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Development, characterization and utilization of genomic microsatellite markers in turmeric (*Curcuma longa* L.)

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ABSTRACT

Development of a robust set of 18 genomic microsatellite markers from turmeric (*Curcuma longa* L.) and its effective utilization in estimating the genetic diversity of 20 turmeric accessions are described. A total of 103 alleles were detected with an average of 5.7 alleles per locus. These markers displayed varied levels of polymorphism as evident from its discriminating power ranging from 0.19 to 0.70. The UPGMA cluster analysis of genetic distance values resolved the 20 turmeric accessions into five main groups. Three sets of genetically identical accessions were detected within the analyzed accessions, suggesting a revisit of the germplasm collection strategy based on vernacular identity. The entire grouping pattern of the entities was loose and independent of their geographical origins. These polymorphic SSR markers would be useful for the population genetic studies and germplasm management of turmeric.

1. Introduction

Turmeric (*Curcuma longa* L. syn. *Curcuma domestica* Val.), family Zingiberaceae, is a pan tropical crop cultivated widely in South East Asia. Due to its multitude use as spice, natural dye, food preservative and therapeutic agent (Sasikumar, 2005), it is gaining high demand in food, cosmetic and pharmaceutical industries. Turmeric is considered to be a triploid [$2n = 3x = 63$; $x = 21$] (Ramachandran, 1961; Islam, 2004), though a recent report based on the flow cytometric data and chromosome counts suggested a new ploidy status [9x] to turmeric by defining a new basic chromosome number [$x = 7$], but without contradicting the triploid status (Skornickova et al., 2007). Though turmeric is propagated clonally, viable sexual reproduction is also reported (Sasikumar et al., 1996). Lack of clear cut morphological traits among turmeric cultivars coupled with vernacular identity of the germplasm collection results in accumulation of duplicates in the germplasm accessions (Shamina et al., 1998), taxing heavily on conservation cost and hampering the crop improvement work. Existence of synonymous/homonymous entities within the crop (Shamina et al., 1998; Syamkumar, 2008) and genus *Curcuma* have been reported (Liu and Wu, 1999; Syamkumar and Sasikumar, 2007; Syamkumar, 2008).

Molecular markers could complement the conventional morphological studies for the discrimination of turmeric cultivars by providing a genetic background for the observed phenotypic variability. Molecular markers employed for characterization studies of turmeric are limited to the application of RAPD (Salvi et al., 2001; Panda et al., 2007; Tyagi et al., 2007), RAPD and or ISSR markers (Nayak et al., 2006; Hussain et al., 2008; Syamkumar, 2008; Vijayalatha and Chezhiyan, 2008), isozyme markers (Shamina et al., 1998) and SSR markers (Sigrist et al., 2010).

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Among the robust class of molecular markers, microsatellites or SSRs (simple sequence repeats) are widely employed for the accurate genotyping in a variety of plant species. These markers are characterized by the presence of 1–6 nucleotide repeats (Gupta et al., 1996), found within the coding and non coding regions (Tautz and Renz, 1984; Toth et al., 2000) of the genome with co-dominant, hyper variable and multiallelic nature (Powell et al., 1996). The development of SSR markers for a new species is time consuming and labour intensive (Zane et al., 2002; Squirrell et al., 2003). However, once developed, these markers provide endless high-throughput applications in molecular breeding by providing accurate, cost effective and reliable genotyping. Genomic SSR markers have been developed in other economically important spice crops such as *Zingiber officinale* (Lee et al., 2007), *Vanilla planifolia* (Bory et al., 2008) and *Piper nigrum* (Menezes et al., 2009). The development of 17 EST-SSR (Siju et al., 2010) and 17 genomic SSR (Sigrist et al., 2010) markers has been recently reported in turmeric. The present study was conducted to generate robust sets of reproducible and polymorphic microsatellite markers from a small-insert genomic library for its effective utilization in the germplasm characterization of turmeric.

2. Materials and methods

2.1. Plant materials and genomic DNA isolation

Genomic DNA was extracted from the fresh leaves of 20 turmeric accessions (Table 1), collected from different geographical locations of India and maintained at the Indian Institute of Spices Research (IISR) Experimental farm, Peruvannamuzhi, Calicut, India following the protocol of Syamkumar et al. (2003). The purity and concentration of the isolated DNA was estimated using Biophotometer (Eppendorf, Hamburg, Germany). The final DNA concentration was adjusted to 25 ng/μl for PCR analysis.

2.2. Construction of a small-insert microsatellite enriched genomic DNA library

A small-insert genomic DNA library enriched for the microsatellite repeat (AG)*n* in turmeric was constructed following the protocol of Glenn and Schable (2005). Briefly, the genomic DNA was digested with *Rsa* I restriction enzyme and ligated into double stranded SuperSNX linkers (SuperSNX24 forward – 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′ and SuperSNX24+4P reverse -5′-pGATTCTGCTAGCTAGGCCTTAAACAAAA-3′). Linker-ligated DNA was denatured and hybridized to 3′ biotinylated oligonucleotide probe – $(AG)_{12}$. The hybridized probe-microsatellite containing DNA fragments were captured on streptavidin coated magnetic beads (Dynal Biotech ASA, Oslo, Norway). The hybridized DNA fragments were eluted from the beads, amplified by PCR using SuperSNX24 forward primer, cloned into pTZ57R/T vector (Fermentas, UAB, Lithuania) and transformed into One shot® TOP10 chemically competent *Escherichia coli* cells (Invitrogen, USA). Recombinant clones were identified using blue/white screening on LB agar plates containing ampicillin, X-gal and IPTG. Presence of inserts in the positive clones was confirmed using colony PCR with vector specific M13 forward (5′-GTAAAACGACGGCCAGT-3′) and M13 reverse (5′-CAGGAAACAGCTATGAC-3′) primers. Plasmid DNA of selected clones was isolated using Fastplasmid mini kit (5 prime, Hamburg, Germany) and sequenced at the Bioserve Biotechnologies, Hyderabad, India.

Table 1
List of turmeric accessions used for genetic diversity analysis indicating their accession number, identity and place of collection from India.

Sl. no:	Accession number	Accession identity	Place of collection (state, location in India)
1	307	Agarthala	Tripura, North-East India
2	1	Alleppey	Kerala, South India
3	2	Amalapuram	Andhra Pradesh, South India
4	203	Arunachal	Arunachal Pradesh, North-East India
5	17	Avanigadda	Andhra Pradesh, South India
6	52	Ayur	Kerala, South India
7	560	Coimbatore	TamilNadu, South India
8	11	Dhagi	Assam, North-East India
9	290	Dibrugarh	Assam, North-East India
10	71	Gaspani	Nagaland, North-East India
11	16	Gorakhpur	Uttar Pradesh. North India
12	10	Jabedi	Orissa, East India
13	75	Jorhat	Assam, North-East India
14	28	Kasturi	Andhra Pradesh, South India
15	12	Katergia	Orissa, East India
16	31	Kuchipudi	Andhra Pradesh, South India
17	326	Lakadong	Meghalaya, North-East India
18	304	Lembucherra	Tripura, North-East India
19	288	Manipur	Manipur, North-East India
20	32	Mananthody	Kerala, South India