bands. The primer (AGG)6 and (GTG)5 produced maximum number of bands(11), while primer (GA9)T produced minimum number of bands (2). The bands were amplified in the range of 190-1700bp. Among these ISSR primers, maximum resolving power (22) was obtained in (GTG)5 primer and minimum Rp (4) was in (GA9)T.

Analysis of data of combined markers

A total of 152 bands were amplified with all the marker out of which 144 were monomorphic, 6 were polymorphic and rest 2 were unique bands (Table-1). All the samples were correlated with each other with an average similarity of 0.976 which ranged between 0.961 to 1.000. The dendrogram constructed using Jaccard's similarity coefficient, separated the 4 populations into two major clusters, one with 7 samples another with rest 5 samples at similarity coefficient of 0.97 (Fig. 2). Each cluster was again subdivided into two sub clusters of which one sub cluster contain single sample being separated from rest of all 2 sub clusters. Cluster I included 7 replicates i.e., 3 from G.Udaygiri, 2 replicate populations from Koraput, one each from Raikia and Phulabani and Cluster II includes 5 replicates i.e., 2 replicates each from Phulabani populations and Raikia populations and one replicate population from Koraput.

Table 1: Details of RAPD, ISSR and combined marker analysis as revealed among four populations of *Alpinia galanga*

Markers	Primer	Sequence of Oligonucleotides	Approx fragment Size(bp)	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	Resolving power
RAPD	OPA4	5'AATCGGGCTG3'	280-2000	6	6	0	0	12
	OPA8	5'AGGTGACCGT3'	825-2800	9	8	1	0	17.166
	OPA9	5'GGGTAACGCC3'	700-2000	4	4	0	0	8
	OPA18	5'AGGTGACCGT3'	500-1400	4	4	0	0	8
	OPC2	5'GTGAGGCGTC3'	790-1900	6	6	0	0	12
	OPC5	5'GATGACCGCC3'	750-2000	3	3	0	0	6
	OPD3	5'GTCGCCGTCA3'	700-3000	9	9	0	0	18
	OPD8	5'GTGTGCCCCA3'	700-2000	4	4	0	0	8
	OPD18	5'GAGAGCCAAC3'	230-1400	11	11	0	0	22
	OPD20	5'ACCCGGTCAC3'	475-2000	6	6	0	0	12
	OPN4	5'GACCGACCCA3'	510-1200	7	7	0	0	14
	OPN16	5'AAGCGACCTG3'	370-900	4	4	0	0	8
	OPN18	5'GGTGAGGTCA3'	250-950	6	6	0	0	12
Total				**79**	**78**	**1**	**0**	
ISSR	SPS 1	(GAC)5	280-900	6	6	0	0	12
	SPS 2	(GTGC)4	300-700	9	7	2	0	14.333
	SPS 3	(GACA)4	330-1500	9	9	0	0	18
	SPS 4	(AGG)6	270-1100	11	8	3	0	18.666
	SPS 5	(GA)9T	290-375	2	2	0	0	4
	SPS 6	T(GA)9	190-775	8	6	2	0	13.5
	SPS 7	(GTG)5	250-1000	11	11	0	0	22
	SPS 8	(GGA)4	280-1250	8	8	0	0	16
	SPS 9	(CAA)5	425-1500	9	9	0	0	18
Total				**73**	**66**	**7**	**2**	0
Grand total				**152**	**144**	**8**	0	

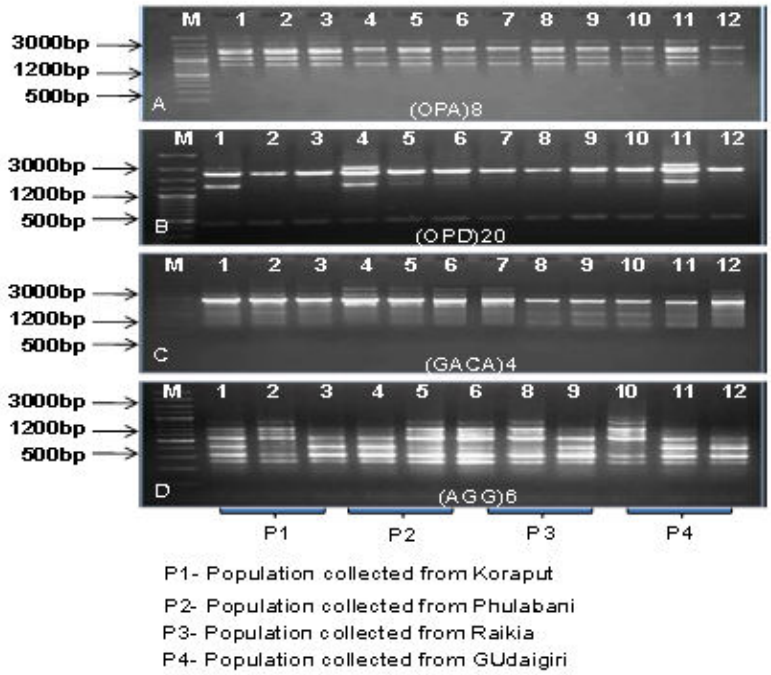

Figure 1. (A, B): RAPD banding pattern and (C, D) ISSR banding pattern of *Alpinia galanga* from different populations (Lane 1-12 and M- marker)

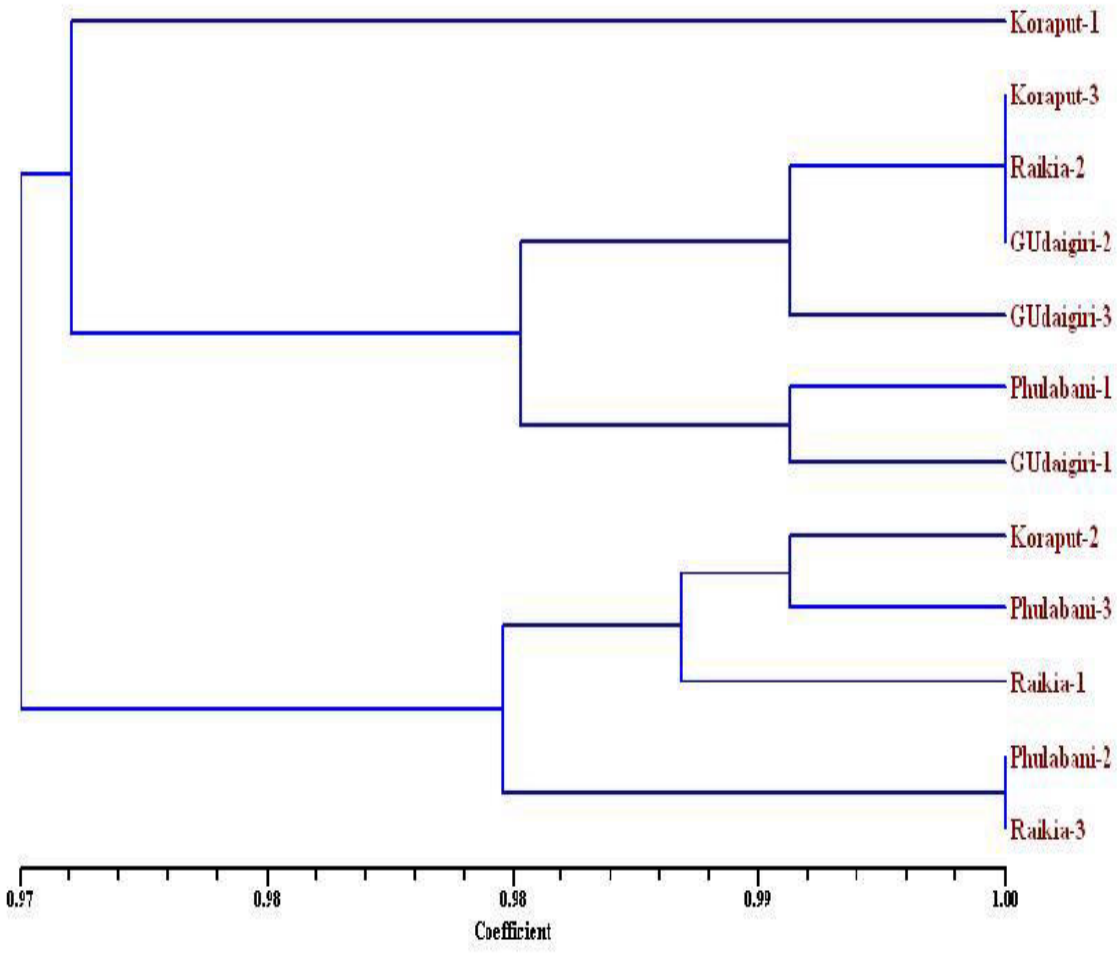

Figure 2: Dendrogram showing genomic relationship within 4 populations in *Alpinia galanga* as revealed from RAPD and ISSR analysis

DISCUSSION

Thirty-seven galanga (*Alpinia spp*.) accessions, 31 cultivated and 6 wild landraces from different areas in Thailand were evaluated for genetic diversity, using random amplified polymorphic DNA (RAPD) primers are reported. In their study Out of 22 random primers used only 8 primers produced a total of 73 polymorphic bands. UPGMA cluster analysis of genetic similarity estimates (Jaccard's coefficient) separated the accessions into 5 major clusters. The dendrogram showed no relation with their morphological characters such as type, color of rhizome and collection sites which were indicated by the regions of Thailand [19]. There are other studies of genetic diversity using ISSR markers [20, 21]. However, this study illustrated that both RAPD and ISSR analysis could be a useful tool to evaluate genetic diversity in galanga accessions. The highly informative primers identified in this study would be available for further genetic analysis of *A. galanga* for plant selection and improvement.

CONCLUSION

Moreover identification of Zingiberaceous taxa through herbarium is very difficult because of problem in preserving thick and fleshy rhizomes. The precise characterization of genetic diversity that exists in available germplasm of different species using molecular markers is one step forward providing accurate genetic information for future breeding programme for improvement of the desired taxa. The present report based on molecular markers was done to characterize genetically different populations in *Alpinia* species. This phylogenetic study could be valuable for species identification of *A. galanga* as being reported for the first time in this genus from eastern India. The resources could also be used as a healthy plant material for supply to industries for commercial exploitation.

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