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Evaluation of genetic diversity in turmeric (*Curcuma longa* L.) using RAPD and ISSR markers

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ABSTRACT

Turmeric (*Curcuma longa* L.) is an industrially important plant used for production of curcumin, oleoresin and essential oil. In the present study we examined the genetic diversity among turmeric accessions from 10 different agro-climatic regions comprising 5 cultivars and 55 accessions. Two DNA-based molecular marker techniques, *viz.*, random amplified polymorphism DNA (RAPD) and inter simple sequence repeat (ISSR) were used to assess the genetic diversity in turmeric genotypes. A total of 17 polymorphic primers (11 RAPDs and 6 ISSRs) were used in this study. RAPD analysis of 60 genotypes yielded 94 fragments of which 75 were polymorphic with an average of 6.83 polymorphic fragments per primer. Number of amplified fragments with RAPD primers ranged from 3 to 13 with the size of amplicons ranging from 230 to 3000 bp in size. The polymorphism ranged from 45 to 100 with an average of 91.4%. The 6 ISSR primers produced 66 bands across 60 genotypes of which 52 were polymorphic with an average of 8.6 polymorphic fragments per primer. The number of amplified bands varied from 1 to 14 with size of amplicons ranging from 200 to 2000 bp. The percentage of polymorphism using ISSR primers ranged from 83 to 100 with an average of 95.4%. Nei's dendrogram for 60 samples using both RAPD and ISSR markers demonstrated an extent of 62% correlation between the genetic similarity and geographical location. The result of Nei's genetic diversity (*H*) generated from the POP gene analysis shows relatively low genetic diversity in turmeric accessions of South eastern ghat (P7), Western undulating zone (P8) with 0.181 and 0.199 value whereas highest genetic diversity (0.257) has been observed in Western central table land (P9). Knowledge on the genetic diversity of turmeric from different agro-climatic regions can be used to future breeding programs for increased curcumin, oleoresin and essential oil production to meet the ever-increasing demand of turmeric for industrial and pharmaceutical uses.

1. Introduction

Turmeric (*Curcuma longa* L. Zingiberaceae), an industrially important crop widely cultivated and highly exportable spice in India, having unique chemical and physical properties and broadly used for numerous industrial applications, such as manufacture of canned beverages, baked products, dairy products, ice cream, yogurt, yellow cakes, orange juice, biscuits, popcorn color, sweets, cake icings, cereals, sauces, gelatins, and curry powders. Turmeric is used as a food additive, preservative and coloring agent in Asian countries (Antunes and Araujo, 2000; Cecílio-Filho et al., 2000). In addition, turmeric has also a variety of pharmacological activities, with recent findings which shows that curcumin, the yellow color pigment of turmeric, is a powerful antioxidant, anti-parasitic, antispasmodic and anti-inflammatory compound, which may also inhibit carcinogenesis (Araujo and Leon, 2001; Ravindran, 2007).

Indian enjoys monopoly in turmeric production and export. Because of its ever-increasing demand in both food and pharmaceutical industries, there is pressing need to further increase the productivity of turmeric. However, to obtain further increases in productivity, information regarding the crop's genetic diversity is essential for breeding programs (Nass, 2001). Turmeric is a cross-pollinated, triploid species ($2n = 3x = 63$), which can be vegetatively propagated using its underground rhizomes (Sasikumar, 2005). Since hybridization is ineffective in most cases, genetic improvement work on turmeric is mostly confined to germplasm selection (Ravindran, 2007). Even though germplasm collections and characterization is prerequisite for genetic improvement of turmeric, such studies are scarce and mostly restricted to the phenotypic evaluation of accessions from different states of India including Orissa, which is the second largest producer of turmeric in India (Chaudhary et al., 2006). The use of phenotypic traits in germplasm characterization is also limited due to the availability of small number of descriptors. Characterization of promising turmeric cultivars/accession by morphological data and qualitative traits like curcumin, oleoresin and essential oil content are not sufficient as

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these characters often change under varying environmental conditions thus raising problem in proper identification and elimination of synonyms.

Predominance of synonyms possesses problems in identification and characterization of germplasm. Many synonyms can be removed by molecular characterization thus reducing the cost of maintenance of redundants in clonal repositories. Due to resurgence of interest in the commercial production of different cultivars of turmeric as new spice crops, it has become necessary to precisely characterize the genetic variations that exist in cultivars, advanced selection and native population. This is one step toward providing accurate genetic information for future breeding program for turmeric improvement. Molecular markers (RAPD, ISSR, SSR, *etc.*) unlike morphological and biochemical markers are not prone to environmental influence and accurately characterize the plants portraying the extent of genetic diversity among taxa (Thimmappaiah et al., 2009; Cheng and Huang, 2009). Of the different molecular markers RAPD and ISSR has been widely used in the last two decades in cultivar identification program (Ebrahimi et al., 2009) and assessing genetic variations among different taxa at DNA level because of their cost effectiveness and simple operation without requiring prior knowledge of species DNA sequences (Williams et al., 1990) and can provide vital information for development of genetic sampling, conservation and improvement strategies (Chalmers et al., 1994). No report has yet been published so far on agro-climatic zone based genetic characterization of existing promising cultivars/accession of turmeric using molecular markers. Available report on detection of genetic variation in 17 promising cultivars of turmeric by (Nayak et al., 2006) necessitate more detail work on genetic characterization of turmeric cultivars and accessions from 10 different agro-climatic regions of Orissa using RAPD and ISSR markers.

2. Materials and methods

2.1. Plant material and DNA extraction

The present investigation deals with 55 turmeric accessions and five cultivars collected from ten different agro-climatic zones (P1–P10) of India including five cultivars collected from High Altitude Research Station, Pottangi of Orissa University of Agriculture and Technology, Koraput (Table 1). They were maintained in the greenhouse of Centre of Biotechnology of Siksha 'O' Anusandhan University. Fresh leaf samples were frozen in liquid nitrogen and preserved in −85 °C freezer for molecular analysis. Genomic DNA was isolated following the protocol of Doyle and Doyle (1990) with little modification. Fresh leaves (2 g) were ground in liquid nitrogen and taken into 50 ml of centrifuge tube. To the ground sample 10 ml of extraction buffer [4% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris–Cl, 4 M NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 2% mercaptoethanol and 2% polyvinyl pyrrolidone, pH 8] was added and incubated at 65 °C for 1 h. Then above sample was extracted with equal volume of chloroform:isoamyl alcohol (24:1), supernatant was treated with RNase at 60 μg ml^{-1} and DNA was extracted with Tris saturated phenol. The supernatant after extraction with Tris saturated phenol was taken and extracted further with chloroform:isoamyl alcohol (24:1) twice more, and the DNA was precipitated with 80% ethanol. The pellet was air dried and resuspended in 100 μl of Tris–EDTA (TE Buffer). Purified total DNA was quantified in 0.8% agarose gel along with known amount of uncut lambda DNA (Bangalore Genei Pvt. Ltd., Bangalore, India) as standard. The sample DNA was diluted as 25 ng μl^{-1} for RAPD and ISSR analysis.

Table 1
Details of the sampling areas of turmeric from different agroclimatic regions of India.

Agro-climatic zone (code)	Place of collection	Accession no.
North western plateau (P1)	Sundargarh	P1-1
	Deogarh	P1-2
	Redhakhol	P1-3
	Titlagarh	P1-4
	Jharsuguda	P1-5
North central plateau (P2)	Patbil	P2-1
	Mayurbhanj	P2-2
	Ghasipura	P2-3
	Padiabeda	P2-4
	Anandpur	P2-5
	Palashpala	P2-6
	Keonjhar	P2-7
North eastern coastal plain (P3)	Panikoili	P3-1
	Boudh	P3-2
	Bhadrak	P3-3
	Nilgiri	P3-4
	Jaleswar	P3-5
	Soro	P3-6
	Hatdihi	P3-7
East and south eastern coastal plain (P4)	Khurda	P4-1
	Athagarh	P4-2
	Niali	P4-3
	Kandarpur	P4-4
	Rahama	P4-5
	Puri	P4-6
	Nayagarh	P4-7
	Ganjam	P4-8
North eastern ghat (P5)	Draingabadi	P5-1
	G. udaigiri	P5-2
	Tikabali	P5-3
	Phulabani	P5-4
	Koraput	P5-5
	Rayagada	P5-6
	Parlakhemundi	P5-7
	Gajapati	P5-8
Eastern ghat highland (P6)	Potangii (cv Surama)	P6-1
	Potangii (cv Roma)	P6-2
	Potangii (cv Ranga)	P6-3
	Pottangi (cv Rasmi)	P6-4
	Pottangi (cv Lakadong)	P6-5
	Nabarangpur	P6-6
South eastern ghat (P7)	Bandaghati	P7-1
	Keonjhar	P7-2
	Malkangiri	P7-3
	Patbil	P7-4
Western undulating zone (P8)	Bhawanipatna	P8-1
	Nuapada	P8-2
	Kalahandi	P8-3
	Kahdhamal	P8-4
Western central table land (P9)	Balangir	P9-1
	Barapalli	P9-2
	Boudh	P9-3
	Sonepur	P9-4
	Sambalpur	P9-5
	Jharsuguda	P9-6
	Kuchinda	P9-7
Mid central (P10)	Angul	P10-1
	Dhenkanal	P10-2
	Cuttack	P10-3
	Jajpur	P10-4

2.2. RAPD amplification

Eleven random decamer primers (Operon Tech, USA) from A, C, D and N series (OPA04, 07, 08, 09, 18; OPC02, 05, 11; OPD08, 16, 18, 20; and OPN04, 06, 16,) were used for RAPD analysis. RAPD were performed in a final volume of 25 μl containing 25 ng of template DNA, 2.5 μl of 10× assay buffer (100 mM Tris–Cl, pH 8.3, 500 mM KCl, and 0.1% gelatin), 15 mM $MgCl_2$, 200 μM of each dNTPs, 15 ng of primer and 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The RAPD analysis was performed as per the methodology described by (Williams et al., 1990) using Gene Amp