



Research Article – Biotechnology

Assessment of genetic diversity of *Curcuma aromatica* from eastern India using ISSR and RAPD markers

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Abstract

Molecular characterization of *Curcuma aromatica* from 4 different populations of Odisha using inter simple sequence repeats and random amplified polymorphic DNA markers to indicate the closeness of species and hybrids quickly and efficiently. A dendrogram was constructed through sequential agglomerative hierarchical and nested (SAHN) clustering and unweighted pair group method with arithmetic mean (UPGMA) analysis using Jaccard's similarity coefficient of combined markers using this particular species. Two major clusters were found i.e., cluster-I (Koraput-1, Koraput-2, Koraput-3, G.Udaigiri-1, G.Udaigiri-2, G.Udaigiri-3 and Phulabani-1, Phulabani-2, Phulabani-3) and cluster-II (Raikia-1, Raikia-2 and Raikia-3). The clustering pattern also revealed moreover the extent of genetic similarity between germplasms collected from four different regions population. The potential of this technique would be further realized to fullest extent for the identification and tagging of important novel gene in different taxa, unexplored yet, thus facilitating the improvement of desired taxa of Zingiberaceae. The findings would be of immense enough significance for complementing the strategies of conservation and characterization of these important taxa of Zingiberaceae following modern biotechnological approach.

Keywords: *Curcuma aromatica*, ISSR, RAPD, Molecular characterization

Introduction

Curcuma aromatica is commonly known as wild turmeric which is an under exploited medicinal plant of family Zingiberaceae. The rhizome is orange yellow in colour and bears yellow colour flower beyond the leaves. The rhizome of the plant has been used for flavouring food and as a colouring agent whereas its rhizome essential oil is being used in aromatherapy. The plant is reported for its various medicinal activities such as promoting blood circulation, treatment of cancer due to presence of curcumin, mosquito repellent activity, anti-dote for snakebite, antimicrobial, antioxidant activity etc. [1-4]. The plant is underexploited due to its presence in wild areas so steps must be taken for proper conservation thereby characterization of the plants genetically for future work. Many molecular markers are used for studying genetic relations, population genetics and genetic characterization in different plant groups and crop cultivars. These markers are not influenced by the external environmental like morphological markers so accurately testify genetic relationship between and among plant groups. RAPD and ISSR have been used for measuring genetic diversity in several plant species [5-9].

Materials and Methods

Plant Material

In the present investigation *Curcuma aromatica* were collected from wild habitats of Koraput, Phulabani, Raikia and G.Udaigiri populations. After collection these medicinal plant species were grown in the medicinal plant garden of Center of Biotechnology, Siksha O Anusandhan University, Bhubaneswar, Odisha.

Molecular analysis

Two types of polymerase chain reaction (PCR) based molecular techniques namely Random Amplified Polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) were utilized for the present study. For RAPD analysis random decamer operon primers (Operon Tech., Alameda, USA) were dissolved in double sterilized T₁₀E₁ buffer, pH 8.0 to the working concentration of 15 ng/μl. Nineteen best selected primers as per the reproducibility and amplification pattern from A, C, D, N and AF series OPA04, OPA07, OPA09, OPA18, OPC02, OPC05, OPC011, OPD03, OPD07, OPD08, OPD12, OPD18, OPD20, OPN04, OPN16, OPN18, AF5, AF14 and AF15 (Operon Tech. Alameda, CA.) were used for RAPD analysis. The RAPD analysis was performed as per the methodology described by [10]. Nine numbers of ISSR primers were used for ISSR analysis. These simple sequence repeats were synthesized and procured from Genei (Bangalore Genei Pvt. Ltd, Bangalore, India). Those primers were (GAC)₅, (GTGC)₄, (GACA)₄, (AGG)₆, (GA)₉T, T(GA)₉, (GTG)₅, (GGA)₄ and (CAA)₅. The ISSR analysis was performed as per the methodology [11].

After the completion of the PCR 2.5 μl of 6X loading dye (MBI Fermentas, Lithuania) was added to the amplified products and were stored at -20°C till further use. Then these PCR products for RAPD were separated in 1.5% agarose gel while those of the ISSR products were resolved in 2% agarose gel alongwith ethidium bromide. The electrophoresis was performed at 60 volt for 3 hours. After electrophoresis, the gel was visualized under the UV-transilluminator (BioRad, USA) and photographed using Gel Documenting System (Bio-Rad, USA) for scoring the bands.

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

Markers	Primer	Sequence of Oligonucleotides	Approx fragment Size(bp)	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	Resolving power	
RAPD	OPA4	5'AATCGGGCTG3'	300-1500	5	5	0	0	10	
	OPA7	5'GAAACGGGTG3'	1200-1500	2	2	0	0	4	
	OPA18	5'AGGTGACCGT3'	250-2600	11	11	0	0	22	
	OPC2	5'GTGAGGCGTC3'	700-2300	7	7	0	0	14	
	OPC5	5'GATGACCGCC3'	1200-1900	3	3	0	0	6	
	OPC11	5'AAAGCTGCGG3'	650-2000	5	3	2	0	9	
	OPD3	5'GTCGCCGTCA3'	900-1300	3	1	2	0	5.166	
	OPD7	5'TTGGCACGGG3'	450-1150	6	4	2	0	10.666	
	OPD8	5'GTGTGCCCA3'	600-2400	8	6	2	0	14.5	
	OPD18	5'GAGAGCCAAC3'	250-1000	7	7	0	0	14	
	OPD20	5'ACCCGGTCAC3'	500-3000	11	11	0	0	22	
	OPN4	5'GACCGACCCA3'	500-1200	3	3	0	0	6	
	OPN16	5'AAGCGACCTG3'	100-1000	5	5	0	0	10	
	OPN18	5'GGTGAGGTCA3'	500-1050	4	2	2	0	6	
	Total				80	70	10	0	
		SPS 1	(GAC)5	220-850	10	10	0	0	20
		SPS 2	(GTGC)4	300-825	8	8	0	0	16
		SPS 3	(GACA)4	340-1510	8	8	0	0	16
	SPS 4	(AGG)6	250-1400	14	14	0	0	28	
	SPS 5	(GA)9T	390-1750	10	10	0	0	20	
	SPS 6	T(GA)9	270-1031	11	11	0	0	22	
	SPS 7	(GTG)5	275-1020	10	10	0	0	20	
	SPS 8	(GGA)4	290-1800	13	13	0	0	26	
	SPS 9	(CAA)5	610-2010	8	8	0	0	16	
Total				92	92	0	0	0	
Grand total				172	162	10	0		

The sizes of the amplicons were determined by comparing them with that of the DNA ladder. The entire process was repeated at least twice to confirm the reproducibility.

Scoring of the Data

The data was scored as '1' for the presence and '0' for the absence of the band for each primer genotype combination for RAPD and ISSR analysis. All the bands were considered under estimation of the genetic similarity [12].

Statistical analysis of the Data

Resolving power (Rp)

Resolving power of the RAPD and ISSR were calculated [13]. Resolving power is: $R_p = \frac{1}{1 - [2 \times (0.5 - P)]}$ (IB (Band informativeness) = $1 - [2 \times (0.5 - P)]$), P is the proportion of the species containing the band.

Primer Index (PI)

The Primer Index was calculated from the polymorphic index. A polymorphic index (PIC) was calculated as $PIC = 1 - \sum P_i^2$, 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 similarity

Jaccard's coefficient of similarity [16] was measured and a phylogram based on similarity coefficients was generated by unweighted pair group method using arithmetic averages (UPGMA) [17] and the SAHN (Sequential Agglomerative Hierarchical and nested) clustering was

obtained. The entire analysis was performed using the statistical package NTSYS-pc 2.02e [18].

Boot strapping

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 analysis

From 25 random decamer oligonucleotide primers 14 primers were selected from A, C, D and N series were amplified. All the 4 populations of *C.aromatica* (Koraput, Phulabani, Raikia and G.Udaigiri) each in triplicates was used which produced distinct reproducible amplicons. The DNA profiles as obtained by RAPD markers were represented in table 1 (fig.2). A total of 80 bands were amplified, 70 of which were found to be monomorphic and only 10 were polymorphic in nature. The highest number of bands (11) were amplified with primer OPA18 (250-2600bp), OPD20 (500-3000bp) and lowest number of band (2) was amplified with primer OPA7 (1200-1500bp). No unique bands were found with all the primers. Average number of bands per primer was found to be 5.7. The resolving power of the primers were varied from 4-22 where the primer with maximum resolution power was OPA18 and OPD20 (22) and the primer with minimum resolution power was OPA7 (4). The details of RAPD banding pattern and amplification with different primers and plant species are presented in table 1.

ISSR analysis

Out of 10 ISSR primers 9 primers resulted in the amplification of 92 fragments. The ISSR banding pattern in 5 species is represented in table 1. The primer (AGG)6 produced maximum number of bands (14), while primer (GTGC)4, GACA)4 and (CAA)5 produced minimum number of bands (8). The bands were amplified in the range of 220- 2010bp and no unique bands were formed. From 92 bands amplified all were monomorphic in nature. Among these ISSR primers, maximum resolving power (28) was obtained in (AGG)6 primer and minimum Rp (16) was in (GTGC)4, GACA)4 and (CAA)5. Details of ISSR banding pattern (fig.2) and bands amplified in different species with different primers have been represented in table 1.

Analysis of data of combined markers

The two types of markers (RAPD and ISSR) were employed for assessment of genetic similarity among 4 populations of *C.aromatica*. A total of 172 bands were amplified with the entire marker out of which 162 were monomorphic and rest 10 were polymorphic (table 1). All the samples were correlated with each other with an average similarity of 0.977 which ranged between 0.949 to 1.000 (table 1). The dendrogram constructed using Jaccard's similarity coefficient, separated the 4 populations into two major clusters, one with 9 samples another with rest 3 samples at similarity coefficient of 0.96 (fig.1). 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 all the replicates of Koraput, GUDAigiri and Phulabani population and Cluster II includes all three replicates from Raikia populations.

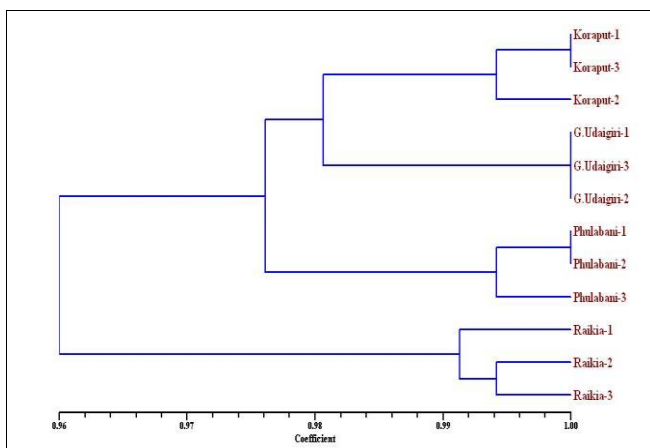


Fig. 1. Dendrogram showing genomic relationship within 4 populations in *C. aromatica* from combined RAPD and ISSR analysis

Discussion

Studies on molecular genetic fingerprints of nine *Curcuma* species from Northeast India using PCR-based markers has been reported [19]. A phylogenetic analysis of the tribe Zingiberaceae was performed using nuclear ribosomal DNA and chloroplast DNA [20]. The study indicated that tribe Zingiberaceae is monophyletic with two major clades, the *Curcuma* clade, and the *Hedychium* clade. Rather very few studies have been done on this particular species and the present work is the first report on using molecular markers in *C. aromatica* from eastern India.

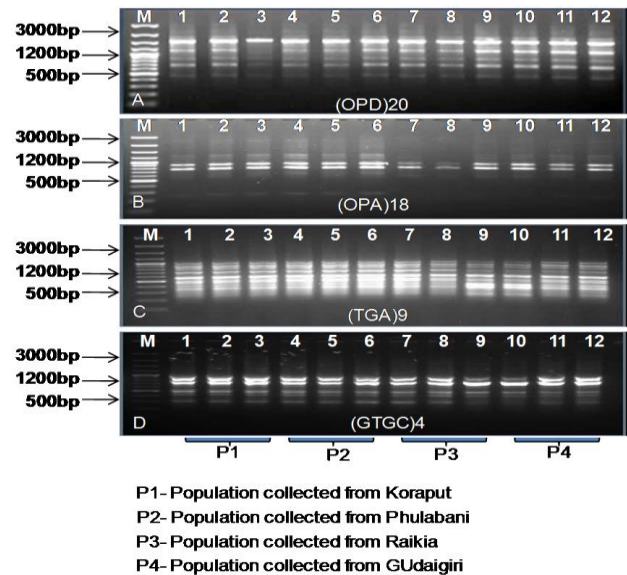


Fig. 2. (A, B) RAPD banding pattern and (C, D) ISSR banding pattern in *C. aromatica* from 4 different populations (Lane 1-12) and M- marker

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Conclusion

The present report based on molecular markers was done to characterize genetically different populations of *Curcuma* species. The resources could be used as healthy plant material for supply to industries for commercial exploitation. Samples present in single clusters represent their closeness with each other, hence could be concluded as intraspecific polymorphism in between the populations. At the same time less number of samples has been analyzed for which there is less chance of variation which needed a further classification.

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Conflicts of interests

There is no conflict of interest

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