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Genetic Diversity of Popular Cultivated Turmeric Genotypes from Telangana Region Using RAPD Markers

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Abstract: ***Turmeric is one of the important spice crops in India and plays a vital role in the Indian economy. Molecular markers based characterization of curcuma species may contribute to analyze taxonomic relationship and intraspecific diversity of the genus. Among molecular markers Random amplified polymorphic DNA (RAPD) analysis is the simplest and least laborious method and has been used to estimate the genetic distance and diversity in awide range of plant especially at the sub-species and cultivar level. The objective of this study was to assess the genetic diversity among 18 popular cultivated genotypes of turmeric, using RAPD markers. The RAPD PCR was performed by using a set of four series of primers namely OPA, OPC, OPD and OPN. The data were analyzed for calculation of various parameters like number of loci, number of polymorphic loci, polymorphism (%), polymorphism information content (PIC) and molecular index (MI)Out of 40 RAPD primers 15 primers which gave scorable banding pattern were used for analysis of all the samples. The amplicons ranged between 80 bp to 2.8 Kb in size. Amplicon number per primer ranged from 15 (OPC 07) to 30 (OPA 18) with an average of 21.4. Polymorphism also varies in different genotype of turmeric with a maximum of thirty bands for the primer OPA 18 and a minimum of eleven bands in both the primers OPD 07 & 18 with a mean of 19.06. PIC values for RAPD primers varied from 0.71(OPD-08) to 0.96(OPA-18) whereas marker indices ranged between 56.3(OPD-18) to 96.0(OPA-18). Based on UPGMA clustering from RAPD, the genotypes were grouped into two major clusters at a similarity index value of 0.48.Pair-wise estimates of coefficient similarity for 18 genotypes ranged from 0.40 to 0.85.***

Keywords: Random amplified polymorphic DNA, Polymerase chain reaction, Genetic diversity, Molecular marker

1. Introduction

Turmeric is one of the important spice crops in India and plays a vital role in the national economy .India is the largest producer and exporter of turmeric in the world and accounts for more than 50% of the world trade (Philip, 1983).Turmeric is a major constituent in curry and is also used in a variety of industrialized products such as sauces, gravies, mustards, dry seasoning, baking mixes, processed cheese, fry soups, beverages and confection (Sasikumar, 2005).Current research has focused on Turmeric's antioxidant, anti-inflammatory, hepato-reprotective, anti-tumor, anti-carcinogenic and anti-microbial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders (Ammon and Wahl, 1991, Kiso*et al.,* 1983).The knowledge of genetic diversity is a pre requisite to study the evolutionary history of a species,as well as for other studies like intraspecific variations, genetic resources conservation, etc. (Islam *et al.,* 2007).Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duwick,1984).Thus, molecular markers based characterization of curcuma species may contribute to analyze taxonomic relationship and intraspecific diversity of the genus. Further, the study will be useful for planning strategies for their conservation and optimal utilization. The usefulness of molecular markers in genetic diversity studies has been convincingly established. (Diwan., *et al* 1997, Prasad *et al.,* 2000, Zhebentyayeva., 2003, belaj*et al.,* 2003, Awasthi., *et al* 2004, Sharma *et al.,* 2008 and Rangan*et al*, 2008).15 Species of Curcuma were grouped into seven clusters based on morphological characters using ISSR markers by Syamkumar*et al* (2007).AFLP analysis provides a high resolution for the detection of genetic diversity and structures between and with species of curcuma was studied by Das *et al.,* (2011). Among molecular techniques, one most progressively more used marker is RAPD markers due to its simplicity and low cost. RAPD analysis has been used to estimate the genetic distance and diversity in awide range of plants especially at the sub-species and cultivar level. (William *et al.,* 1990, Rathnaparkhe*et al.,*1995,Shasany*et al.,* 2002, Fico*et al.,*2003, nayak*et al.,* 2005 and Saritnum*et al.,* 2005). The objective of this study was to assess the genetic diversity among 18 popular cultivated varieties of turmeric, using RAPD markers.

2. Materials and Methods

2.1 Plant Material

In the present study eighteen popular cultivated genotypes of turmeric were collected and maintained from Turmeric Research Center, Kammerpally, Nizamabad,Telengana State, India for studying the genetic diversity .The sampling was done from February 2013 to September 2013. The research work conducted at University College of Science, Saifabad, Osmania University, Telangana state in India.

2.2 DNA isolation

Fully opened fresh tender leaves of the 18 popular Turmeric varieties were used for the isolation of DNA. The genomic DNA was isolated by CTAB method (Doyle and Doyle, 1987)with minor changes. The extraction buffer contains 2% CTAB, 1.5 M Nacl, 100mMTris, 20mM EDTA and 0.1% mercaptoethnol.The quantity and quality of the extracted DNA was confirmed to be consistent both spectro-photometrically and by running the extracted DNA on 0.8% agarose gels stained with ethidium bromide.

2.3 RAPD analysis

PCR amplification of the genomic DNA was carried outusing RAPD PCR performed by using a set of four series of primers namely OPA,OPC,OPD and OPN (Operon tech, USA).The reaction mixture of 25 µl contained 50ng/µl of template DNA 1X assay buffer (100mM Tris sulfonic acid, pH 8.8, 15mM Mgcl2,500 mMKcl, 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.PCR amplification was carried out in a mini thermal cycler (Applied Bio systems 9700). Thermal cycling conditions were as follows. Pre-denaturing step of 5 min at 94°C, followed by 35 cycles each of 45 s at 94°C, annealing for 1 min at 32°C, extension for 1 min at 72°C and followed by one final extension cycle of 7 min at 72°C.The amplification products were electrophored in 2% agarose gel in 0.5x TBE (10X stock contained 0.8 M Tris, 0.8 Mboric acid, 0.5 M EDTA). The gels were photographed under a UV transliuminator. All the amplifications were performed more than thricefor each sample /primer combination and only those primers giving reproducible patterns were used for scoring.

Numerical analysis of PCR data: The banding pattern of each strain was coded in binary form, 1 representing the presence and 0 the absence of each band. The data were analyzed for calculation of various parameters like number of loci, number of polymorphic loci, polymorphism (%), polymorphism information content (PIC)Table (2). The PIC values were calculated based on the following formula

$$PIC = 1 - \sum_{j=1}^{n} Pij2$$

WherePij = frequency of the jth pattern of the ith band. Alternatively, PIC was calculated using online PIC calculator software (http://www.genomics.liv.ac.uk/animal/Pic1.html).

Dendrograms were generated within the SAHN program of NTSYS-pc (Exeter Software, Setauket, NY) using the unweighted pair-group method with arithmetic averages (UPGMA) method. Similarity matrices were calculated using Dice coefficient with the SIMQUAL program.

3. Results and Discussion

Cultivated varieties of turmeric are the result of several thousands of years of human selection from the available genetic diversity in various natural environments and human cultures. Modern breeding in the last century has done little more than to control the process of hybridization and selection in a more efficient way and the result has been to adapt varieties to better controlled and fertilized environments.

The extent of genetic diversity among the genetic materials has been estimated by adopting various methods over a period of time using a wider range of simply and complexly inherited traits. Though morphological and isozyme markers have been employed in assessing the underlying genetic diversity of a species, the accuracy of the assessment is questionable. The availability of low number of morphological and biochemical markers, their poor or unknown genetic control, environmental influence on the phenotypic expression, stage specific identification and procedural difficulties are known impediments in using these as genetic markers in genetic diversity analysis. Considering the problems associated with morphological and isozyme markers, the researches involved in diversity analysis searched for the alternative tools. Among the tools identified, usage of RAPD markers attained a wider range not only in turmeric but also in every living organism. RAPD markers generated at DNA level are known for their technical simplicity and high throughput (Williams *et al.,* 1990, Welsch & McClelland, 1990).

In the present investigation, RAPD markers were employed to assess the genetic diversity among 18 genotypes of turmeric (Table-1). The employment of RAPD markers in genetic diversity analysis helped in grouping the genotypes according to their morphological characters like rhizome size (8-12 cm), color(Yellow mixed Red) and dry yield (Clusters A&B). The RAPD markers gave more clusters with fewer genotypes in each cluster, indicating more variation within each cluster.When more clusters are obtained with fewer genotypes in each, the lower significance inclustering is because of the smaller differences and smaller sample sizes for each cluster.The genetic diversity among the 18 popular cultivated turmeric genotypes were evaluated by 40 selected RAPD primers. Initially, 40 primers from four series of (OPA,OPC, OPD and OPN) RAPD primers were screened with a subset of samples. 15 primers which gave scorable banding pattern were used for analysis of all the samples. The amplicons ranged between 80bp to 2.8 Kbin size. Amplicon number per primer ranged from 15 (OPC 07) to 30 (OPA 18) with an average of 21.4. Polymorphism also varies in different genotype of curcuma with a maximum of thirty bands for the primer OPA 18 and a minimum of eleven bands in both the primers OPD 07&18 with a mean of 19.1 (Table.2). RAPD profile of eighteen popular cultivated Turmeric genotypes analyzed showed the polymorphic index value of 87.74%across all the genotypes examined in the current study. The details of Primers, amplification products, polymorphic fragments generated, PIC and MI values for each primer were showed in Table -2. PIC values for RAPD primers varied from 0.71(OPD-08) to 0.96(OPA-18) whereas marker indices ranged between56.3(OPD-18) to 96.0(OPA-18).

Figure 1 depicts a representative electrophoretic pattern of RAPD-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 80bp to 2.5 kb. The electrophoretic profile with this primer is highly distinct and polymorphic.

The genetic relationships among eighteen genotypes of Turmeric were analyzed by 15 RAPD primers on the basis of Dice genetic distance. Resulting clusters were expressed as UPGMA dendrogram constructed using SHAN neighbor-joining tree separately for each molecular marker used. The coefficients on the x-axis represent the similarity indices (DICE) of the different genotypes chosen for the study. Based on UPGMA clustering algorithm from RAPD, the genotypes were grouped into two major clusters at a similarity index value of 0.48(fig.2)UPGMA cluster can be divided into two major clusters A and B at 0.48 similarity levels.

Clusters A had eight genotypes. Cluster A showed a high level of genetic variation among the genotypes and was further sub-divided into two sub-clusters. The sub cluster A1 showed a 51% similarity level with the sub cluster A2 genotypes. Sub cluster A1 had four genotypes (PTB,EGT, TGT and MNP) While sub cluster A2 also contained four genotypes (SHL,KTA,TDP and ARM).Sub clusters A1 and A2 both were further sub-divided into two cluster and both were showing 59% similarity level and each sub cluster comprised of two genotypes. All of them belonged to the high yielding cultivated genotypes.

The cluster B had a ten genotypes and further sub –divided into three major sub clusters,B1,B2 and B3 at 50% similarity level, sub clusters B1(DGR,TKP,MDK and KSR) and B2 (JTL,KDR,CLI and SMJ) contained four Genotypes each. Among four genotypes of B1 sub cluster2 genotypes DGR and TKP were exhibited 100% similarity according to the morphological and low yielding characters. Two genotypes (CMP and DPG) belonging to sub cluster B3 showed a 61% similarity level with B1 and B2.

This study showed that pair-wise estimates of similarity for 18 genotypes belonging to popular cultivated turmeric genotypes ranged from 0.40 to 0.85 (Table-3). Four pairs of Genotypes; TGT and EGT, MNP and EGT, DGR and ARM, CLI and JTL were the closest genotypes with the same highest similarity index of 0.63. It was followed by another two pairs of Genotypes; KDR and SHL and KDR and ARM with the same similarity index of 0.40 were the closest genotypes belonging to low yielding cultivated genotypes indicating the highest genetic variability from other populations of indigenous turmeric from Telangana. Similarity, Ullah Jan et al (2011) found out that turmeric genotypes two pairs of genotypes B2,B3 and B4, B5 were the closest genotypes and highly associated with each other by a similarity coefficient of 0.71.

In all the 18 genotypes analyzed, the similarity coefficient of each pair of high and low yielding cultivated turmeric genotypes of KTA and SHL, TKP and DGR were showed highest similarity level at 0.82 and 0.85 .Each genotype of JTL and ARM, these two are locally cultivated genotypes belong to low yielding cultivated genotypeswere showed a similarity coefficient of 0.45 to 0.73 and 0.45 to 0.74 with all the genotypes. Knowledge about genetic relationships will be useful to avoid the chance of using genetically similar genotypes /landraces and will also be supportive in future breeding programs to select genetically diverse parents of turmeric genotypes.

4. Conclusion

Random amplified polymorphic DNA (RAPD) analysis is the simplest, very quick, easy to develop and least laborious method and has been used to estimate the genetic distance and diversity in awide range of plant especially at the sub-species and cultivar level.Our investigate affirmed the ability of RAPD markers to differentiate between genotypes of turmeric and using RAPD markers as a means for the assessment of genetic diversity of indigenous turmeric genotypes in Telangana state in India.

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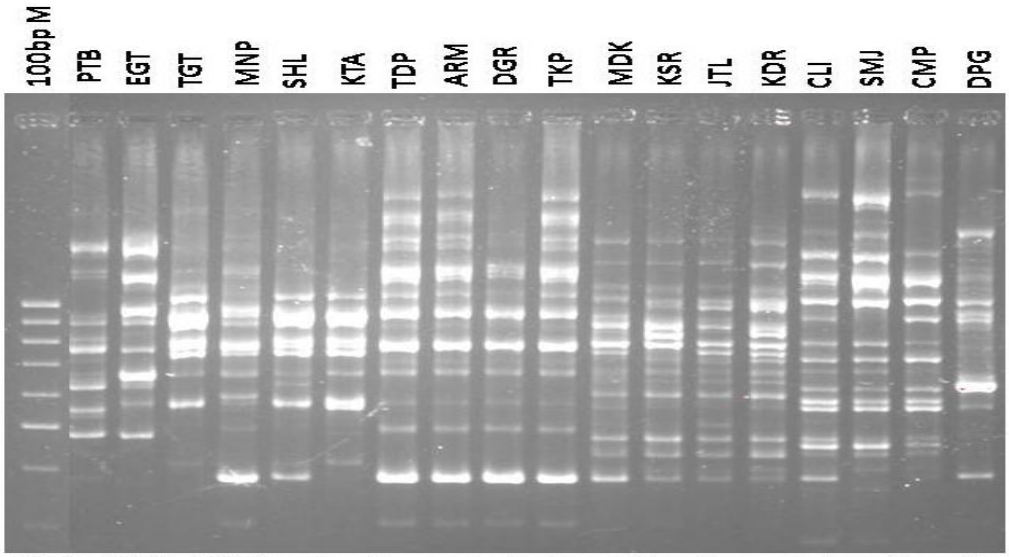

Fig.1: RAPD PCR based polymerase chain reaction fingerprints pattern for genomic DNA of Turmeric genotypes from the Telangana.

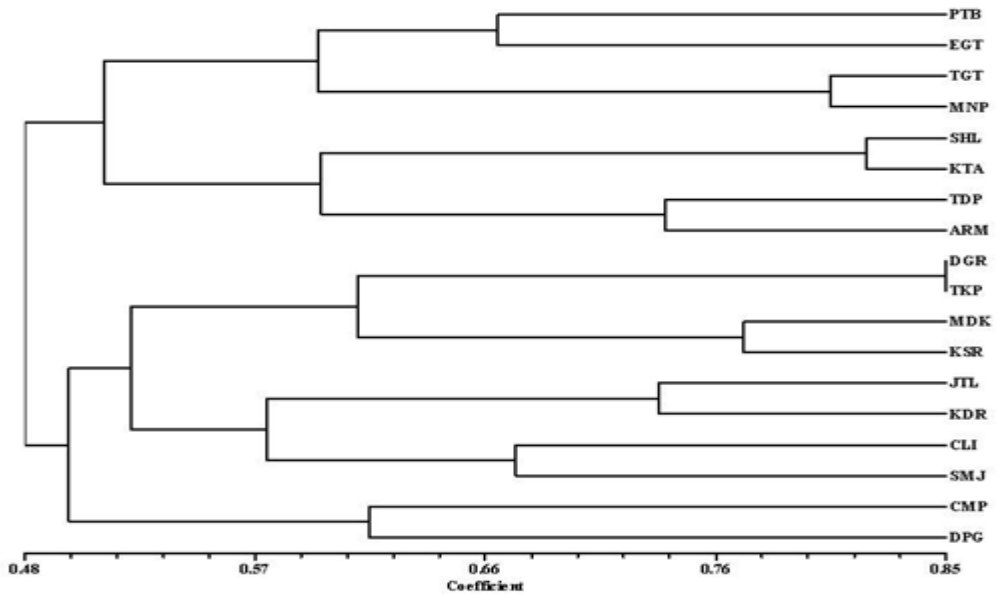

Figure 2: UPGMA Dendrogram showing relatedness among Turmeric genotypes using 15 RAPD Primers

Table 1: List of popular cultivated Turmeric genotypes used in this study

S. No	*Genotypes*	*Original Designation*		*Place of Collection*	*State*
1	PTB	Prathiba	High yielding genotypes	Turmeric Research Center (TRC), Kammer pally, Nizamabad	Telangana, India
2	EGT	Eraagunturu		TRC, Nizamabad	Telangana, India
3	TGT	Tellagunturu		TRC, Nizamabad	Telangana, India
4	MNP	Manapasupu		TRC, Nizamabad	Telangana, India
5	SHL	Shylam		TRC, Nizamabad	Telangana, India
6	KTA	Kasturiavidi		TRC, Nizamabad	Telangana, India
7	TDP	Thudapuja	Low yielding genotypes	TRC, Nizamabad	Telangana, India
8	ARM	Armoor local		TRC, Nizamabad	Telangana, India
9	DGR	Duggirala		TRC, Nizamabad	Telangana, India
10	TKP	Terkupeta		TRC, Nizamabad	Telangana, India
11	MDK	Mydukur		TRC, Nizamabad	Telangana, India
12	KSR	Kesari		TRC, Nizamabad	Telangana, India
13	JTL	Jagityal local		TRC, Nizamabad	Telangana, India
14	KDR	Kedaram		TRC, Nizamabad	Telangana, India
15	CLI	CLI-Rajampet		TRC, Nizamabad	Telangana, India
16	SMJ	Somajuli		TRC, Nizamabad	Telangana, India
17	CMP	Casampet		TRC, Nizamabad	Telangana, India
18	DPG	Deepangudapeddapasupu		TRC, Nizamabad	Telangana, India

Table 2: List of 15 RAPD primers,polymorphism and banding patterns of 18 genotypes of Turmeric

S. No	RAPD Primers	Sequence (5'--3')	No of amphlified bands	No of Polymorphic bands	Polymorphism (%)	PIC Values	MI Values
1	OPA-07	GAAACGGGTG	25	20	80.00	0.94	75.2
2	OPA-08	GTGACGTAGG	26	26	100.00	0.89	89.0
3	OPA-10	GTGATCGCAG	27	27	100.00	0.93	93.0
4	OPA-11	AAAGCTGCGG	23	21	91.30	0.94	85.5
5	OPA-18	AGGTGACCFT	30	30	100.00	0.96	96.0
6	OPC-02	GTGAGGCGTC	25	25	100.00	0.95	95.0
7	OPC-05	GATGACCGCC	23	19	82.60	0.74	60.6
8	OPC-07	GTCCCGACGA	15	15	100.00	0.90	90.0
9	OPD-03	GTCGCCGTCA	20	15	75.00	0.93	69.7
10	OPD-07	TTGGCACGGG	17	11	64.70	0.82	56.3
11	OPD-08	GTGTGCCCCA	16	15	93.70	0.71	66.0
12	OPD-18	GAGAGCCAAC	16	11	68.70	0.92	62.5
13	OPD-20	ACCCGGTCAC	17	12	70.50	0.93	65.1
14	OPN-05	ACTGAACGCC	22	21	95.40	0.93	88.3
15	OPN-06	GAGACGCACA	19	18	94.70	0.92	86.4
	Total		321	286	—	—	—
	Mean		21.4	19.1	87.74	—	—
	Range		15-30	11-30	64.7-100	0.71-0.96	56.3-96.0

Table 3: Dice coefficients of similarity based on RAPDs showing the relationship between turmeric genotypes

Genotypes	PTB	EGT	TGT	MNP	SHL	KTA	TDP	ARM	DGR	TKP	MDK	KSR	JTL	KDR	CLI	SMJ	CMP	DPG
PTB	1.00																	
EGT	0.67	1.00																
TGT	0.56	0.63	1.00															
MNP	0.56	0.63	0.80	1.00														
SHL	0.48	0.53	0.55	0.58	1.00													
KTA	0.47	0.50	0.54	0.54	0.82	1.00												
TDP	0.51	0.50	0.49	0.55	0.64	0.62	1.00											
ARM	0.45	0.49	0.49	0.52	0.57	0.56	0.74	1.00										
DGR	0.53	0.55	0.52	0.55	0.50	0.54	0.57	0.63	1.00									
TKP	0.48	0.48	0.48	0.49	0.48	0.53	0.57	0.62	0.85	1.00								
MDK	0.47	0.48	0.45	0.46	0.48	0.52	0.45	0.47	0.61	0.61	1.00							
KSR	0.49	0.48	0.49	0.47	0.47	0.53	0.46	0.44	0.61	0.62	0.77	1.00						
JTL	0.50	0.49	0.50	0.49	0.45	0.46	0.45	0.44	0.53	0.52	0.60	0.67	1.00					
KDR	0.51	0.46	0.47	0.47	0.40	0.41	0.41	0.40	0.46	0.43	0.56	0.58	0.73	1.00				
CLI	0.56	0.51	0.48	0.47	0.49	0.49	0.49	0.49	0.56	0.55	0.49	0.52	0.63	0.66	1.00			
SMJ	0.48	0.45	0.45	0.43	0.44	0.44	0.45	0.48	0.50	0.51	0.44	0.45	0.49	0.52	0.68	1.00		
CMP	0.49	0.44	0.47	0.49	0.48	0.49	0.42	0.45	0.50	0.49	0.42	0.45	0.46	0.47	0.55	0.56	1.00	
DPG	0.48	0.45	0.43	0.48	0.47	0.44	0.45	0.45	0.53	0.53	0.45	0.46	0.50	0.47	0.56	0.56	0.62	1.00