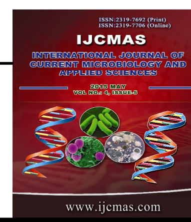
Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 485-490

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 4 Number 5 (2015) pp. 485-490
http://www.ijcmas.com



Original Research Article

DNA Isolation and PCR Amplification of Turmeric Varieties from Telangana State

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A B S T R A C T

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. Of the different molecular markers RAPD and ISSR has been widely used. A marker system called Inter-Simple Sequence Repeats (ISSRs) has only recently been developed. Extremely high variability and high “mapping density” as compared with RFLP and RAPD data make these new dominant, microsatellite-based molecular markers ideal for producing genetic maps of individual species. PCR amplification of the genomic DNA was carried out using ISSR PCR performed by using a set of 20 primers namely UBC 801-UBC 82. Initially, 20 primers (UBC 801-UBC 820) were screened with a subset of 18 samples. 8 primers which gave scorable banding pattern were used for analysis of all the samples. Majority of amplification products are in the form of strong and well-defined bands in the range of 70 bp to 2.0 kb. ISSR profile of eighteen popular cultivated Turmeric genotypes analyzed showed the polymorphic index value of 87.27% across all the genotypes examined in the current study. The primers 809, 810, 811, and 816 exhibited higher polymorphism percentage among these 809 were exhibited 100.00 polymorphism.

Keywords

Rhizomes, Genetic diversity, RFLP, RAPD

Introduction

Turmeric is an important medicinal plant cultivated exclusively in India. Turmeric is a cross pollinated, triploid species (2n = 3x = 63), which can be vegetatively propagated using its underground rhizomes (Sasikumar, 2005). Rhizomes are used as a condiment, dye, drug and cosmetic since Vedic age and it exhibits germicidal, aromatic, carminative, antihelmentic, antioxidant, anti-tumor and cholesterol lowering properties (Satyavati *et al.,* 1976). The rhizomes are usually boiled, cleaned, and dried, yielding a yellow powder. Dried rhizome of *Curcuma longa* is the source of the spice turmeric, the ingredient that gives curry powder. The curry powder of Turmeric is characteristic

yellow color. Turmeric is used extensively in foods for both its flavor and color. The demands for the rhizome have been increased rapidly, due to its medicinal application of turmeric and curcumin, a major constituent of rhizome (Chattopadhyay *et al.,* 2004) *Curcuma* molecular biology studies, so far, are confined to few isozyme-based characterization of germplasm accession/species (Shamina *et al.,* 1998: Apavatjrut *et al.,* 1999; Paisooksantivatana *et al.,* 2001). Anatomical characterization of Curcuma species and cultivars have been attempted, not much has been done on molecular characterization (Sasikumar, 2005)

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.

A marker system called Inter-Simple Sequence Repeats (ISSRs) has only recently been developed as an anonymous, RAPDs-like approach that accesses variation in the numerous microsatellite regions dispersed throughout the various genomes (particularly the nuclear genome) and circumvents the challenge of characterizing individual loci that other molecular approaches require. Microsatellites are very short (usually 10–20 base-pair) stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. They are characterized by mono-, di- or trinucleotide repeats, e.g., AA…, or AG…, CAG…, that have 4–10 repeat units side-by-side. In ISSRs, we specifically target the di- and trinucleotide repeat types of microsatellite, because these are characteristic of the nuclear genome (mononucleotide types are found in the chloroplast genome and we don't want these). Extremely high variability and high "mapping density" as compared with RFLP and RAPD data make these new dominant, microsatellite-based molecular markers ideal for producing genetic maps of individual species (Nagaoka and Ogihara, 1997). These features, combined with greater robustness in repeatability experiments and less prone to changing band patterns with changes in constituent or DNA template concentrations, make them superior to other readily available marker systems in investigations of genetic variation among very closely related individuals and in crop cultivar classification (Fang and Roose, 1997; Nagaoka and Ogihara, 1997).

Materials and Methods

Plant materials

Turmeric popular 18 cultivated genotypes from North Telangana region were collected during 2013 from Turmeric Research Center, Kammerpally, Nizamabad, Telengana, India and maintained at University College of Science, Saifabad, Osmania University, Hyderabad. Young seedlings of Turmeric cultivars (18 genotypes) were grown under greenhouse conditions (at 24°C under fluorescent light and a photoperiod of 16 h day/8 h night) for 4 wk for the preparation of DNA samples for studying the PCR amplification of DNA

using ISSR Primers. The research work conducted, at University College of Science, Saifabad, Osmania University, Hyderabad Telangana state in India.

DNA extraction

The protocol for DNA extraction was as described by Edwards *et al.* (1991). Turmeric leaves (3 g) were ground in liquid nitrogen with a mortar and pestle and incubated at 65°C for 1 h in 20 mL of SDS extraction buffer (100 mM Tris-HCl [pH 8.5], 100 mM NaCl, 50 mM EDTA, 2% SDS). After extraction with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1) and centrifugation at 2500*g* for 15 min, the upper phase was extracted with chloroform once again. Genomic DNA was precipitated out of the upper phase by adding 0.6 vol of isopropanol and keeping at less than 4°C for several hours. The precipitate was carefully picked out, washed several times with 75% ethanol, and then air-dried and dissolved in 1 mL of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). RNase (Sigma) was added to the DNA samples to eliminate RNA by incubation at 37°C for 1 h. Next, genomic DNA was quantified with use of λDNA as a reference.

ISSR analysis

PCR amplification of the genomic DNA was carried out using ISSR PCR performed by using a set of 20 primers namely UBC 801-UBC 820. A single primer was used in each PCR reactions, the PCR each reaction mix of 25 ml contained 3 ml of genomic DNA (30 ng/µl), 3.3 ml of primer (0.2 mM concentration), 2.5 ml of 10x buffer (0.1 M *Tris*pH 8.3; 0.5 M KCl; 7.5 mM $MgCl_2$; 0.1% gelatin), 1.0 ml of 200 mM dNTPs and 1.0 unit of Taq polymerase. Then 0.5 ml of 2% formamide and 1.2 ml of spermidine (10 mM) were added to increase the stringency of the reaction. PCR amplifications were performed in 96-well plates on a (Applied Bio systems 9700) thermal cycler under the following conditions: a hot start of 94°C for 5 min, followed by 44 cycles of denaturing at 94°C for 1 min, annealing for 45 s, extension at 72°C for 2 min, and final extension at 72°C for 5 min. The annealing temperature was 50°C contained 25 µl of 50ng/µl of template DNA 1X assay buffer (100mM Tris sulfonic acid, pH 8.8, 15mM $MgCl_2$, 500 mM KCl, and 0.1% gelatin) 0.2 mM each dNTPs (B Genei, India), 5 pmol of each primer and 1 U of Taq polymerase. The reaction was performed in 0.2 ml microfuge tubes. The gels were photographed under a UV transilluminator. All the amplifications were performed more than thrice for each sample /primer combination and only those primers giving reproducible patterns were used for scoring.

Results and Discussion

ISSR–PCR is a simple, cost-efficient, robust, multi-locus marker method for determining genetic variability among the germplasm accessions/isolates of pathogens. Though, there are large numbers of reports of use of ISSRs for studying diversity of crop germplasm, its use in pathogen diversity analysis is limited. In the present study, we have studied the PCR amplification was done using ISSR Markers with 18 popular varities of Turmeric. Several parameters that could affect pattern quality and reproducibility of ISSR fingerprints, the genomic DNA at 30 ng was found to be optimum for PCR amplification. More spurious products were amplified at lower concentration with low intensity and were difficult to score. Some products that were amplified at 30 ng were not amplified at higher concentration (50 ng). Many studies showed that 5–500 ng template DNA could offer an amplification result (Huang and Sun, 2000).

Table.1 Sequence of ISSR primers, number of bands generated by each primer and percentage of polymorphism

ISSR Primers	Primer sequence	No. of Amplified bands	No. of Polymorphicbands	Polymorphism (%)
UBC 807	$(AG)_8T$	11	9	81.81
UBC 808	$(AG)_8C$	14	12	85.71
UBC 809	$(AG)_8G$	13	13	100.00
UBC 810	$(GA)_8T$	09	08	88.88
UBC 811	$(GA)_8C$	10	09	90.00
UBC 812	$(GA)_8A$	14	11	78.57
UBC 816	$(CA)_8T$	8	7	87.50
UBC 817	$(CA)_8A$	8	6	85.71

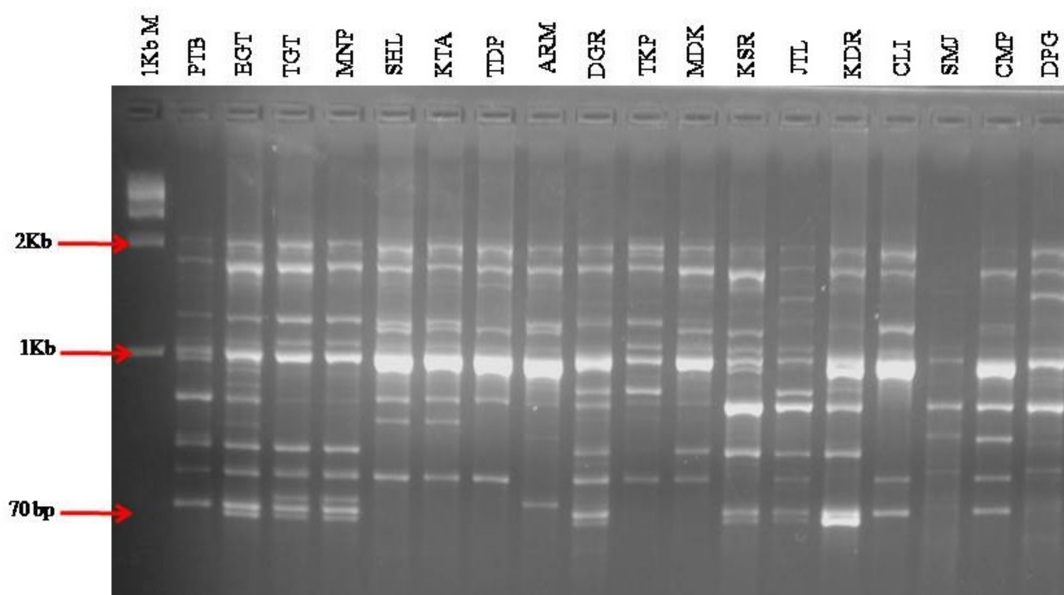

Figure 1. ISSR marker profiles of 18 Turmeric Genotypes generated by primer UBC809 in 2.0 per cent agarose gel.

The sequence details of the ISSR primers were obtained from University of British Columbia website. Initially, 20 primers (UBC 801-UBC 820) were screened with a subset of 18samples.Each amplification product was considered as an ISSR marker in ISSR–PCR. Consistency of the bands was checked by running the reaction twice, and only the reproducible bands were scored in all samples for each of the 8 primers, separately. Bands were recorded as present (1) or absent (0). 8 primers which gave scorable banding pattern were used for analysis of all the samples. The representative electrophoretic pattern of ISSR-PCR amplified products from Turmeric. As seen in the figure, majority of amplification products are in the form of strong and well-defined bands in the range of 70 bp to 2.0 kb. Most of these primers which produced polymorphic bands were based on AG or GA repeats followed by AC

or CA repeats. An especially attractive feature of ISSR analysis is its flexibility in terms of experimental design: the number of amplicons generated may be optimized by changing the number of the core repeat units and anchoring bases (Liu and Wendel, 2001). The electrophoretic profile with this primer is highly distinct and polymorphic amplicon number per primer ranged from 6 (UBC 817) to 13 (UBC 09) with an average of 9.37. Polymorphism also varies in different genotype of curcuma with a maximum of 14 bands in both the primers UBC 808 & 812 and a minimum of 8 bands in both the primers UBC 816 & 817 with a mean of 10.87 (Table 1). ISSR profile of eighteen popular cultivated *Curcuma longa* genotypes analyzed showed the polymorphic index value of 87.27% across all the genotypes examined in the current study. The details of Primers, amplification products, polymorphic fragments generated, were showed in table 1. The polymorphism percentage ranged from 78.57 (UBC 812) to 100.00 (UBC 809). The primers 809, 810, 811, and 816 exhibited higher polymorphism percentage among these 809 were exhibited 100.00 polymorphisms and therefore were more informative (Table 1).The primers 807,808,812 and 817 were exhibited medium range polymorphism (Table 1). All these primers individually could distinguish 18 out of 18genotypes of Turmeric with distinct profile.

To date, the very small number of published papers using ISSR markers has focused on identification or genotyping of variants in agriculturally important plants, e.g., barley (Nagaoka and Ogihara 1997) and blueberry (Levi and Rowland 1997). Tsumura *et al.* 1997 reported the Studies of ISSR locus heritability have demonstrated an exceedingly close approximation to classic Mendelian ratios.

In conclusion, ISSR markers were chosen because the technique is very simple, fast, cost effective, highly discriminative, and reliable and require small quantity of sample DNA. They also do not need any prior primer sequence information and are non-radioactive.

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