

# Assessment of genetic diversity in *Costus pictus* accessions based on RAPD and ISSR markers

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**Abstract** *Costus pictus*, belonging to the family *Costaceae*, is one of the valuable medicinal plants with its anti-diabetic property. Despite ever-increasing demand from the pharmaceutical industry, this species is being less exploited at molecular level. Hence, an effort has been made in the present study to characterize the 15 accessions of *C. pictus* collected from different geographical regions of India through random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. A total of 25 RAPD and 20 ISSR primers were used in the present study. The RAPD analysis generated 343 loci, of which 124 were polymorphic with an average of 4.96 loci per primer. While, ISSR primers produced 177 loci, of which 77 were polymorphic with an average of 3.85 loci per primer. The similarity coefficients ranged from 0.86–0.99, 0.84–0.95 and 0.86–0.96 for RAPD, ISSR and combined RAPD-ISSR, respectively. The UPGMA dendrogram generated using these data showed low level of divergence among the accessions from South and West regions. Further, accession-specific bands were also revealed by RAPD and ISSR markers which might be contributed to specific trait. This investigation was an understanding of genetic variation within the *C. pictus* accessions. The present finding indicates that both the marker tools RAPD and ISSR combined or individually can be used in determining the genetic

relationship between the accessions. It may be concluded that data of hereditary differences appeared among the *C. pictus* accessions could be utilized for their conservation and reproducing programs.

**Keywords** *Costus pictus* · Genetic diversity · RAPD · ISSR · Polymorphism

## Introduction

Interest in the utilization of medicinal and aromatic plants as pharmaceuticals, perfumes and cosmetics, flavoring, herbal and alternative natural products has been significantly expanded in the recent years (Ayensu 1996). *Costus pictus* D. Don is an important medicinal plant that belongs to family *Costaceae*, normally known as Spiral flag or Insulin plant. This plant is widely used by the tribal people of Kolli hills of Tamilnadu, to treat diabetes (Elavarasi and Saravanan 2012). *C. pictus* recently introduced in India from the American countries as an herbal care for diabetes and, therefore, commonly called as ‘insulin plant’ (Benny 2004). The monocot family *Costaceae* has close to seven genera and containing about 143 known species (Christenhusz and Byng 2016). This is believed to be native of tropical climates of Asia, Central America, South America and Africa. Recently, this plant gains very high medicinal interest due to its medicinal potential and shows various pharmacological properties such as anti-diabetic, diuretic effect, anti-oxidant, anti-cancers and putative activity (Hegde et al. 2014). Particularly, in south Indian region it is grown as ornamental plant.

Various important phytochemical properties have been reported from *C. pictus* plant including ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, steroids, triterpenoids, alkaloids,

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tannins, saponins and flavonoids (Devi and Urooj 2010). It is also reported that leaves of this plant contain 21.2% fibers including significant amounts of the elements K, Ca, Cr, Mn, Cu, and Zn (Hegde et al. 2014). Because of its prospective use as valuable medicine, integration of *pictus* species into the agricultural forestry organizations would not only make accessible to majority of the backward populace that uses it but also pay attention towards its genetic conservation.

For improvement of the breeding and cultivation of plants, understanding its genetic assortment is very important. Morphological inspection is a very simple and direct way to study diversity, from which we cannot get significant genetic diversity among accessions (Nag et al. 2015). Cataloging of the various genera and species is largely based on morphological characteristics. However, these characters may not be significantly diverse and usually require growing plants by farmers up to the maturity prior to identification and also influenced by various environmental factors. Thus, it is essential to determine genetic diversity of such species so that only promising accessions can be multiplied and preserved.

Rapid advances in molecular marker systems have proven to be powerful tools in the assessment and evaluation of genetic diversity and in the interpretation of genetic relationships within and among species could help to breeders for genetic improvement of important medicinal plants (Grover and Sharma 2016). With the molecular approach, identification of particular plant accessions/genotypes seems to be more effective as compared to traditional morphological marker system as it directly accesses the plants hereditary information and makes it possible to understand the genetic relationship between

individuals (Paterson et al. 1991). Random amplified polymorphic DNA (RAPD) marker system has been extensively used to distinguish the genetic variation at molecular level in several medicinal and aromatic plants (Ganie et al. 2012; Kumar et al. 2013; Khanna et al. 2014; Prajapat et al. 2015; Bhattacharyya and Kumaria 2015).

The advantages of RAPD markers are that they do not require prior sequence information, less expensive and can efficiently differentiate taxa below the species level, since it targets both coding and non-coding regions of the genome (Ganie et al. 2015). Other marker systems, namely inter-simple sequence repeats (ISSR) are similarly PCR-based molecular marker that amplifies genomic regions flanked by the SSR sequences repeat oriented in opposite orientation. RAPD and ISSR studies have been widely used in medicinal plants for genetic diversity analysis (Patel et al. 2015; Sarwat et al. 2008; Tripathi et al. 2012). This marker is highly polymorphic, reproducible and widely used for genetic diversity, gene tagging, phylogeny, evolutionary biology and genome mapping studies (Godwin et al. 1997).

As per our knowledge and literature survey, there is a lack of information on the molecular characterization of the *Costus pictus* plant. Thus, the present study was carried out with different accessions of *C. pictus* collected from various geographical regions of India using RAPD and ISSR markers.

## Materials and methods

### Plant material and DNA extraction

A total of fifteen *C. pictus* samples were collected from different geographical regions of India (Table 1). Two

**Table 1** Details of *Costus pictus* accessions collected from different regions of India for genetic diversity analysis

S. no.	Accessions name	Place of collection	State	Latitude (°N)	Longitude (°E)
1	CP-1	Howrah	West Bengal	22°33'38.4"N	88°17'20.1"E
2	CP-2	Bhopal	Madhya Pradesh	23°15'35.7588"N	77°24'45.414"E
3	CP-3	Panji	Goa	15°29'07.4"N	N73°49'49.0"E
4	CP-4	Hyderabad	Telangana	17°23'6.1584"N	78°29'12.0156"E
5	CP-5	Navsari	Gujarat	20°56'48.1272"N	72°57'7.326"E
6	CP-6	Daang	Gujarat	20°49'31.3608"N	73°42'2.5524"E
7	CP-7	Vellore	Tamilnadu	12°55'13.8"N	79°07'32.3"E
8	CP-8	Thekkady	Kerala	9°36'11.1924"N	77°9'41.2488"E
9	CP-9	Bangalore	Karnataka	12°58'17.7564"N	77°35'40.4268"E
10	CP-10	Nashik	Maharashtra	19°59'50.8308"N	73°47'23.2872"E
11	CP-11	Vansada	Gujarat	20°45'28.5552"N	73°21'50.5368"E
12	CP-12	Chennai	Tamilnadu	13°4'57.648"N	80°16'14.5848"E
13	CP-13	Palode	Kerala	8°43'27.9624"N	77°1'29.2116"E
14	CP-14	Dharwad	Karnataka	15°27'32.1264"N	77°0'0.0000"E
15	CP-15	Nagpur	Maharashtra	21°8'44.88"N	79°5'17.358"E

grams of fresh and young leaves was collected from each accession and rapidly frozen in liquid nitrogen for DNA extraction. DNA was isolated using the CTAB method as described by Doyle and Doyle (1990) with minor modification. Further, purity and concentration were measured using the Nanodrop (Thermo Scientific, USA) software.

### RAPD and ISSR amplification

RAPD amplification was performed using 10-mer short oligonucleotide primers randomly from operon series (Eurofins Genomic, India). PCR amplification was carried out with 20 µl reaction volume containing 1X Taq-PCR buffer with MgCl<sub>2</sub> (Finnzymes, Thermo scientific, USA), 5 unit Taq Polymerase (Finnzymes, Thermo scientific, USA), 30 ng genomic DNA, 10 pM of primer (Eurofins Genomic, India), 200 µM dNTPs (Finnzymes, Thermo scientific, USA), in 200 µl PCR tubes (Axygen, USA) in thermocycler instrument (Eppendorf, Germany). RAPD-PCR mixture was initially denatured at 94 °C for 6 min, followed by 40 cycles of 94 °C for 1 min, 36–38 °C (depending on primer composition) for 1 min, 72 °C for 1 min, and final extension at 72 °C for 8 min. ISSR primers used in the present study were designated from the previous reports of Mohanty et al. (2014) and Verma et al. (2015). ISSR-PCR mixture was subjected to initial denaturation at 94 °C for 10 min followed by 35 cycles of denaturation at 94 °C for 1.3 min, annealing at 40–68 °C (depending on primer composition) for 1 min and extension at 72 °C for 2 min with a final extension at 72 °C for 10 min.

### Agarose gel electrophoresis

Amplified PCR products of RAPD and ISSR were fractionated on 1.5% agarose in 1X Tris–borate–EDTA buffer for 1.5 h. DNA bands were visualized by staining with ethidium bromide (EtBr) and photographed on a UV transilluminator using gel documentation system (Bio-Rad, USA). Molecular size of the amplified product was estimated using a known molecular 100 + 500 bp DNA Ladder (Thermo fisher, USA). The experiment was repeated twice with each primer and only those results were considered in which repeatable patterns (DNA fingerprint) were observed.

### Data analysis

Qualitative scoring of bands was done from gel photographs obtained from RAPD and ISSR analysis. Clearly reproducible bands from each accession were scored as unit character and data were recorded in a binary matrix ('0' for absence and '1' for presence) sheet. The binary data obtained from the marker systems were analyzed

individually using Dice's coefficient (Dice 1945) to determine pairwise comparison to estimate the genetic relationship among the accessions. Genetic similarity coefficient was computed using the Dice's coefficient with the NTSYS-pc software (Rohlf 1998). Dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) algorithms in the NYSYSpc 2.02 based on the genetic distance of Nei and Li (1979). To judge primer efficiency, polymorphism information content (PIC) was calculated according to the formula described by Garcia et al. (2004)  $PIC = 1 - \sum f^2$ , where "f" is the frequency of "ith" allele. EIGEN module of NTSYSpc 2.02 software was used to analyze principal component analysis (PCA) and results were graphically expressed in the form of 3D plots. The Mantel matrix correspondence test for RAPD and ISSR markers was carried out using the MXCOMP function of the NTSYSpc 2.02 (Mantel 1967).

## Results

### Performance of marker systems

#### RAPD marker analysis

A total of 35 primers were initially screened for their ability to produce polymorphic patterns and only 25 of them were selected which gave reproducible and distinct polymorphic amplified products. The data collected from random amplification of polymorphic DNA (RAPD), detected total of 343 loci in 15 accessions, out of which 124 (35.57%) were polymorphic (Table 2). The molecular size of the amplified PCR products ranged from 110 (OPM-14; OPN-02) to 3190 bp (OPN-16). Moreover, the RAPD primer OPK-16 produced the maximum number of 24 loci, while OPN-08 produced the minimum number of 7 loci. The average polymorphic loci were found to be 4.96 per primer. The RAPD primer OPK-20 gave the highest polymorphism, while the lowest polymorphism was detected by the OPK-07 primer (Table 2). The average PIC (Polymorphism Information Content) value for RAPD was 0.91. The highest PIC value obtained was 0.95 for OPK-16 marker and the lowest PIC value was 0.85 for OPN-8. In the present study, a total of 25 RAPD primers examined, from which five primers (OPK-18; OPN-6; OPN-7; OPM-9; OPN-10) produced accession-specific amplicons (Fig. 1a; Table 4).

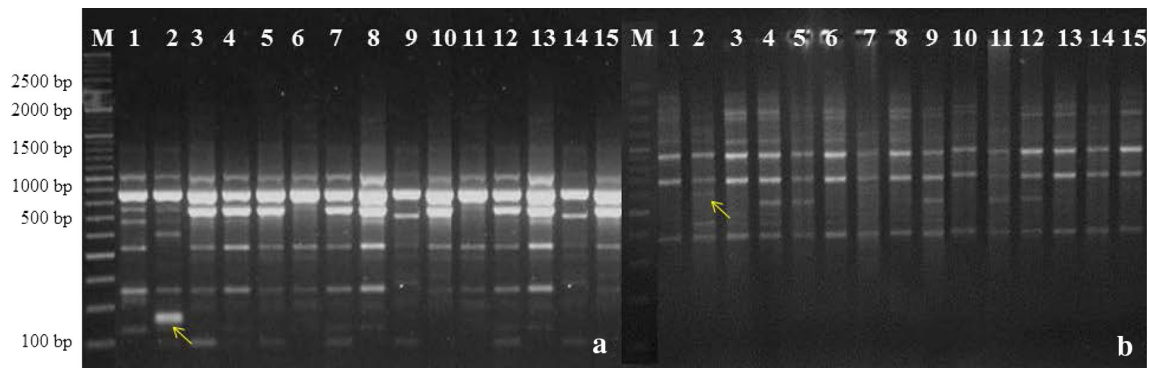
#### RAPD-based cluster analysis

Genetic distances were obtained with UPGMA algorithm using Dice coefficient (Table 3). The results observed in the present study showed similarity coefficient ranged from

**Table 2** Numerical data as obtained from PCR amplification by RAPD primers among *Costus pictus* accessions

Sr. no.	Primer name	Primer sequence 5'-3'	GC content (%)	Annealing temperature	Total number of bands	Total number of loci	Monomorphic loci	Polymorphic loci	Polymorphic %	Polymorphism	PIC	Molecular weight (bp)
1	OPK-07	AGCGAGCAAG	60	36	133	10	8	2	20.00	0.89	220–1500	
2	OPK-16	GAGCGTCGAA	60	36	283	24	15	9	37.50	0.95	160–2670	
3	OPK-18	CCTAGTCGAG	60	36	159	14	8	6	42.86	0.92	190–2580	
4	OPK-20	GTGTCGGGAG	70	38	154	16	8	8	50.00	0.91	280–2200	
5	OPM-01	GTTGGTGGCT	60	36	184	17	10	7	41.18	0.93	230–2640	
6	OPM-02	ACAACGCCTC	60	36	128	12	7	5	41.67	0.90	220–1720	
7	OPM-03	GGGGGATGAG	70	38	121	13	7	6	46.15	0.89	260–2930	
8	OPM-04	GGCGGTGTC	70	38	144	11	8	3	27.27	0.91	160–1800	
9	OPM-05	GGGAACGTG	67	37	156	14	8	6	42.86	0.90	120–2200	
10	OPM-06	CTGGGCAACT	60	36	139	12	8	4	33.33	0.90	130–3125	
11	OPM-07	CCGTGACTCA	60	36	138	12	7	5	41.67	0.90	245–2065	
12	OPM-08	TCTGTTCCCC	60	36	151	13	7	6	46.15	0.91	240–2100	
13	OPM-09	GTCTTGCGGA	60	36	162	13	10	3	23.08	0.91	160–2900	
14	OPM-14	TCTGGCGCAC	70	38	115	9	7	2	22.22	0.88	110–1350	
15	OPN-02	ACCAGGGGCA	70	38	109	9	6	3	33.33	0.87	110–2200	
16	OPN-04	GACCGACCCA	70	38	245	19	14	5	26.32	0.94	141–1600	
17	OPN-06	GAGACGCACA	60	36	128	12	7	5	41.67	0.90	190–3110	
18	OPN-07	CAGCCCAGAG	70	38	198	19	11	8	42.11	0.93	140–1050	
19	OPN-08	ACCTCAGCTC	60	36	92	7	5	2	28.57	0.85	390–1410	
20	OPN-09	TGCCGGCTTG	70	38	154	15	9	6	40.00	0.91	240–2480	
21	OPN-10	ACAAC TG GGG	60	36	239	20	14	6	30.00	0.94	140–1860	
22	OPN-13	AGCGTACTC	60	36	102	9	6	3	33.33	0.87	330–2160	
23	OPN-15	CAGCGACTGT	60	36	166	14	10	4	28.57	0.92	200–2420	
24	OPN-16	AAGCGACTTG	60	36	149	14	8	6	42.86	0.91	250–3190	
25	OPN-18	GGTGAGGTCA	60	36	194	15	11	4	26.67	0.93	170–3000	
Total					3943	343	219	124	–	–	–	–
Average/primer					157.72	13.72	8.76	4.96	35.57	0.91	110–3190 <sup>a</sup>	

<sup>a</sup> Range



**Fig. 1 a** RAPD profile in *Costus pictus* accessions generated by OPM-09 and **b** ISSR profile in *Costus pictus* accessions generated by UBC-809. (M 100 + 500 bp DNA ladder, 1 CP-1, 2 CP-2\*, 3 CP-3, 4

CP-4, 5 CP-5, 6 CP-6, 7 CP-7, 8 CP-8, 9 CP-9, 10 CP-10, 11 CP-11, 12 CP-12, 13 CP-13, 14 CP-14, 15 CP-15; \*accession-specific band)

**Table 3** Dice's similarity coefficient based on RAPD analysis in 15 *Costus pictus* accessions

Accessions	CP-1	CP-2	CP-3	CP-4	CP-5	CP-6	CP-7	CP-8	CP-9	CP-10	CP-11	CP-12	CP-13	CP-14	CP-15
CP-1	1.00														
CP-2	0.92	1.00													
CP-3	0.92	0.92	1.00												
CP-4	0.91	0.90	0.94	1.00											
CP-5	0.90	0.89	0.93	0.94	1.00										
CP-6	0.88	0.88	0.92	0.93	0.95	1.00									
CP-7	0.87	0.87	0.90	0.92	0.90	0.92	1.00								
CP-8	0.88	0.87	0.89	0.90	0.90	0.92	0.94	1.00							
CP-9	<b>0.86</b>	0.87	0.88	0.90	0.90	0.91	0.92	0.94	1.00						
CP-10	0.89	0.88	0.89	0.91	0.90	0.90	0.91	0.92	0.94	1.00					
CP-11	0.89	0.89	0.92	0.91	0.94	0.98	0.91	0.91	0.91	0.89	1.00				
CP-12	0.88	0.88	0.90	0.90	0.89	0.91	0.97	0.93	0.92	0.90	0.92	1.00			
CP-13	0.88	0.87	0.89	0.90	0.90	0.91	0.94	<b>0.99</b>	0.94	0.91	0.91	0.94	1.00		
CP-14	0.87	0.87	0.88	0.89	0.90	0.90	0.91	0.93	0.98	0.94	0.91	0.92	0.93	1.00	
CP-15	0.89	0.89	0.89	0.91	0.89	0.90	0.89	0.91	0.93	0.97	0.90	0.90	0.91	0.93	1.00

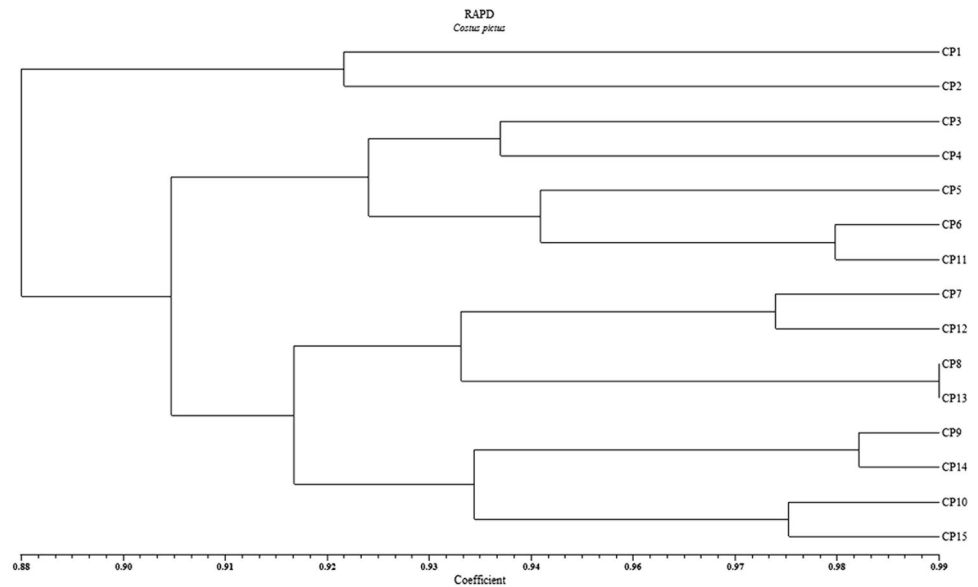
Bold values indicate higher and lower values of similarity index

0.86 (between OC-1 and OC-9) to 0.99 (between OC-8 and OC-13) with an average value of 0.91. The UPGMA cluster analysis based on RAPD data grouped 15 accessions into two main groups (Fig. 2). The cophenetic correlation (Matrix correlation) for RAPD markers was computed and demonstrated a very good fit ( $r = 0.90$ ). Group A consisted of single cluster I with two accessions CP-1 and CP-2. Group B sub divide into two clusters, cluster I consisted of 5 accessions and cluster II which enclosed 8 accessions illustrated similarity indices 92 and 91% respectively. Cluster I (B) consisted of two sub-clusters, I and II. Sub-cluster II includes CP-5, CP-6 and 11, which are of Gujarat region and CP-6 and CP-11 illustrated 97% similarity indices. Cluster II (B) include two sub-clusters each with 4 accessions, where in sub-cluster I accessions CP-8 and CP-13 showed 99% similarity coefficient.

#### ISSR marker analysis

Among the 26 ISSR primers tested in the study, 20 primers based on di, tri or tetranucleotide repeats were further used to characterize *C. pictus* accessions. The present study showed that out of 177 loci, 77 (42.47%) loci were polymorphic showing an average of 3.85 polymorphic loci per primer tested. The maximum polymorphic loci were generated by primer UBC 818 and UBC 889. A total number of 177 loci, ranged from 200 bp (UBC 826) to 3070 bp (UBC 847), were produced using the 20 primers previously screened (Table 5). Among the primers studied, UBC 818 generated the highest 13 loci, while UBC 812 generated the lowest 5 loci with an average of 8.85 loci per primer. The percentage of polymorphism among tested primers was ranged from 20% for primers UBC 812 and 63.64% for

**Fig. 2** UPGMA cluster analysis of 15 *Costus pictus* accessions with Dice's similarity coefficient of RAPD



primers UBC 889 with an average of 42.47% polymorphism per primer (Table 5). In this study, the highest PIC value of 0.91 for primer UBC 818 and the lowest PIC value of 0.77 for primer UBC 812, with an average value of PIC per primer 0.87, were obtained. Three ISSR primers viz., UBC-847, UBC-848 and UBC-808 produced accession-specific amplicons (Fig. 1b; Table 4).

#### ISSR-based cluster analysis

Similarity coefficient obtained by ISSR profile is shown in Table 6. Similarity coefficient was ranged from 0.84 to 0.95 with an average value of 0.88. In the present ISSR cluster analysis, the two most closely related accessions were found to be CP-8 and CP-13 (0.95), and the two most distantly related accessions were CP-1 and CP-15 with lowest similarity index (0.84). Cluster analysis of ISSR data based on the similarity matrix generated a dendrogram with two major groups (Fig. 3). The cophenetic correlation (Matrix correlation) for ISSR markers was computed and demonstrated a good fit ( $r = 0.74$ ). Group B consisted of only one cluster with two populations CP-10 and CP-15 with 91% similarity indices. First group A was divided into two clusters I and II with 87% similarity; cluster I has two accessions and cluster II has 11 accessions. Second cluster (A) was divided into two sub-clusters: the first sub-cluster has 5 accessions and the second sub-cluster has 6 accessions with 91% similarity indices.

#### Combined RAPD and ISSR analysis

Further, to have an overview of the genetic similarities between the accessions, data of both the primers RAPD and ISSR were used to generate genetic diversity (Table 7).

**Table 4** Accession-specific amplicons as revealed by RAPD and ISSR markers

Accession code	Characterized by the RAPD markers (bp)	Characterized by the ISSR markers (bp)
CP-4	OPK-18 (494)	UBC-847 (727)
CP-5	OPN-6 (166)	–
CP-2	OPN-7 (331)	UBC-848 (1,024)
	OPM-9 (188)	UBC-808 (1391)
CP-1	OPN-10 (1855)	–

Based on the combined data, dendrogram indicated that all the accessions were divided into two major groups: A and B with 2 and 13 accessions, respectively (Fig. 4). The first group A had only one cluster with two accessions CP-1 and CP-2 having 92% similarities, while group B was divided into two major clusters, cluster I and cluster II with 89% similarities. Cluster I (B) and cluster II (B) both have two sub-clusters with the similarity indices, 91 and 90%. Similarity coefficient for combined RAPD-ISSR ranged from 0.86 to 0.96 with an average value of 0.90 (Table 7). The cophenetic correlation (Matrix correlation) was also calculated based on the combined data and indicated a very good fit ( $r = 0.90$ ). The results showed that the two most closely related accessions were CP-9 (Karnataka) and CP-14 (Karnataka) with the highest similarity index (0.96). On the other hand, the most distinct accessions were CP-1 (Kolkata) and CP-15 (Maharashtra) at 0.86 similarity index.

#### Principal component analysis

Result of the PCA based on similarity coefficient of RAPD markers was comparable with its cluster analysis (Fig. 5).

**Table 5** Numerical data as obtained from PCR amplification by ISSR primers among *Costus pictus* accessions

St. no	Primer name	Primer sequence 5'-3'	GC content %	Annealing temperature	Total number of bands	Total number of loci	Monomorphic loci	Polymorphic loci	Polymorphic %	Polymorphism	PIC value	Molecular weight (bp)
1	UBC 808	AGAGAGAGAGAGAGC	53	60	99	8	3	5	62.50		0.87	370–2025
2	UBC 809	AGAGAGAGAGAGAGG	53	60	104	11	6	5	45.45		0.88	380–2190
3	UBC 811	GAGAGAGAGAGAGAC	53	63	60	6	3	3	50.00		0.79	450–2515
4	UBC 812	GAGAGAGAGAGAGAA	47	51	63	5	4	1	20.00		0.77	250–1700
5	UBC 818	CACACACACACACAG	53	53	253	13	6	7	53.85		0.91	260–2500
6	UBC 826	ACACACACACACACC	53	53	102	11	8	3	27.27		0.90	200–2220
7	UBC 834	AGAGAGAGAGAGAGYT	44	52.5	113	10	6	4	40.00		0.88	320–1880
8	UBC 835	AGAGAGAGAGAGAGYC	50	52.5	91	7	5	2	28.57		0.85	400–1790
9	UBC 841	GAGAGAGAGAGAGAY	47	52.5	88	7	4	3	42.86		0.84	640–2830
10	UBC 842	GAGAGAGAGAGAGAYG	50	53	122	9	4	5	55.56		0.89	355–2400
11	UBC 847	CACACACACACACARC	50	53	86	8	4	4	50.00		0.85	610–3070
12	UBC 848	CACACACACACACARG	50	52.5	101	8	6	2	25.00		0.86	335–2190
13	UBC 852	GTGTGTGTGTGTGYC	50	52	116	10	4	6	60.00		0.89	305–2725
14	UBC 866	CTCCTCCTCCTCCTC	67	57.5	94	9	4	5	55.56		0.87	305–2830
15	UBC 880	GGAGAGAGAGAGAGA	60	55.5	115	9	6	3	33.33		0.88	400–2580
16	UBC 883	TATATATATATABVB	0	36.8	105	8	5	3	37.50		0.87	400–2180
17	UBC 888	CACACACACACABDB	41	50.5	105	8	6	2	25.00		0.87	390–2000
18	UBC 889	ACACACACACACACDBD	42	51.5	108	11	4	7	63.64		0.89	385–2560
19	UBC 890	CTCTCTCTCTCTVHV	41	51	115	9	6	3	33.33		0.88	305–2770
20	UBC 891	TGTGTGTGTGTGHVH	41	51	121	10	6	4	40.00		0.89	310–2680
Total					2161	177	100	77	—		—	—
Average/primer					108.05	8.85	5	3.85	42.47		0.87	200–3070 <sup>a</sup>

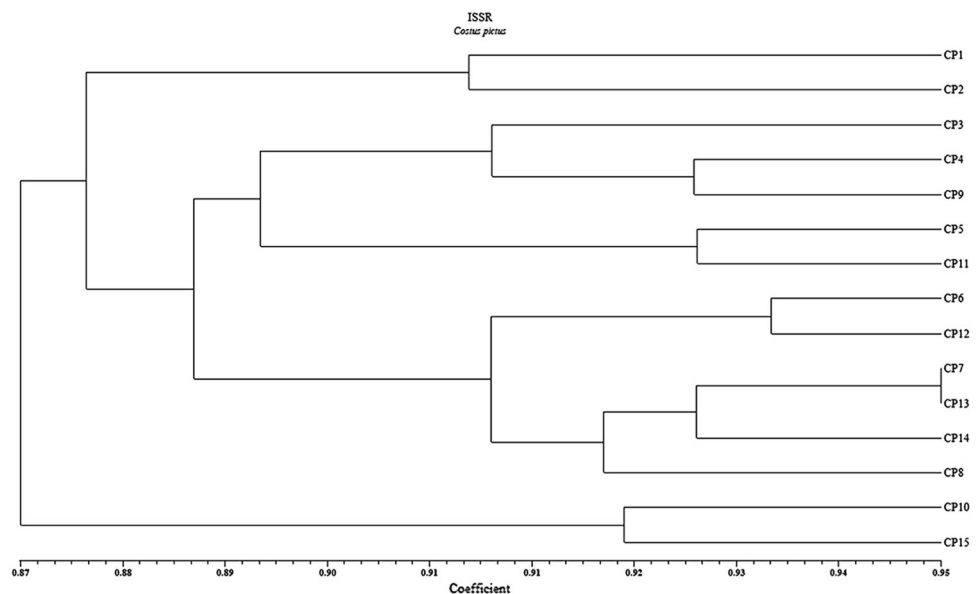
<sup>a</sup> Range

**Table 6** Dice's similarity coefficient based on ISSR analysis in 15 *Costus pictus* accessions

Accessions	CP-1	CP-2	CP-3	CP-4	CP-5	CP-6	CP-7	CP-8	CP-9	CP-10	CP-11	CP-12	CP-13	CP-14	CP-15
CP-1	1.00														
CP-2	0.91	1.00													
CP-3	0.87	0.86	1.00												
CP-4	0.89	0.88	0.91	1.00											
CP-5	0.87	0.86	0.89	0.86	1.00										
CP-6	0.87	0.88	0.86	0.85	0.91	1.00									
CP-7	0.87	0.87	0.88	0.88	0.88	0.92	1.00								
CP-8	0.89	0.88	0.91	0.91	0.88	0.88	0.93	1.00							
CP-9	0.91	0.91	0.91	0.93	0.89	0.90	0.89	0.91	1.00						
CP-10	0.85	0.86	0.89	0.87	0.85	0.84	0.88	0.88	0.85	1.00					
CP-11	0.86	0.85	0.91	0.91	0.93	0.88	0.88	0.89	0.89	0.87	1.00				
CP-12	0.87	0.87	0.87	0.86	0.89	0.94	0.92	0.89	0.87	0.87	0.89	1.00			
CP-13	0.88	0.89	0.87	0.86	0.88	0.93	<b>0.95</b>	0.90	0.89	0.87	0.88	0.94	1.00		
CP-14	0.89	0.88	0.90	0.88	0.88	0.91	0.93	0.93	0.92	0.88	0.90	0.89	0.93	1.00	
CP-15	<b>0.84</b>	0.85	0.89	0.89	0.88	0.87	0.89	0.88	0.85	0.92	0.91	0.88	0.88	0.87	1.00

Bold values indicate higher and lower values of similarity index

**Fig. 3** UPGMA cluster analysis of 15 *Costus pictus* accessions with Dice's similarity coefficient of ISSR



The PCA based on RAPD data revealed that the accessions belonging to a particular cluster were grouped together in the PCA plot (Fig. 5a). Interestingly, it showed that the accessions belong to same place state were clustered together, whereas some accessions also separated in PCA as presented by dendrogram. Similarly, ISSR-based PCA analysis revealed that the majority of accession was grouped as illustrated by the dendrogram (Fig. 5b). It is also evident from data of both the marker tools (RAPD + ISSR) that accessions were impartially distributed on PCA plots, which imitate a good genetic relatedness among the *C. pictus* accessions (Fig. 5c).

## Discussion

Although work on biochemical (pharmacological properties) and morphological characterization of *C. pictus* plant has been attempted extensively, studies on genetic diversity are scanty. Therefore, efforts have been made in the present study to characterize fifteen accessions of *C. pictus* collected from different part of India, using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers.

*Costus pictus* is introduced from the America due to its rich indigenous medicinal properties, so that information

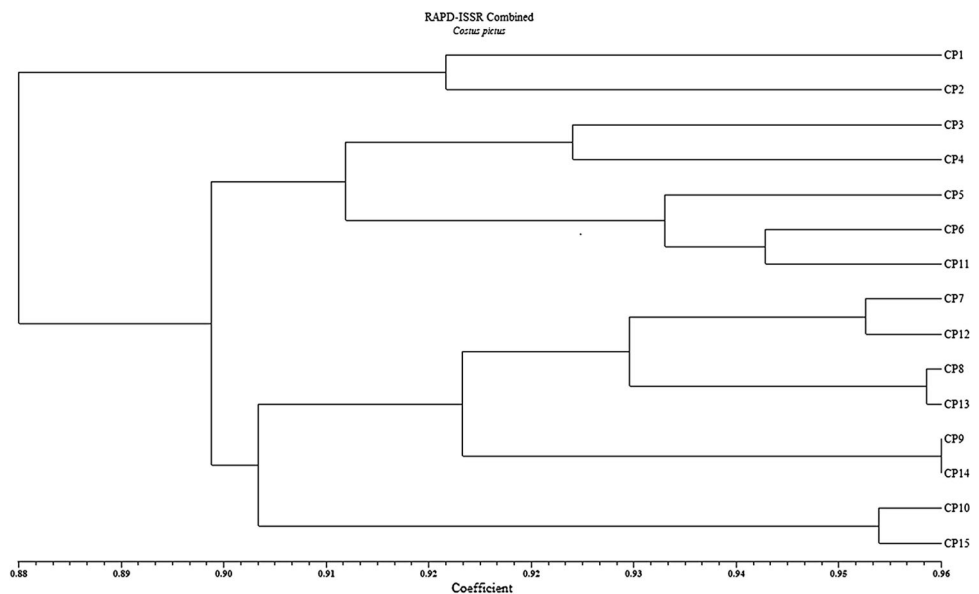


**Table 7** Dice's similarity coefficient based on combined RAPD-ISSR analysis in 15 *Costus pictus* accessions

Accessions	CP-1	CP-2	CP-3	CP-4	CP-5	CP-6	CP-7	CP-8	CP-9	CP-10	CP-11	CP-12	CP-13	CP-14	CP-15
CP-1	1.00														
CP-2	0.92	1.00													
CP-3	0.90	0.90	1.00												
CP-4	0.90	0.89	0.93	1.00											
CP-5	0.89	0.88	0.91	0.91	1.00										
CP-6	0.88	0.88	0.90	0.90	0.94	1.00									
CP-7	0.87	0.87	0.89	0.90	0.90	0.92	1.00								
CP-8	0.89	0.87	0.90	0.90	0.89	0.90	0.94	1.00							
CP-9	0.88	0.88	0.89	0.91	0.90	0.90	0.91	0.93	1.00						
CP-10	0.87	0.88	0.89	0.89	0.88	0.88	0.90	0.90	0.91	1.00					
CP-11	0.88	0.87	0.91	0.91	0.93	0.95	0.90	0.90	0.90	0.89	1.00				
CP-12	0.88	0.88	0.89	0.88	0.89	0.92	0.95	0.91	0.90	0.89	0.91	1.00			
CP-13	0.88	0.87	0.89	0.89	0.89	0.92	0.94	0.95	0.92	0.90	0.90	0.94	1.00		
CP-14	0.88	0.87	0.89	0.89	0.89	0.91	0.92	0.93	<b>0.96</b>	0.92	0.91	0.91	0.93	1.00	
CP-15	<b>0.86</b>	0.87	0.89	0.90	0.89	0.89	0.89	0.90	0.90	0.95	0.90	0.89	0.90	0.91	1.00

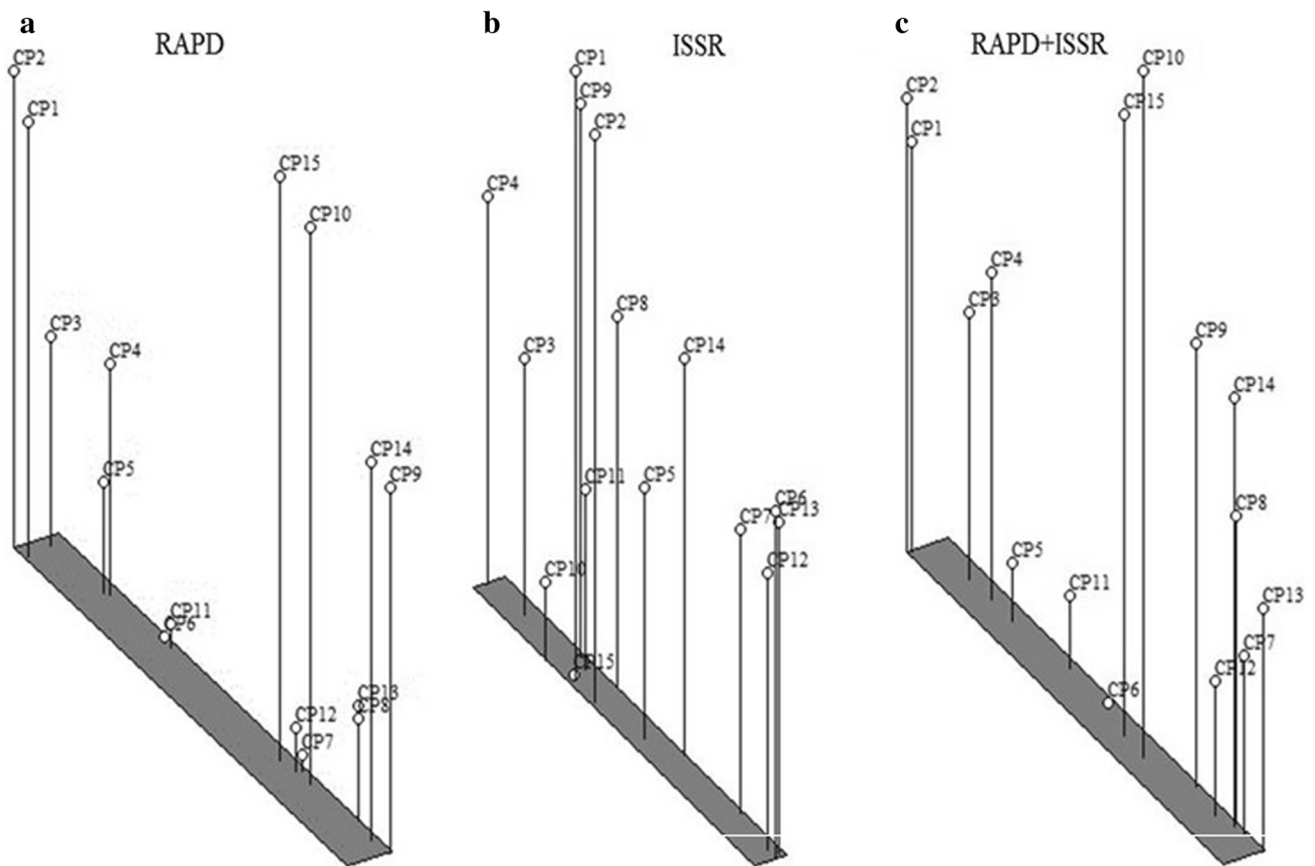
Bold values indicate higher and lower values of similarity index

**Fig. 4** UPGMA cluster analysis of 15 *Costus pictus* accessions with Dice's similarity coefficient combined RAPD + ISSR



on genetic structure and the diversity of populations is essential for their conservational program (Ganie et al. 2015). With the emergence of molecular biology techniques, DNA-based markers are now very useful tool for characterization of germplasm, assessment of genetic diversity, varietal identification, hybrid testing, clonal fidelity testing, genetic relationship, systemic and evolutionary biology, phylogenetic studies, gene tagging and marker-assisted selection (Huang and Han 2014). Molecular data can help the conservationists to investigate many tissues of concern such as gene pool fragmentation and genetic erosion (Barrett and Kohn 1991).

The present study on intrinsic genetic diversity analysis with both marker systems revealed that the higher level of polymorphism was obtained with ISSR (42.47%) than that of RAPD (35.57%). It was found that the degree of genetic diversity or polymorphism was relatively lower in *C. pictus* accessions, which indicates narrow gene pool. Our results are in accordance with the findings of Thomas and Palni (2016) who reported only 9.09% of polymorphism in six *C. pictus* accessions with RAPD markers. Another study conducted by Mandal et al. (2007) found 73.33% of polymorphism in 14 closely related accessions of *Costus speciosus* with RAPD markers. Moreover, low-level



**Fig. 5** Three-dimensional plot of principal component analysis of *Costus pictus* accessions based on **a** RAPD, **b** ISSR and **c** combined (RAPD + ISSR) analysis

polymorphism in ISSR and RAPD markers was also reported in many previous studies, for examples, (26.43% RAPD; 24.36% ISSR) in *Bruguiera gymnorrhiza* and (14.4% RAPD; 12.73% ISSR) in *Heritiera fomes* (Dasgupta et al. 2015). Higher reproducibility and stability of ISSR system than RAPD were found by Zietkiewicz et al. (1994). However, it was noted in the present study that high reproducibility for both RAPD and ISSR markers can be achieved when the PCR conditions are well defined.

The present study showed low level of polymorphism in *C. pictus* accessions. This may be due to the less number of primers and/or accessions used in this study which may not sufficient to capture the diversity. In addition, we can assumed that *C. pictus* had been travelled from one location to another than shifted to longer distance, then adapted and correlated with that environmental conditions prevailed over there. For instance, adaptability and evolution of any species are based on the level of genetic diversity present in populations, which reflect the richness of diverse germplasm in a specific environment or location. The percentage of polymorphic loci can be used as indicator to measure the degree of genetic diversity in *C. pictus*. From the genetic perspective, a higher level of genetic diversity

results in a greater ability to adapt and evolve (Li and Chen 2004). Low genetic variation could result in decreased adaptability and elevates the occurrence of less beneficial genes leading to eventual extinction of the species and thus the lower level of diversity within the populations should be paid special attention (Dasgupta et al. 2015).

The distribution of accessions to the dendrogram based on banding patterns of RAPD, ISSR and combined RAPD-ISSR may significantly differ because each technique amplifies different regions of genome. Interestingly, both the marker tools divided all *C. pictus* accessions into two main clusters. The combined RAPD-ISSR banding patterns increase the validity of the results as it allows to screen more segments site in the genome. Still, many studies concluded that ISSR would be a superior tool than RAPD for phylogenetic study (Marotti et al. 2007).

A close genetic similarity was observed in some of the accessions analyzed as shown by high values of similarity index. In this study, the average similarity index for *Costus* accessions using RAPD accessions was ranged from 0.87 to 0.99. While similarity index of the ISSRs ranged from 0.84 to 0.95. High range of similarity indices in both the samples for RAPD and ISSR markers clearly indicated that

there was low genetic variability between accessions collected from different geographical regions. The similarity index for combined RAPD-ISSR markers ranged from 0.87 to 0.96, which is higher than the ISSR-based markers indicating less diversity, while it was lower than the RAPD marker. This finding is consistent with the study in *Artemisia capillaris* (Shafie et al. 2011).

Accessions collected from the Gujarat (CP-5, CP-6 and CP-11) regions showed very low genetic diversity and grouped into same cluster with 93% similarities. Same kind of results was observed from the accessions collected from the Tamilnadu (CP-7 and CP-12), Kerala (CP-8 and CP-13) and Karnataka (CP-9 and CP-14). Although, accessions collected from Kolkata (CP-1) and Madhya Pradesh (CP-2) were genetically distant from other accessions collected from South-West regions of India. Similar results were also reported in many plant species (Rabbani et al. 1998; Dias et al. 1993). It was noted that accession collected from South regions of India showed high similarities among them and formed single cluster II with 90% similarities.

Due to the same geographical location, a high genetic similarity was expected among *C. pictus* accessions in the southern part of country. But the broad genetic base observed in this study indicates an earlier introduction of this species to south, and subsequently leading to accumulation of variation. The clusters in dendrogram might be correlated some extent of the geographical distribution, as revealed in the present results. These results are in agreement with some previous study, which concluded that the accessions from diverse geographical regions can be genetically similar (Jain et al. 2003). Additionally, recent introduction of the *C. pictus* accessions in the collection site which are of same ancestor could be the reason for genetic similarities between accessions (Jose and Reddy 2010). Mantle matrix correspondence test revealed high matrix correlation in all the cases. Correlation values for RAPD, ISSR and combined data were 0.90, 0.74 and 0.90, respectively, which represent good to fit. The PCA was used to demonstrate the multiple dimension distribution of the accessions in a scatter-plot. Some differences were observed in the separation of individual accessions to their respective clusters as obtained from the UPGMA dendrogram as well as from PCA for both the markers. This multivariate approach was used to complement the information obtained from the cluster analysis methods because it is more informative regarding distances among major groups (Taran et al. 2005).

Because of its valuable medicinal properties, *C. pictus* needs more attention toward the conservation program. Sustainable management strategies can be developed by the understanding of the population structure of *C. pictus*. Even though there are no more molecular data to support the present analysis, it appears that identification based on

the morphology alone cannot correctly designate different accessions of the same species. Studies at the molecular level are necessary to understand genetic diversity and relationships among accessions. Such discrepancies were also observed in rice (Virk et al. 1995).

## Conclusion

On the basis of findings of present study, it can be concluded that genetic variability in the studied accessions of *C. pictus* is low, though accessions were collected from a diverse geographical regions. This might be due to the deployment of a small set of markers and accessions to capture the diversity. However, accession-specific amplicons generated by RAPD and ISSR primers could be converted to co-dominant SCAR marker. As significant research has not been performed, there is a scope of research at genetic level to understand the genetic pools, to develop conservation strategies and breeding programs for this newly introduced plant. Thus, above results can be the beginning for future research to understand the genetic variability of *C. pictus*.

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**Author contributions** AN Collected samples and conducted main experiment; PP Analyzed the data and critically reviewed the manuscript; RK Reviewed the manuscript and provided all the facilities to conduct this experiment; JMP Helped in experimental setup and analyzed the data.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they do not have any conflict of interest.

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