Journal of Biological Engineering Research and Review, 2015; 2(1): 20-24

ISSN: 2349-3232 Online
Available online at www.biologicalengineering.in

Research Article

Genetic Diversity Analysis in Turmeric (*Curcuma Longa* L.) Based on SSR Markers

Ashwani Kumar Singh[1*], Priyanka Nanda[2], Anupam Singh[3] and Bijendra Singh[4]

[1]*Department of Biotech., Faculty of Life Sci. and Tech., Shri Venkateshwara Univ., Amroha, Meerut, U.P., India*
[2]*Molecular Biology and Tissue Culture Laboratory, The Energy and Resources Institute (TERI), Lodhi Road, New Delhi*
[3]*Department of Biotechnology, Bansal College of Engineering and Technology, Lucknow, U.P., India*
[4]*Department of Horticulture, Sardar Vallabhbhai Patel Uni. of Agriculture and Tech., Meerut, U.P., India*
[*]***Email: ashwani307@gmail.com***

RECEIVED: 03/06/2015 ***REVISED: 30/06/2015*** ***ACCEPTED: 08/07/2015*** ***PUBLISHED: 15/07/2015***

ABSTRACT

Turmeric being an economically important crop due to its use in the food, ayurvedic medicine and pharmaceutical industries, attracts the attention in many areas of research work. In the current study, genetic diversity of 10 turmeric genotype was investigated using SSR primers. DNA was extracted from young leaves using modified CTAB method. The banding pattern was analyzed using UPGMA based Jaccard's similarity coefficient. The results revealed that SSR markers showed distinct polymorphism among the genotypes. The dendrogram revealed 2 major distinct clusters, which showed a significant genetic variation ranging between 0.60 and 0.98 among the different genotypes. Based on this study, the larger range of similarity values for related cultivars using SSR provide a tool for the assessment of genetic diversity and relationships. The highest PIC value of 0.98 for the SSR loci was associated with higher level of polymorphism. The findings distinctly identified and characterized 10 genotypes using 10 different SSR markers which can be used in background selections during backcross breeding programs.

Keywords: Genetic diversity, SSR marker, Polymorphism, Dendrogram, Genotypes.

INTRODUCTION

Turmeric (*Curcuma longa* L.) is one of the important perennial spice crops popularly known as “Indian saffron”. It belongs to family Zingiberaceae and can be vegetatively propagated using its underground rhizomes [1]. While originated in South East Asia, India has the predominant position as the largest producer of turmeric in the world. Besides India, it is also grown in China, Taiwan, Indonesia, Srilanka and other tropical countries. The highest diversity is concentrated in India and Thailand [2]. Andhra Pradesh, Orissa, Tamil Nadu, Assam, Maharashtra and Karnataka are the major turmeric producing states in India. Over 80 species are reported in the genus Curcuma (Zingiberaceae) from Indo Malayan region and about 40 of them are indigenous to India [3]. A comprehensive global taxonomic revision of the genus has not yet been attempted. Conventional taxonomic techniques in conjunction with molecular biology tools may go a long way in resolving the taxonomic confusion prevailing in the genus. Though a few studies on morphological and anatomical characterization of Curcuma species and cultivars have been attempted, not much has been done on molecular characterization [1].

Molecular marker based study of turmeric can be used in improving the yield of valuable metabolites in more quantity through marker assisted selection and genetic manipulation. Since hybridization is ineffective in most cases, genetic improvement is often limited to germplasm selection and mutation breeding. Because of its increasing demand in kitchen and pharmaceutical industries, turmeric growing techniques have been the focus of several studies [6,7]. However, to obtain further increases in productivity, information regarding the crop’s genetic diversity is required for breeding programs [8]. DNA marker technology has provided an efficient tool to facilitate plant genetic resource conservation and management. The current study utilizes SSR marker system due to the fact that they are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature and provide highly polymorphic fingerprints [9,10]. The study is focused on genetic diversity and characterization of the genotypes. This might lead to genetic improvement, selection of high yielding germplasm and evaluation of accessions from different geographical regions of Indian continent that increase the efficiency of selection in breeding programs.

MATERIALS AND METHODS

Plant material

Ten genotypes of turmeric (*Curcuma longa* L.) were used in the current study. Leaf samples were collected from Sardar Vallabhbhai Patel University of Agriculture and Technology, U.P., India, India (Table 1).

Table:1 List of turmeric genotypes used in investigation.

Name of Genotypes	Origin / Source of Sample Collection	Characteristic Feature
ISSR Alleppey	IISR Experimental Farm, Kozhikode, Kerala.	Mean yield 5.58 dry t/ha, Curcumin content 5.5%
Suguna	IISR Experimental Farm, Kozhikode, Kerala.	Short duration variety, thick and plumpy rhizomes, high yield potential. Tolerant to rhizome rot.
Suroma	High Altitude Research Station, Orissa University of Agriculture and Technology, Orissa.	Round, plumpy rhizome, Tolerance to leaf blotch, leaf spot and rhizome scales.
Ranga	High Altitude Research Station, Orissa University of Agriculture and Technology, Orissa.	Crop duration 250 days, Curcumin content 6.3%, essential oil 4.4%.
Roma	High Altitude Research Station, Orissa University of Agriculture and Technology, Orissa.	Crop duration 250 days, Curcumin content 9.3%, essential oil 4.2%.
Rasmi	High Altitude Research Station, Orissa University of Agriculture and Technology, Orissa.	Crop duration 240 days, curcumin content 6.4%, Essential oil 4.4%.
Rajendra Sonia	Department of Horticulture, Tirhut College of Agriculture, Rajendra Agricultural University, Bihar.	Crop duration 225 days, Curcumin content 8.4%, essential oil 5%.
Krishna	Maharashtra Agricultural University, Maharashtra.	Crop duration 240 days, Curcumin content 2.8%, essential oil 2%.
Sugandham	Spices Research Station, Gujarat Agricultural University, Gujarat.	Crop duration 210 days, Curcumin content 3.1%, essential oil 2.7%.
BSR I	Department of Spices and Plantation Crops, Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.	Attractive rhizomes, yellow fingers, closer internodes, high Curcumin content (4.2%), suitable for water logged conditions.

DNA extraction

Total DNA was extracted from fresh leaves by the modified Cetyl Tri-methyl Ammonium Bromide (CTAB) method. The quality and concentration of extracted DNA were estimated by using a UV-Vis spectrophotometer. The DNA was spooled out, washed twice with 70% ethanol and dissolved in TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) containing 25 µg/ml RNase-A, incubated at 37°C for 30 min and extracted with chloroform:*iso*-amyl alcohol (24:1 v/v). DNA was re-precipitated and dissolved in TE buffer. DNA was checked for its quality and quantity by 0.8% agarose gel electrophoresis.

PCR analysis and gel electrophoresis

A set of 10 SSR markers were used (Table 2). The PCR reaction was carried out using Taq polymerase in 20 µl reaction volume containing 1.5X PCR buffer, 2 mM $MgCl_2$, 0.02 mM of each dNTPs, 1 mM of forward and reverse primers each, 0.5µl (3 unit) Taq polymerase and 50 ng genomic DNA. The PCR reaction profile was used as follows: an initial hot start and denaturing step at 95°C for 5 min followed by 35 cycles at 94°C for 1 min, annealing at 55°C for 1 min, primer elongation at 72°C for 2 min and final extension step at 72ºC for 7 min were performed. The SSR-PCR products were analyzed on 4% agarose gel, visualized by staining with ethidium bromide and transillumination under short-wave UV light. DNA ladder used in the electrophoresis was of 100 bp.

Table 2. List of SSR Primers and their sequences used in the genetic diversity analysis of ten turmeric genotypes.

S. N.	Marker	Make	Primer sequences 5'→ 3'	
			FORWARD	REVERSE
1	CuMiSat -19	Merck	CATGCAAATGG AAATTGACAC	TGATAAATTGAC ACATGGCAGTC
2	CuMiSat -20	Merck	CGATACGAGTC CATCTCTTCG	CCTTGCTTTGGT GGCTAGAG
3	CuMiSat -21	Merck	TCATTCAAAGT CCGATGGAA	TTCGAGTGCAGA AGGAGAATTA
4	CuMiSat -22	Merck	AATTTATTAGC CCGGACCAC	AAGAAAGTGAGT AGAAACCAAAGC
5	CuMiSat -23	Merck	CGTGGAAGGTG AGTTTGAC	CAGAAGGGAACT GAGATGG
6	CuMiSat -24	Merck	AGGTATTCTAC TCGACCAAG	AAATTCATATAG CCCCATC
7	CuMiSat -25	Merck	TACATGAGAAA CAACAAAGCCC	AGTTAGCCAAGT CCCAATTTAGC
8	CuMiSat -26	Merck	CATTCCGATGA ATTGTATG	GCAGTTGTTTTG CTTCAG
9	CuMiSat -27	Merck	TATAGATAGCC ATGCTGAAG	CCATTTTAGTTC ATTACGTG
10	CuMiSat -28	Merck	TTCAACTTCTCC TCGCTCAG	GCAAGGTCTGCA TCTATTTCTC

Data analysis

Pair wise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate Jaccard's similarity coefficient by NT-SYS-pc version 2.02e software [11]. The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA) according to [12]. A combined analysis was performed by using dendrogram along with Jaccard's similarity coefficient matrix. The polymorphism information content (PIC) value described by Botstein *et al.* (1980) [13] and modified by Anderson *et al.* (1993)[14] for self-pollinated species was calculated as follows:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where, p_i equals the frequency of the i^{th} allele and p_j the frequency of the allele. Only the data from polymorphic loci were used for this analysis. The above mentioned methods were used for estimating the result. Markers which did not amplify any allele were shown as (-) symbol.

RESULTS AND DISCUSSION

Number of alleles

Among 10 SSR markers, 7 SSRs were with polymorphic loci which were fully distributed across turmeric genomes, a total of 65 alleles were detected among the 10genotypes of turmeric. The overall size of amplified products ranged from 150 to 290 bp (Table 3.).

Table 3. Polymorphism Information Content (PIC) of SSR Loci across various genotypes of Turmeric analyzed in the investigation

S. N.	Primer code	Repeat motif	GenBank Accession number	Molecular wt. of amplified product	Total No. of alleles	No. of polymorphic alleles	No. of monomorphic alleles	Diversity in value of PIC
1.	CuMiSat-19	(AC)16 (AT)6	HQ154119	200-260	10	3	0	0.98
2.	CuMiSat-20	(AC)6	HQ154120	170-250	12	2	0	0.97
3.	CuMiSat-21	(AAG)9	HM438970	-	-	-	-	-
4.	CuMiSat-22	(CTT)10	HM438971	200-240	17	3	0	0.96
5.	CuMiSat-23	(AAG)4	HM438972	170-240	4	4	0	0.75
6.	CuMiSat-24	(AAC)10	HM438973	-	-	-	-	-
7.	CuMiSat-25	(AAC)7	HM438974	180-290	5	5	0	0.43
8.	CuMiSat-26	(AAC)9	HM438975	150-250	6	1	0	0.97
9.	CuMiSat-27	(AC)8	HM438976	180-230	11	5	0	0.95
10.	CuMiSat-28	(AAG)7(GAT)5	HM438977	-	-	-	-	-

The highest number of alleles were observed in CuMiSat-22 (seventeen alleles) followed by CuMiSat-20 (twelve alleles), CuMiSat-27, (eleven alleles), CuMiSat- 19 (ten alleles), CuMiSat- 26 (six alleles) and so on. The lowest number of alleles was observed in CuMiSat-23, (four alleles) which provided the summarized data regarding the number of unique alleles and their distribution in various entries. No result (-) were obtained from three SSR primers namely CuMiSat-21, CuMiSat-24, CuMiSat-28.

Polymorphism of SSR markers

The alleles revealed by SSR markers showed a high degree of polymorphism with as many as 7 markers were produced 100% polymorphic bands.The average numbers of 6.5 bands were calculated per primer. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all of the SSR loci tested. The PIC value for the SSR loci ranged from 0.43 to 0.98. The highest PIC value was observed for markers CuMiSat-19 (0.98), followed by CuMiSat-20, CuMiSat-26 (0.97) for both, CuMiSat-22 (0.96), CuMiSat-27 (0.95), CuMiSat-23 (0.75). The lowest PIC value 0.43 was observed for the marker CuMiSat-25 (0.43) (Figure 1). Marker CuMiSat-21, CuMiSat-24, CuMiSat-28 provided (-) result.

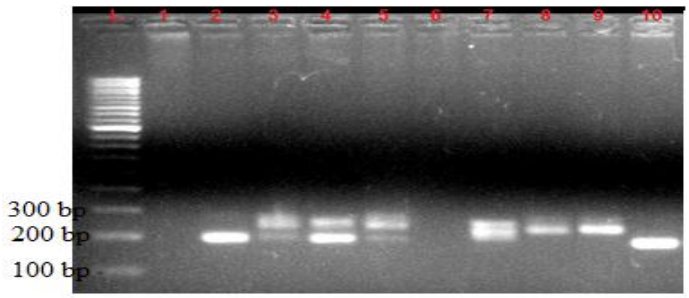

Figure 1. Agarose gel electrophoresis of PCR products of the microsatellite marker CuMiSat-25 for 10 Turmeric genotypes; M=DNA ladder (100bp)

Similarity vs Dissimilarity Analysis

Out of 65 scorable amplified bands 24 were found polymorphic. The percentage of polymorphism showed maximum of 100% showed by 7 SSR primers (Table 3). Based on dendrogram the genotypes of turmeric belong to genetically diverse clusters. The UPGMA dendrogram (Figure 2) showed two main distinct clusters of turmeric genotypes which were also genetically diverse amongst themselves. Clusters I comprised of two genotypes with similarity coefficient 0.67. Jaccard's coefficient of similarity (Table 4) revealed that high degree of similarity to the extent of 98 % existing between the varieties Ranga and variety Suguna. They were almost genetically similar.

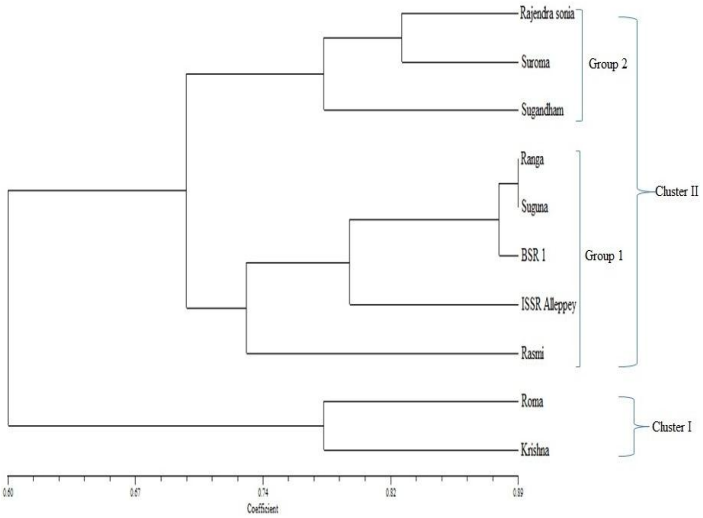

Figure 2. Dendrogram showing clustering of 10 genotypes of Turmeric constructed by using UPGMA cluster analysis of genetic similarity based on SSR data.

The cluster I consists of 2 genotypes of turmeric including Roma and Krishna. The similarity coefficients of this cluster ranged between 0.65 and 0.78 to the other members of the Cluster II. Cluster II was the largest and included 8 genotypes of turmeric with similarity coefficients ranging between 0.62 and 0.98. It is interesting to note that the Cluster II is further divided into two diversified groups to simplify their comparative study. Group I includes 5 turmeric genotypes namely Ranga, Suguna, BSR 1, ISSR Alleppey and Rasmi with similarity coefficient ranging from 0.62 to 0.98. Likewise group II included 3 turmeric genotypes viz. Rajendra Sonia, Suroma and Sugandham with the similarity coefficient ranging between 0.70 to o.86. Figure 2 reveals that in cluster I the genotype Rajendra Sonia was distantly related to Krishna with similarity coefficient 0.60. High degree of similarity was found between variety Ranga and variety Suguna with similarity coefficient 0.98. Based on study, the large range of similarity values for related cultivars using microsatellites provided greater confidence for the assessment of genetic diversity and relationships.

Table 4. Jaccard's coefficient of similarity derived from SSR data obtained by 10 genotypes of Turmeric.

GENOTYPES	Rasmi	ISSR Alleppey	Sugandham	Ranga	BSR-1	Rajendra sonia	Suguna	Suroma	Roma	Krishna
Rasmi	1									
ISSR Alleppey	0.77	1								
Sugandham	0.82	0.77	1							
Ranga	0.66	0.69	0.75	1						
BSR-1	0.66	0.71	0.71	0.77	1					
Rajendra	0.82	0.82	0.70	0.62	0.66	1				
Suguna	0.622	0.69	0.62	0.98	0.80	0.75	1			
Suroma	0.77	0.68	0.73	0.65	0.65	0.86	0.80	1		
Roma	0.68	0.68	0.75	0.78	0.65	0.75	0.73	0.71	1	
Krishna	0.75	0.71	0.75	0.78	0.73	0.60	0.82	0.87	0.67	1

DISCUSSION

The 10 SSR markers were distributed throughout the turmeric genotypes that required more study to reveal gene information and its product. The seven identified markers are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate at a specific locus in turmeric.The study revealed that the markers CuMiSat-21 had 17 alleles as compared to other markers belonging to SSR's with lesser allele numbers. Many studies have also reported a significantly greater allelic diversity of microsatellite markers than other molecular markers [15]. Turmeric similarity ratio revealed that high degree of similarity to the extent of 98% exists between genotype Ranga and Suguna, whereas very low level of similarity exists between Rajendra sonia and Krishna.

The suggested markers are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate at a specific locus. The main cause for a high level of polymorphism could be intra-specific variation as reported by Nayak e*t al.* (2006)[16] who demonstrated that high number of polymorphic loci revealed profound intra-specific variation among turmeric cultivars. The dendrogram (Figure 2) resulting from UPGMA analysis could reveal allelic richness of 2 clusters for various sizes. UPGMA cluster analysis of the SSR based genetic similarity matrix resulted in the classification of genotypes into separate clusters, sub-clusters and groups. CuMiSat-22, CuMiSat-20, CuMiSat-27 and CuMiSat-19 weregenerated higher levels of polymorphism and any two of them can be used to differentiate between the 10 turmeric genotypes. SSR data sets generated from two or three primer combinations are sufficient for estimation and additional data sets do not change the relationships among the 10 genotypes.

According to Akkaya and Buyukunal-Bal (2004)[17], high PIC value can be attributed to the use of more informative markers. Highest PIC values were observed for SSR marker CuMiSat-19 (0.98), CuMiSat-20 (0.97), CuMiSat-26 (0.97) and CuMiSat-22 (0.96) CuMiSat-27 (0.95). PIC value is reflection of allele diversity and frequency among the genotypes. The markers showed an average PIC of 0.86, which confirms that SSR markers used in this study were highly informative because markers with PIC values of 0.56 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus [18]. This indicated that the genotypes used in the present study were more diverse due to differences in origin, ecotype and speciation. Microsatellite markers exhibit high PIC values because of their codominant expression and multiallelism [19].

CONCLUSIONS

A comparison of these values of allelic diversity among the genotypes, clearly emphasize the scope for introgression of genes through hybridization among the cultivars for increasing genetic diversity in the cultivated turmeric pool. This also reiterates the need for genetic diversity evaluation among the principal genotype classes and cataloguing them for the benefit of the future. Based on the study a large range of similarity values for related cultivars using microsatellites provides greater confidence for the assessment of genetic diversity as the results revealed showed distinct polymorphism among the genotypes. Such fingerprinting makes identification and characterization of genotype very easy and further it will be of greater help in background selections during back cross breeding programme. Varietal profiling based on SSR markers will be more reliable as compared to other markers, since SSR markers detect finer levels of variations among closely related lines.

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About Author

Mr. Ashwani Kumar Singh received BSc. from University of Allahabad, Allahabad, U.P., B.Tech. and M. Tech. from Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P. He has worked as a Senior Research Fellow at Sardar Vallabhbhai Patel University of Agriculture and Technology, Research Associate (Trainee) and Project Associate at The Energy & Resources Institute (TERI, New Delhi). He has published four books and various research papers in National and International journals of repute.