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RESEARCH ARTICLE

RELATIONSHIPS AMONG SIX MEDICINAL SPECIES OF CURCUMA ASSESSED BY RAPD MARKERS

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ABSTRACT

The genus *Curcuma* is a member of the ginger family (Zingiberaceae) they are native to Southeast Asia, southern China, the Indian Subcontinent, New Guinea and northern Australia, tropical Africa, Central America and Florida. The aim of this study was to assess the genetic variation and relationships between six varieties of *curcuma* species using PCR-based molecular markers (RAPD). Random amplified polymorphic DNA (RAPD) markers were applied to detect the genetic relationships and diversity among six *Curcuma* species, which have medicinal properties. Two polymorphic primers (OPC-4, OPC-7) were evaluated for genetic diversity studies. DNA was extracted using CTAB method was used directly in PCR DNA at 30 ng, primer concentration of ~30 pic moles, 200 µm dNTPs and 2 units of Taq DNA Polymerase at 35 PCR cycles gave better result for developing RAPDs in *Curcuma* species. Among the two primers, highest number of bands were amplified in *Curcuma longa* (S 1) (primer OPC-7). Matching coefficients produced a total of Six groups in the *Curcuma* species studied using the RAPD markers with maximum similarity between *C. longa* and *C.amada*. The study was undertaken to identify different *Curcuma* species using RAPD markers. The original Gawl and Jarret protocol for DNA extraction was modified to get good quality of DNA from rhizome and PCR conditions for good amplification was standardized with DNA from rhizome. This investigation was an understanding of genetic variation within the accessions. It will also provide an important input into determining resourceful management strategies and help to breeders for ginger improvement program.

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INTRODUCTION

The genus *Curcuma* is a member of the ginger family (Zingiberaceae) comprises more than 80 species of rhizomatous perennial herbs and has a widespread occurrence in the tropics of Asia and extends to Africa and Australia (Purseglove, et al., 1981). It is endowed with widespread adaptation from sea level to an altitude as high as 2000 m in the Himalayas. A total of 40 species of *Curcuma* occur in India. Commonly known as turmeric, *Curcuma longa* L. (syn. *C. domestica* Val.) is a commercially important spice crop (Velayudhan et al., 1994) and curcumin is also used in medicine (Maheshwari et al., 2006). *Curcuma* is gaining importance worldwide as a potential source of new drug(s) to combat a variety of ailments as the species contain molecules credited with anti-inflammatory, hypocholestraemic, choleric, antimicrobial, insect repellent, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic and antihepatotoxic properties, as well as anticancerous properties (Sasikumar B et al., 2005).

Many workers have been reported the genetic diversity among zingiber and curcuma species but the studied species are area

specific based on their availability in that region (Das A et al., 2011; Jatoi SA et al., 2006; Syamkumar S et al., 2007; Sasikumar B et al., 2005). DNA-based molecular markers show differences in nucleotide sequences of DNA, which are now well established as powerful and versatile tools in the fields of Plant Breeding, Taxonomy, Physiology and Genetic Engineering (Kesawat MS et al., 2009).

Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and population. The random amplified polymorphic DNA (RAPD) technique is a popular tool in genetic studies (Williams JGK et al., 1990). RAPD markers provide a rapid, inexpensive and effective system for studying plant genetic relationships.

RAPD have been used to detect some *Curcuma* plants (Xiao XH et al., 2000; Syamkumar S et al., 2007). The objectives of this paper are to estimate the genetic diversity of six medicinal species of *Curcuma* distributed in Andhra Pradesh by using RAPD analysis and provide a basis for identifying these species shown in Table 1.

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MATERIAL AND METHODS

Plant material

The plant materials used in present study was collected from (Gudala, Allavaram and Amalapuram) Andhra Pradesh. Freshly collected plant materials were dried under shade and the dried material was milled to obtain a coarse powder. Genomic DNA was extracted from all the samples as per the protocol (Remya *et al.*, 2004).

Table 1 The origin of materials used in this study

S.No.	Taxon	Origins	Notes wild
1.	<i>Curcuma longa</i>	Gudala, Amalapuram	Wild
2.	<i>Curcuma longa</i>	Gudala, Amalapuram	Wild
3.	<i>Curcuma amada</i>	Allavaram, Amalapuram	Cultivated
4.	<i>Curcuma aromatic</i>	Allavaram, Amalapuram	Cultivated
5.	<i>Hedychium coronarium</i>	Allavaram, Amalapuram	Wild
6.	<i>Curcuma zedoaria</i>	Allavaram, Amalapuram	Wild

DNA extraction

Fresh rhizomes of the *Curcuma* varieties were used for the extraction of DNA. Tiny pieces of curcuma (100 to 200 mg) were transferred to a 1.5 ml reaction tube containing 500µl extraction buffer (10mM tris HCl.ph8.0, 20mM EDTA and 1.4M NaCl) and 20 µl Proteinase K (20 mg/ml). Incubation was at 52 °C for 1 hour on a thermo block. After incubation DNA was extracted using CTAB method. DNA was either used directly in PCR. This DNA sample was used for subsequent PCR reactions.

PCR amplification

The PCR is set up with 20pM of RAPD primers. The reaction is setup in 20ul of reaction volume with final concentration of 50mM KCl, 10mM Tris, and 200µM dNTP's, 1.5mM MgCl₂ and 2.5µg template DNA. Reaction were setup under cycling parameters 95⁰ C for 5min, 95⁰ C for 1min, 42⁰ C for 45sec, 72⁰ C for 45sec and final extension 72⁰ C for 10 min. the number of PCR cycles was 60. After PCR, the samples were subjected to 2% agarose gel electrophoresis at 100V for 20-30 minutes. Ethidium Bromide stained gels were visualized under UV light and were documented. Band sizes were compared to molecular weight marker of 100-1000bp for confirmation.

RAPD data analysis

Photographs were used to score the RAPD data. For each material of the x primer combination, the presence (1) or absence (0) of an amplified fragment was treated as an independent character without considering the quantitative aspects of the results, that is, band intensity. The data matrix was entered into the NTSYS-pc program (Rohlf FJ *et al.*, 1993).

RESULTS AND DISCUSSION

The method of DNA extraction from fresh rhizome samples of *Curcuma* species included two major steps, (i) treatment by DNA extraction buffer, and (ii) grinding of fresh leaves in the buffer, after incubation. Using this method of DNA extraction,

we got DNA yield of 1.0~2.0 µg per single sample. The quality and quantity of DNA was obtained with normal methods. The purity of DNA determined from the A260/280 ratio ranged from 1.63 to 2.58 and averaged 2.32 for all the samples. The Random Amplified Polymorphic DNA method is based on the Polymerase Chain Reaction (PCR) using short (usually ten nucleotides) primers of arbitrary sequences. Polymorphism of amplified fragments is caused by: (a) base substitution or deletion in the priming sites, (b) insertion that renders priming sites too distant to support amplification, or (c) insertion or deletion that changes the size of the amplified fragment (Williams JGK *et al.*, 1990). Our results are in conformity with those reported by (Thaikert R *et al.*, 2009; Angle GR *et al.*, 2008). For *Curcuma* species, efficacy of isozyme markers for diversity analysis has been reported among six early flowering species of *Curcuma* (Apavatjirut, P., *et al.*, 1999). Two RAPD primers were used for the present work. Among the two primers highest number of bands were amplified in *Curcuma longa* (S 1) (primer OPC-7) and lowest number of bands were amplified in primer OPC-7 in *Curcuma amada* (S3) (primer OPC-7). Of the 2 primers tested, 21 (70%) produced polymorphic fragments in OPC-4 primer. Matching coefficients produced a total of Six groups in the *Curcuma* species studied using the RAPD markers with maximum similarity between *C. longa* and *C. amada*. Slight similarity was also observed in *C. longa* and *H. coronarium* with OPC-4 primer shown in Figure 1.

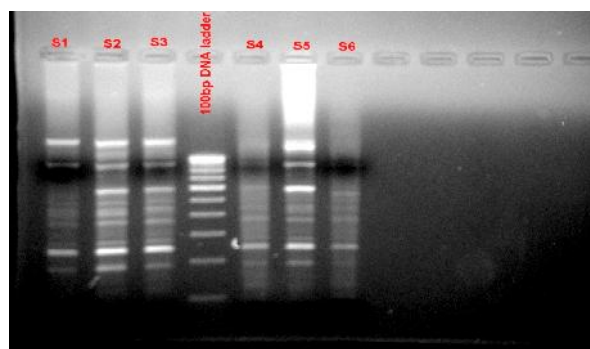


Fig 1 RAPD banding pattern of *Curcuma* species with OPC-4 primer. Lane 1 = *Curcuma longa*, Lane 2 = *Curcuma longa*, Lane 3 = *Curcuma amada*, lane M = molecular weight marker 100bp ladder DNA, Lane 4 = *Curcuma aromatic*, Lane 5 = *Hedychium coronarium*, Lane 6 = *Curcuma zedoaria*

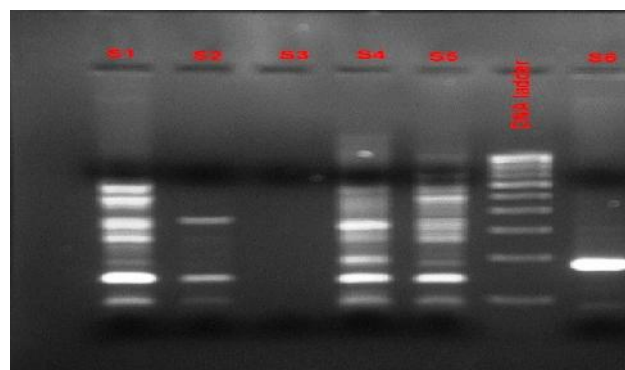


Fig 2 RAPD banding pattern of *Curcuma* species with OPC-7 primer. Lane 1 = *Curcuma longa*, Lane 2 = *Curcuma longa*, Lane 3 = *Curcuma amada*, Lane 4 = *Curcuma aromatic*, Lane 5 = *Hedychium coronarium*, lane M = molecular weight marker 100bp ladder DNA, Lane 6 = *Curcuma zedoaria*

Various primers produced different bands from the same template DNA with OPC-7 primers as shown in Figure 2 shows the results of amplification. The OPC-7 primers produced bands ranging from 3 to 8 bands shown in Figure 2. The molecular size of the bands ranged from 500 to 3400 bp. The number of bands generated per primer ranged from five in primer OPC-4 to 10 in primers OPC-7; all the bands generated by each primer were polymorphic. The molecular size of the bands ranged from 10kb to 50kb. The numbers of bands produced by each primer, the number of polymorphic bands and the percentage of polymorphism are shown in Figure 1 and 2. It was observed that total bands were observed highest for OPC-04(86.00) followed by OPC- 07(48.00).

For developing a molecular marker based technology for the identification of the seed rhizome, another modified CTAB protocol was developed to isolate good quality DNA from fresh mature rhizomes of turmeric. The yield of the DNA from fresh mature rhizomes ranged from 10.75 - 14.44 µg DNA with a purity ranged from 1.83-1.87. Two dominant molecular markers viz. RAPD were used for the molecular characterization of Six *Curcuma* species. This suggest that these cultivars are genetically very similar or got collected as distinct accessions based on vernacular names. *Curcuma* species was a vegetatively propagated crop; rhizomes are used as planting material. All the varieties/cultivars are not easily discriminated based on aerial or rhizome morphology. This could lead to unscrupulous seed trade practice in this crop. A protocol based on rhizome DNA for varietal identification could be thus more relevant. In this study, the same species that came from different localities reveal that the genetic relationships of these *Curcuma* species are not associated with their geographical distribution, and there is no separation of cultivated populations from wild populations.

The RAPD analysis in the present study has proven to be successful in revealing the diversity within and among the species of *Curcuma* as also reported earlier in *Curcuma* species (Syamkumar S *et al.*, 2007; Sasikumar B *et al.*, 2005). Detailed knowledge about genetic relationships between wild and cultivated species of *Curcuma* will enhance the utilization value of wild species for any future study. The present investigation clearly demonstrated that the Six *Curcuma* varieties could be distinguished by these RAPD primers, showing a high level of polymorphism with the RAPD technique, which suggests that this marker amplification technique can be a useful and serve as a potentially powerful tool for genotyping studies in *Curcuma*.

CONCLUSION

Matching coefficients produced a total of Six groups in the *Curcuma* species studied using the RAPD markers with maximum similarity between *C. longa* and *C. amada*. Cultivars Gudala, Amalapuram and Allavaram, Amalapuram showed maximum similarity in the bands constructed using RAPD markers. RAPD markers confirm genetic relationships among *Curcuma* species and the genera previously reported. Therefore, RAPD is also a reliable method for estimating phylogenetic relationships since it reflects coding and non-coding regions of the genome and it could well be used in

aiding identification as well as classification of the Zingiberaceae using more species in each genera. The present investigation was an effort to use RAPD markers as a tool to study the interrelationships between different species of *curcuma*. It was understood that each location varied with respect to environmental factors and genetic parameters. Results showed that the accessions whose cultivation regions are very close shows maximum similarity among them as compared to accessions which are farther apart. This outcome is supported by who established that main cause of polymorphism could be intraspecific variation among different cultivars (Nayak S., *et al.*, 2006).

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