

Genotyping of High Yielding Varieties and Genotypes of Turmeric (*Curcuma longa* L.) using RAPD Markers

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ABSTRACT

In the study four released varieties and six high yielding turmeric clones were genotyped using fifteen RAPD markers to see band amplification patterns and genetic variations. Jaccard's coefficient method was used as an estimator of genetic identity and similarity among the genotypes and UPGMA (un-weighted pair group method, arithmetic average) utilized for clustering the genotypes. All the fifteen RAPD primers produced clear polymorphic amplification for the turmeric genotypes used in the study resulting 68 RAPD bands of which 59 bands were polymorphic with average polymorphism of 86.76%. Jaccard's similarity between genotypes pairs ranged between 0.36 and 0.93 with a mean of 0.62. Prominent difference was observed between the genotypes with BSR-1 and PTS-8. The genotype CL-101 and CL-193 recorded the highest similarity index (0.93) followed by the combination BSR-2 and CL-101 (0.89). The lowest average similarity index was recorded by the variety BSR-1 (0.49) followed by PTS-8 (0.54) and CL-219 (0.58). The RAPD marker result depicted that the use of such molecular marker tool could be a viable option in the presence of a validated marker system developed for identification of the genotypes.

Keywords: Turmeric, genetic diversity, RAPD markers

INTRODUCTION

Turmeric (*Curcuma longa* L.), probably a native of Southeast Asia, is an ancient spice and modern medicine is gaining pristine importance worldwide (Roses, 1999). It is widely used as a dietary spice, coloring agent in food, source of phenolic compounds, curcuminoids and is widely used as a medicine in most Asian countries. Recently, it has attracted much attention due to its significant medicinal potential due to the presence of several potent antioxidant compounds (Cousins *et al.*, 2007). Due to the crop economic importance several studies on its genetics has been implemented for successful breeding objectives and conservation of plant genetic resources efficiently.

Morphological studies by several authors have indicated a high degree of variability in turmeric germplasm in India (Chandra *et al.*, 1997; Kurian and Nair 1996; Philip and Nair 1983). However, the use of only such classical approaches to study variability has several inherent limitations leading to an advent exploitation of molecular markers to analyze intra-specific diversity in many crop species. As a result, the use of molecular markers in species delimitation and study of varietal divergence in the genus RAPD markers assume great significance (Sasikumar and Syamkumar, 2007). In this regard significant genetic variations by RAPD markers have also been reported in other species at cultivar level (Huang *et al.*, 2003). Nayak *et al.* (2006) demonstrated the utility of using RAPD markers to characterize genetic diversity among 17 promising cultivars of *C. longa*. Similar trends in plant species of tropical origin are also indicated by Hamrick and Loveless (1989) maintaining high genetic diversity within populations.

Hence, the current study was conducted with the objective of investigate the varietal difference among ten high yielding turmeric clones under production in Tamil Nadu state, India. RAPD molecular markers were used for the purpose of this study to discriminate the varietal difference among the turmeric clones.

MATERIAL AND METHODS

The study was conducted at Tamil Nadu Agricultural University, Horticultural College and Research Institute using four released varieties and six high yielding turmeric clones that are collected from Tamil Nadu area. Three plants were raised on pots under greenhouse condition and unrolled young leaf tissues were used for DNA extraction.

DNA Extraction and Purification

The DNA was isolated from unrolled young leaves weighing 3g using CTAB method (Doyle and Doyle, 1987) with modification (Harini *et al.*, 2008). The CTAB extraction buffer contained 3% (w/v) CTAB, 3M NaCl, 100 mM Tris.HCl (PH 8.0), 20mM EDTA (PH 8.0) and a freshly added 0.2 % 2-mercaptoethanol. Qualitative analyses of the isolated DNA was done by running the samples on 1% agarose gel to visualize the intensity of bands and the samples were stored at 4°C for RAPD marker studies.

RAPD Amplification

PCR amplification was performed using fifteen random decamer primers in a BIORAD (Bio-Rad Laboratories, Inc. USA). Reaction volume of 25- μ l containing 2.5 μ l 10x PCR reaction buffer, 2.0 mM MgCl₂, 10 mM each dNTP, 20 pmol RAPD primer, 50 ng genomic DNA, and 0.3 U Taq DNA polymerase were used for amplification. Hot start PCR (95°C) with an initial denaturation at 94°C for 4 min and 40 cycles at 94°C for 1 min, 37°C for 1 min, 72°C for 1½ min, followed by 10 min at 72°C. Then amplified products were separated on 2% agarose gel in 1x TBE buffer (100 mM Tris-HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 100 V. The gels were stained with 0.5 μ g/ ml ethidium bromide solution and visualized under alpha imaging system by comparing to 500bp of DNA ladder.

Table1. List of RAPD decamer primers used for molecular variation study

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPA 02	TGCCGAGCTG	OPF 08	GGGATATCGG
OPA 04	AATCGGGCTG	OPF 09	CCAAGCTTCC
OPB 03	CATCCCCCTG	OPF 10	GGAAGCTTGG
OPF 03	CCTGATCACC	OPF 13	GGCTGCAGAA
OPF 04	GGTGATCAGG	OPF 14	TGCTGCAGGT
OPF 05	CCGAATTCCC	OPF 15	CCAGTACTCC
OPF 06	GGGAATTCGG	OPF 16	GGAGTACTGG
OPF 07	CCGATATCCC		

Data Scoring and Analysis

The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. Data analyses were performed using the NTSYS-pc (Numerical Taxonomy System, version 2.02, Rohlf, 2000). Analysis utilized the SAHN clustering programme of the NTSYS-pc package to construct an UPGMA (unweighted pairgroup method, arithmetic average) dendrogram for the genotypes. The SIMQUAL program was used to calculate the Jaccard's coefficient, a common estimator of genetic identity and was calculated as follows:

$$\text{Jaccard's coefficient} = \frac{N_{AB}}{(N_{AB} + N_A + N_B)}$$

Where, N_{AB} is the number of bands shared by samples, N_A represents amplified fragments in sample A, and N_B represents fragments in sample B. Similarity matrices based on these indices were calculated.

RESULT AND DISCUSSION

Pattern of Polymorphic Bands

All the fifteen RAPD primers produced clear polymorphic amplification for the turmeric genotypes used in the study resulting 68 RAPD bands of which 59 bands were polymorphic with average polymorphism of 86.76%. The polymorphism of the RAPD primers ranged from 50% in OPA2 and OPF-14 to 100% in primers OPB 03, OPF 04, OPF 05, OPF 07, OPF 08 and OPF 09 while OPF 16 showed 83.33 % polymorphism (Table 2).

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The total number of markers amplified by the 15 primers ranged from 34 in genotype BSR-1 to 53 in genotype CL-213. The other genotypes showed a comparable number of amplification with genotypes CL-206, CL-219, PTS-8, PTS-10, BSR-2, CL-15, CL-101, and CL-193 recorded 42, 44, 45, 46, 49, 49, 49 and 49 markers respectively. The OPF series primers OPF-5 and OPF-9 failed to show any amplification for the genotype BSR-1. Similarly, the primers OPF-8 and OPF-4 did not produce band for the genotypes CL-219 and CL-206.

Table2. Sequence of RAPD primers with their amplification pattern for turmeric genotypes in the study

Primer	Sequence (5'-3')	Total no. number of bands	No. of polymorphic bands	Polymorphism (%)
OPA 02	TGCCGAGCTG	4	2	50
OPA 04	AATCGGGCTG	4	3	75
OPB 03	CATCCCCCTG	2	2	100
OPF 03	CCTGATCACC	4	4	100
OPF 04	GGTGATCAGG	3	3	100
OPF 05	CCGAATTCCC	6	6	100
OPF 06	GGGAATTCGG	3	2	66.67
OPF 07	CCGATATCCC	5	5	100
OPF 08	GGGATATCGG	3	3	100
OPF 09	CCAAGCTTCC	3	3	100
OPF 10	GGAAGCTTGG	4	3	75
OPF 13	GGCTGCAGAA	8	5	62.5
OPF 14	TGCTGCAGGT	6	3	50
OPF 15	CCAGTACTCC	6	4	66.67
OPF 16	GGAGTACTGG	6	5	83.33

Similarity Patterns among Turmeric Clones

Jaccard’s similarity between pairs of accessions ranged between 0.36 and 0.93 (Table 3) with a mean of 0.62. Prominent differences existed in the genetic similarities of the genotypes with BSR-1 and PTS-8 showed the least similarity index followed by BSR-1 and CL-101, 193 and 206 pair wise similarity indexes. The genotype CL-101 and CL-193 recorded the highest similarity index (0.93) followed by the combination BSR-2 and CL-101 (0.89). The lowest average similarity index was recorded by the variety BSR-1 (0.49) followed by PTS-8 (0.54) and CL-219 (0.58) (Table 3).

Table3. Jaccard’s pair wise similarity in 10 *C. longa* (turmeric) genotypes.

	BSR -1	BSR -2	PTS -8	PTS -10	CL- 15	CL- 101	CL- 193	CL- 206	CL- 213	CL- 219	Average
BSR-1	1.00	0.44	0.36	0.61	0.65	0.43	0.44	0.45	0.55	0.54	0.49
BSR-2		1.00	0.55	0.58	0.67	0.89	0.87	0.73	0.67	0.58	0.66
PTS-8			1.00	0.50	0.53	0.57	0.58	0.58	0.54	0.54	0.54
PTS-10				1.00	0.80	0.60	0.61	0.55	0.76	0.67	0.61
CL-15					1.00	0.62	0.64	0.68	0.69	0.57	0.61
CL-101						1.00	0.93	0.68	0.69	0.57	0.64
CL-193							1.00	0.70	0.70	0.58	0.65
CL-206								1.00	0.64	0.55	0.64
CL-213									1.00	0.66	0.65
CL-219										1.00	0.58
Total No. Of bands	34	49	45	46	49	49	49	42	53	44	

Clustering of the Turmeric Clones

The present RAPD studies revealed relatively distinct clustering pattern among the genotypes. The variety BSR-1 was grouped distinctly different from other genotypes, while the rest of the genotypes viz. CL-206, CL-219, PTS-8, PTS-10, BSR-2, CL-15, CL-101, CL-193 and CL-213 were classified in the second cluster. The second cluster was further divide in to 3 groups. The genotype PTS-8 was grouped alone. The genotypes PTS-10, CL-15, 213 and 219 were grouped together. In the same cluster variety BSR-2, CL-101, CL-206 and CL-193 were grouped together.

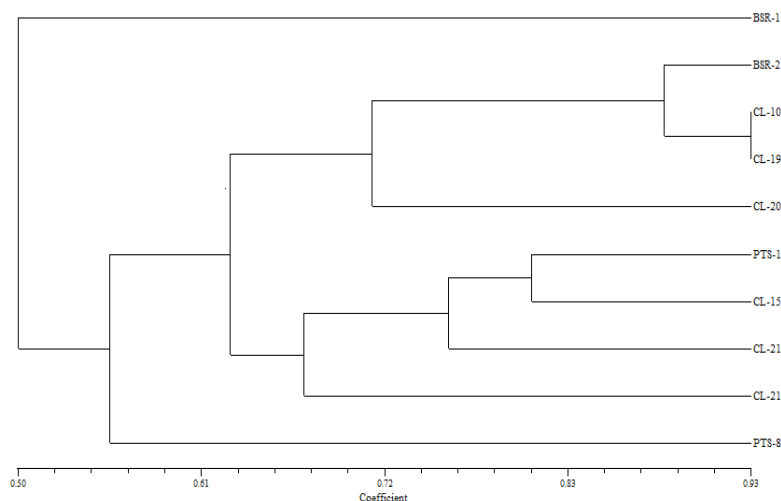


Figure 1. Dendrogram of 10 turmeric genotypes resulting from a UPGMA cluster analysis obtained from 15 RAPD primers.

Diversity Analysis with RAPD Marker

The evaluation of genetic diversity and construction of linkage maps would promote the efficient use of genetic variations in the breeding program (Paterson *et al.*, 1991) and provides an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983).

The presence of high polymorphism in the study is in line with several documented results by the use of RAPD primers indicating the robustness of the marker system used for genetic variation analysis among the turmeric clones. Polymorphic nature of RAPD bands accounts for the intra-specific genetic variation known to occur in the *C. longa L.* (Sasikumar and Syamkumar, 2007; Islam *et al.*, 2005; Paisooksantivatana *et al.*, 2001; Nayak *et al.*, 2006; Siju *et al.*, 2010 and Paisooksantivatana and Thaikert, 2009) and significant genetic variations by RAPD markers have also been reported in other species at cultivar level (Huang *et al.*, 2003). The causes of these genetic variations in the genus *Curcuma* is suggested by different authors: aneuploidy is reported for some *Curcuma* species (Eksomtramage *et al.*, 2002) and geographically isolated population also accumulates genetic differences as they adapt to different environments (Paisooksantivatana *et al.*, 2001). Amsalu *et al.*, (2000) also concluded that the clustering pattern of the regions of origin by RAPD markers was broadly concordant with cultivars grown in similar agro-climatic conditions. Since the difficulties pertaining to hybridization in turmeric, it can be assumed that the difference in genetic distance between the genotype PTS-8, supposed to be originated from Potangi region as opposed to Erode region with the rest of the genotypes might be attributed to differentiation through adaptation phenomenon.

CONCLUSION

The RAPD marker result depicted that the use of such molecular marker tool could be a viable option in the presence of a validated marker system developed for identification of the genotypes. In the present study the presence and absence of some specific markers were observed in the primer OPF 05 for distinguishing the genotypes PTS-8 and PTS-10 which are believed to arise from the same region. The marker size ~1kb and ~400bp were present for the genotype PTS-8 which were absent in the genotype PTS-10 in a reverse manner a marker size ~200bp was present in the genotype PTS-10 while not PTS-8 indicating that the use of these primers is a very viable option for identification of single genotypes.

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