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Evaluation of Intra and Inter-specific Variability among Species of *Curcuma* using Microsatellite Markers

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Abstract

Microsatellite markers such as ISSRs and SSRs were used to study the genetic variability among 15 selected accessions in 8 species of *Curcuma* germplasm comprising of two accessions in each of the seven *Curcuma* species and one of *C. longa.* Of the primers tested, 10 (out of 10) ISSR and 7 (out of 10) SSR primers were selected for their reproducibility and high polymorphism. A total of 166 polymorphic ISSR bands were detected out of 176 bands and a total of 123 polymorphic SSR bands were detected out of 135 bands. Results demonstrated an extensive genetic variability within the selected accessions as well as the value of ISSR and SSR markers in characterization of *Curcuma* species. Marker scoring data was used to construct UPGMA dendrogram and Pearson Correlation Coefficient. For both markers a high similarity in dendrogram clustering was obtained. All dendrograms, including that obtained by the combined use of both the ISSR and SSR marker data, depicted the genetic relationship/variability among the accessions, depending upon their species similarity/ dissimilarity.

Key words: *Curcuma*, microsatellite markers, genetic similarity, UPGMA dendrogram, Pearson correlation coefficient

Introduction

The genus *Curcuma* belongs to the family Zingiberaceae within the sub family Zingiberoideae and tribe Zingiberae (Kress et al. 2002) and the include about 120 species distributed in tropical and subtropical Asia (Skornickova et al. 2007). The generic name *Curcuma* is the Latinized version derived from the Arabic word 'Karkum' meaning yellow (Purseglove et al. 1981). The genus *Curcuma* was first established by Carl Linnaeus, in his book 'Species Plantarum'.

The genus *Curcuma* is considered to have originated in the Indo-Malayan region and is broadly distributed in the tropics of Asia to Africa and Australia (Purseglove, 1968). It has a wide spread occurrence from sandy coastal habitat to an altitude as high as 2000 m in the Western Ghats and Himalayas. The greatest diversity of the genus occurs in India (Ravindran et al. 2007). *Curcumas* are found to occur in diverse tropical conditions, margins of forests, secondary forests, plantations, open grasslands, plains in coconut and arecanut groves. *Curcuma* species diversity in India is concentrated in north eastern India, south India and Andaman and Nicobar Islands. The other countries rich in *Curcuma* species diversity are Indonesia, Malaysia, China, Thailand, Philippines, Vietnam, Singapore, Brunei, and Papua New Guinea (Sabu, 1991).

Morphological characterization is done in each accession using a set of standard descriptors. According to Kaplan (2001), for the comprehension of life cycles, geographical distribution, evolution, conservation status as well as species delimitation, morphological data are very important. However, the phenotypic variation of plants does not always follow the genetic pattern of variation and diversity of plant populations. Traditional methods

to characterize and identify species in *Curcuma* are based on phenotypic observations, but this approach is slow and subject to environmental influences which may mislead characterization of the species. Some authors cautioned about the wisdom of solely relying floral or vegetative traits for characterizing the *Curcuma* species (Larsen and Smith, 1978, Santapau, 1952). Hence, new methods based on studies at the DNA level must be included into breeding programs in order to accelerate and optimize genotype fingerprinting and to study genetic relationships among species.

In this study, ISSR and SSR markers based on the polymerase chain reaction (PCR) were used. The ISSR molecular marker based analysis in *Curcuma* species is already known (Syamkumar, 2008) but the originality of species used for the study was doubtful.

Simple sequence repeat (i.e. SSR or microsatellites) are currently becoming the preferred technique for the molecular characterization of different plant species (Rafalski et al. 1996; Gupta et al. 1996; Wunsch and Hormaza, 2002). SSRs are based on tandem repeats of short (2-6 bp) DNA fragments scattered throughout the genome that lie between conserved sequences (Litt and Luty, 1989) and have been isolated and characterized in large number of animal and plant species (Tautz, 1989; Powell et al. 1996; Lefort et al. 1999).

This study aimed to molecular characterization of selected accessions of *Curcuma* species and thus understand the intra and inter-specific relationship using PCR-based molecular markers ISSR and SSR.

Materials and Methods

Plant material

The study was conducted at the Molecular Biology Laboratory, Division of Crop Improvement, ICAR-CTCRI, Thiruvananthapuram. The experimental material comprised of 15 accessions belonging to 8 species of the genus *Curcuma* L. (2 accessions in each of *C. amada* Roxb., *C. angustifolia* Roxb., *C. aromatica* Salisb., *C. decipiens* Dalz., *C. malabarica* Velay., *C. raktakanta* Mangaly and Sabu, *C. zedoaria* Rosc. and one accession of *C. longa* Linn.) maintained in the field gene bank of ICAR-CTCRI farm (Table 1).

Genomic DNA extraction

Fresh tender leaves of the *Curcuma* species accessions were used for the isolation of DNA. The *Curcuma* genomic DNA was isolated using the method of Syamkumar (2008). 1.5 g young leaves were ground in liquid nitrogen to fine powder and extracted with hot cetyltrimethylammoniumbromide (CTAB) extraction buffer. The extraction buffer contains 3% CTAB, 5 M NaCl, 1M Tris, 0.5 M EDTA and 0.2% β

Table 1. Passport data of the 15 accessions in 8 species of *Curcuma* L.

Sl. No.	Species	Identity Number	Local name	Place	District	State
1	*C. amada*-1	AKI/BT/2017-1	Inji Manga	Cheenikuzhy	Idukki	Kerala
2	*C. amada*-2	AKI/BT/2017-2	Manga Inji	Amboori	Trivandrum	Kerala
3	*C. angustifolia*-1	AKI/BT/2017-3	Tikhur	Moglai	Bastar	Chhattisgarh
4	*C. angustifolia*-2	AKI/BT/2017-4	Tikhur	Shemaljodi	Raipur	Chhattisgarh
5	*C. aromatica*-1	AKI/BT/2017-5	Kasthoori Manjal	Cheenikuzhy	Idukki	Kerala
6	*C. aromatica*-2	AKI/BT/2017-6	Kasthoori Manjal	Vellayani	Trivandrum	Kerala
7	*C. decipiens*-1	AKI/BT/2017-7	Kuzhi Koova	Manchikkal	Idukki	Kerala
8	*C. decipiens*-2	AKI/BT/2017-8	Kuzhi Koova	Uppukunnu	Idukki	Kerala
9	*C. malabarica*-1	AKI/BT/2017-9	Neelakkoova	Oliviruppu	Idukki	Kerala
10	*C. malabarica*-2	AKI/BT/2017-10	Kattukoova	Nanminda	Kozhikode	Kerala
11	*C. raktakanta*-1	AKI/BT/2017-11	Koova	Athirappally	Thrissur	Kerala
12	*C. raktakanta*-2	AKI/BT/2017-12	Koova	Neerickode	Ernakulam	Kerala
13	*C. zedoaria*-1	AKI/BT/2017-13	Manjakkoova	Chanthavila	Trivandrum	Kerala
14	*C. zedoaria*-2	AKI/BT/2017-14	Manjakkoova	Cheenikuzhy	Idukki	Kerala
15	*C. longa*	AKI/BT/2017-15	Manjal	Malayinchi	Idukki	Kerala

mercaptoethanol. The mixture was incubated at 65°C for 1 h, followed by two extractions with chloroform/ isoamyl alcohol (24:1). Ice cold isopropanol was used to precipitate nucleic acids, and the pellet obtained was washed with 70% ethanol dissolved in Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH = 8.0 and 1 mM EDTA, pH = 8.0). RNA was removed by digestion with RNase A at 37°C in waterbath. The isolated total DNA was quantified by spectrophotometry and its quality verified by gel electrophoresis.

PCR amplification

ISSR reaction was carried out in 15 µl reaction volume containing 7.5 µl EmeraldAmp® GT PCR master mix (2X), 1.3 mM $MgCl_2$, 0.5 µl primer (0.3 ìM), 3 µl genomic DNA (40 ng), 2.7 µl sterile distilled water. PCR reaction done with initial denaturation at 94°C for 5 minutes followed by 38 cycles with denaturation at 94°C for 30 seconds, primer annealing at 56.3°C for 1 minute and extension at 72°C for 1 minute. The final extension was performed at 72°C for 10 minutes followed by its holding at 4°C.

SSR reaction was carried out in 15 µl reaction volume containing 7.5 µl EmeraldAmp® GT PCR master mix (2X), 1 mM $MgCl_2$, 0.5 µl primer (forward and reverse) (0.3 µM), 4 µl genomic DNA (50 ng), 2.0 µl sterile distilled water. Amplification done with initial denaturation at 94°C for 5 minutes followed by 38 cycles with denaturation at 94°C for 30 seconds, primer annealing at appropriate temperature for 45 seconds and extension at 72°C for 1 minute. The final extension was performed at 72°C for 20 minutes followed by its holding at 4°C. Both the PCR was carried out in Proflex™ Thermalcycler. The amplification products were stored at 4°C before analysis.

The amplified products were resolved in a 2% agarose gel using 100 bp (GeNei) and 1 kb (NEB) ladder for checking amplification, to identify molecular weight of obtained bands and for polymorphism studies. All PCR reactions were repeated to ensure the reproducibility of bands. The electrophoretic products were visually analysed and DNA bands were scored as present (1) or absent (0). The matrix obtained was entered in to the R package 3.5.0 and UPGMA dendrogram was constructed based on Euclidean distance. Pearson Correlation Coefficient (PCC) data were obtained and intra and inter-specific genetic relationship is estimated.

Results and Discussion

ISSR data analysis

Ten UBC series primers were selected for ISSR analysis. The sequence of the ISSR primers selected for the molecular genetic fingerprinting of the 15 accessions of *Curcuma* species is shown in the Table 2. The total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table 3.

Among 10 primers, 7 primers gave 100% polymorphic bands. As highly polymorphic primers were used for the analysis, a relatively large number of polymorphic markers were detected by these ISSR primers. A total of 7 primers produced 176 markers out of which 166 (94.31%) were polymorphic. Uniqueness of bands produced for each-species-accessions indicated that ISSR markers were efficient for assessment of intra and inter species diversity of *Curcuma* spp. The ISSR polymorphism obtained with primer UBC-808 for the selected *Curcuma* accessions is shown in the Fig. 1.

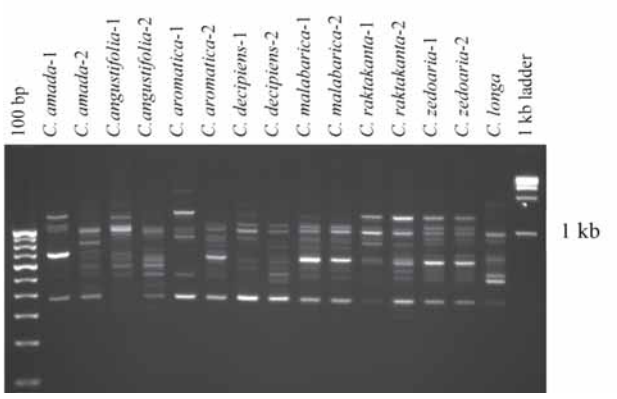

Fig. 1. ISSR banding patterns of UBC 808 primer with 15 *Curcuma* accessions

Table 2. ISSR primers selected for the genetic fingerprinting of *Curcuma* species

Sl. No.	Primer name	Sequence
1	UBC-807	$(AG)_8T$
2	UBC-808	$(AG)_8C$
3	UBC-809	$(AG)_8G$
4	UBC-811	$(GA)_8C$
5	UBC-825	$(AC)_8T$
6	UBC-836	$(AG)_8YA$
7	UBC-841	$(GA)_8YC$
8	UBC-842	$(GA)_8YG$
9	UBC-864	$(ATG)_6$
10	UBC-873	$(GACA)_4$

Table 3. Analysis of ISSR primers

Sl. No.	Primer	Total number of bands	Number of polymorphic bands	Percentage polymorphism (%)
1	UBC-807	19	14	73.6
2	UBC-808	30	30	100.0
3	UBC-809	15	15	100.0
4	UBC-811	19	19	100.0
5	UBC-825	20	19	95.0
6	UBC-836	15	15	100.0
7	UBC-841	12	8	66.6
8	UBC-842	15	15	100.0
9	UBC-864	15	15	100.0
10	UBC-873	16	16	100.0
	Total	176	166	94.3

SSR data analysis

Seven Curcuma specific SSR primers were selected for SSR analysis (Table 4.). The sequence of the SSR primers used for the molecular genetic fingerprinting of the 15 accessions of *Curcuma* species and the total number of bands produced by each primer, number of polymorphic bands and percentage of polymorphism produced by each primer are presented in Table 5.

Seven SSR primers produced a total of 135 scorable bands in the 15 accessions of 8 species studied out of which 123 (91.1%) were polymorphic. CuMiSat-19 gave 100% polymorphic bands. The ISSR polymorphism obtained with primer CuMiSat-26 for the selected *Curcuma* accessions is shown in the Fig. 2.

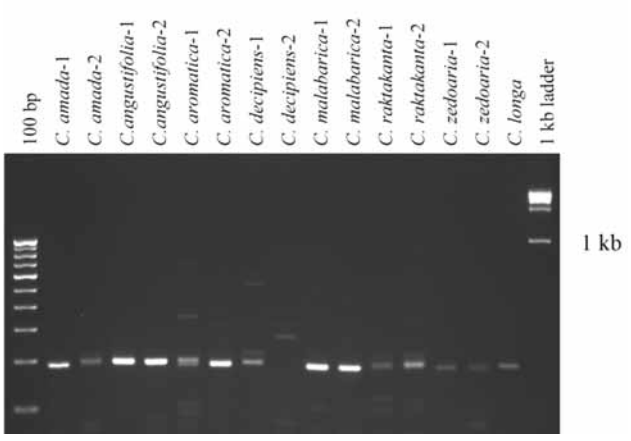

Fig. 2. SSR banding patterns of CuMiSat-26 primer with 15 *Curcuma* species accessions

Table 4. SSR primers selected for the molecular fingerprinting of *Curcuma* species

Sl. No.	Primer name	Sequence	Annealing temperature
1	CuMiSat-19 F:	CAT GCA AAT GGA AAT TGA CAC	
	CuMiSat-19 R:	TGA TAA ATT GAC ACA TGG CAG TC	65°C
2	CuMiSat-20 F:	CGA TAC GAG TCC ATC TCT TCGCCT TGC	
	CuMiSat-20 R:	TTT GGT GGC TAG AG	65°C
3	CuMiSat-22 F:	AAT TTA TTA GCC CGG ACC AC	
	CuMiSat-22 R:	AAG AAA GTG AGT AGA AAC CAA AGC	64°C
4	CuMiSat-24 F:	AGG TAT TCT ACT CGA CCA AG	
	CuMiSat-24 R:	AAA TTC ATA TAG CCC CAT C	58°C
5	CuMiSat-25 F:	TAC ATG AGA AAC AAC AAA GCC	
	CuMiSat-25 R:	CAGT TAG CCA AGT CCC AAT TTA GC	65°C
6	CuMiSat-26 F:	CAT TCC GAT GAA TTG TAT	
	CuMiSat-26 R:	GGCA GTT GTT TTG CTT CAG	58°C
7	CuMiSat-28 F:	TTC AAC TTC TCC TCG CTC AG	
	CuMiSat-28 R:	GCA AGG TGC TGC ATC TAT TTC TC	65°C

Cluster analysis

a) ISSR marker

UPGMA cluster analysis grouped the 15 accessions into 5 major clusters shown as in the dendrogram (Fig. 3). The coefficients on the y-axis represent the dissimilarity indices of the different species chosen for the study. Based on UPGMA clustering from ISSR, the genotypes were grouped into 5 major clusters. *C. decipiens* accessions were grouped as a distant cluster. Clusters II and V consisted of only one group having *C. zedoaria* and *C. decipiens* respectively. Within cluster III, three subgroups were evident, containing *C. amada*, *C. aromatica* and *C. angustifolia* accessions. *C. longa* alone formed cluster IV. *C. malabarica* and *C. raktakanta* were grouped under cluster I.

b) SSR marker

UPGMA cluster analysis was done based on Euclidean distance in which the fifteen accessions were grouped into 6 major clusters shown as in the dendrogram (Fig. 4). Based on UPGMA clustering from SSR data, the genotypes were grouped into 6 major clusters. Here also, the *C. decipiens* accessions were grouped as a distant cluster. Clusters II, IV and VI consisted of only one group having *C. aromatica*, *C. zedoaria* and *C. decipiens* respectively. Within cluster I, subgroups of *C. amada* and *C. angustifolia* were placed and within cluster III, subgroups of *C. malabarica* and *C. raktakanta* were included.

The Pearson correlation coefficient obtained using the ISSR scoring data and thus the intra-specific variation within accessions of same species and inter-specific variation between the species were estimated. The intra- and inter-specific similarity among the selected accessions are shown in the Table 6.

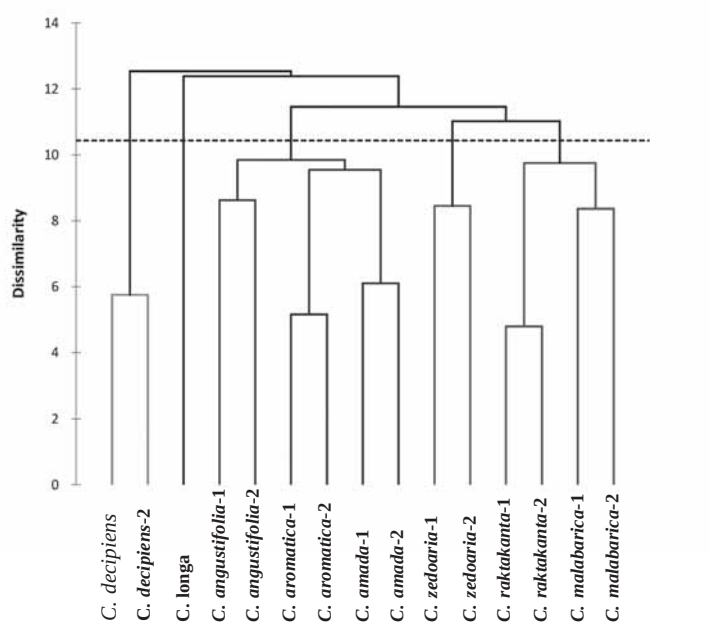

Fig. 3. Cluster dendrogram based on ISSR markers

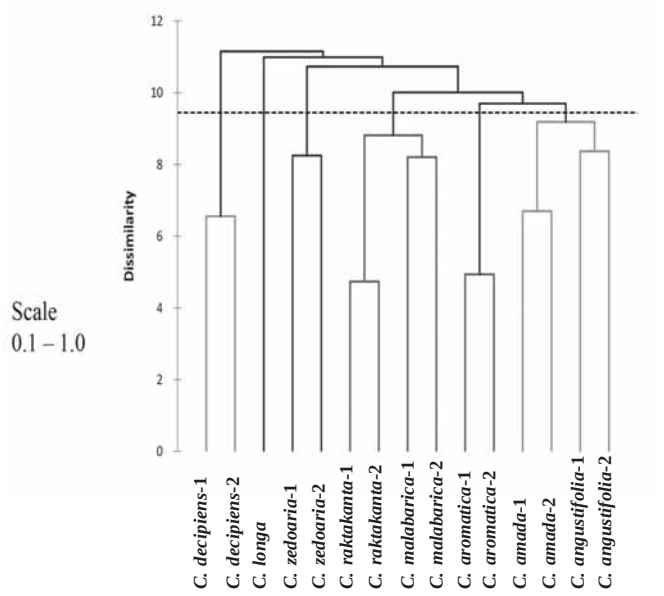

Fig. 4. Cluster dendrogram based on SSR markers

Table 5. Analysis of SSR primers

Sl. No.	Primer	Total number of bands	Number of polymorphic bands	Percentage Polymorphism (%)
1	CuMiSat-19	19	19	100.0
2	CuMiSat-20	24	23	95.8
3	CuMiSat-23	8	6	75.0
4	CuMiSat-24	20	17	85.0
5	CuMiSat-25	13	12	92.3
6	CuMiSat-26	20	19	95.0
7	CuMiSat-28	31	27	87.0
	Total	135	123	91.1

Table 6. Comparison of results of ISSR and SSR molecular marker data

Marker	Similarity	Intra-specific	Inter-specific	With *C. longa*
ISSR	Highest	*C. raktakanta*-1	*C. aromatica*-1	
		C. raktakanta-2 (0.86)	*C. amada*-2 (0.51)	*C. aromatica*-2 (0.22)
	Lowest	*C. angustifolia*-1	*C. decipiens*-1	
		C. angustifolia-2 (0.55)	*C. raktakanta*-1 (-0.027)	*C. decipiens*-1 (-0.13)
SSR	Highest	*C. raktakanta*-1	*C. malabarica*-1	
		C. raktakanta-2 (0.83)	*C. raktakanta*-1 (0.49)	*C. angustifolia*-1 (0.25)
	Lowest	*C. angustifolia*-1	*C. decipiens*-2	
		C. angustifolia-2 (0.47)	*C. raktakanta*-1 (0.043)	*C. decipiens*-1 (-0.13)

Curcuma species show diversity in habitat, morphology and use, Detailed knowledge about genetic relationship/ variability among *Curcuma* species will improve its utilization value for any future study. A few studies based on morphological, anatomical, and biochemical characterization of *Curcuma* species have been attempted before (Das et al. 2011, Prasanth et al. 2015, Saha et al. 2016, Syamkumar, 2008). Relying much on the morphological characters alone in species differentiation has its own limitations since they are environmental sensitive and are not always representative of the entire genetic structure. Conventional taxonomic techniques in conjunction with molecular biology tools may provide accurate and powerful ways of analyzing genetic relationship in the genus *Curcuma*. (Syamkumar and Sasikumar, 2007). Earlier, diversity studies have been reported in *Curcuma* species all over India by using only RAPD and ISSR markers (Das et al. 2011). However, not much study on *Curcuma* species has been done using SSR markers.

The present work is the first attempt to assess the intra- and inter-specific genetic relationship of 8 indigenous species of *Curcuma*. The percentage of polymorphism revealed by ISSR markers was found highest (94.3) as compared to SSR (91.1) in the studied *Curcuma* species accessions. This indicates that the efficiency of ISSR markers in terms of amplification of a large number of fragments is high, compared to SSR. This is in agreement with Manimekalai et al. (2007) and Goulao and Oliviera, (2001). Yang et al. (1994) also stated that ISSR assay can provide more informative data than other techniques.

PCC data of both ISSR and SSR markers have shown that a less variation exists within accessions of same species and a high variation exists between accessions of different species. Among the 15 accessions the lowest intra-specific similarity was observed between *C. angustifolia* accessions (0.55) and the highest in *C. raktakanta* accessions (0.86). The highest inter-specific similarity was observed between *C. aromatica* and *C. amada* accessions (0.51) and the lowest between *C. raktakanta* and *C. decipiens* accessions (0.043). Similar results have been observed in the morphological characterization also. The dendrogram constructed based on scoring data of both the markers did not vary much from the individual dendrograms. The two species *C. malabarica* and *C. raktakanta*, which clustered together in both dendrograms share many common vegetative, rhizome, floral traits and this may be contributing to the identical banding pattern and similarity of the two species This is similar to the observation of Sabu (1991). The same has been noticed in the case of *C. amada* and *C. angustifolia*. However, they are morphologically distinct. *C. decipiens* clustered as a separate group in both ISSR and SSR clusters. This was in congruence with the report of Syamkumar (2008). The most cultivated species *C. longa* showed little similarity to *C. angustifolia* (0.29) and *C. aromatica* (0.22) and this is in contrary to Sabu (1991) who observed *C. longa* and *C. amada* share some similarities.

Conclusion

The present study revealed that there exists a good genetic variability among all the selected 15 accessions of *Curcuma*. The variability among the accessions within species was found to be less than as compared between the variability among different species. The study also suggests that relying solely the morphology may be ambiguous and it would be wise to use ISSR/SSR markers along with morphological tools. The ISSR/SSR primers selected for the study have shown high polymorphism

and this study suggests that both these markers are efficient tools in the intra and inter-specific characterization of *Curcuma* species and they could be employed in future genetic variability studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Das, A., Kesari, V., Satyanarayana, V. M., Parida, A. and Rangan, L. 2011. Genetic Relationship of Curcuma Species from Northeast India Using PCR-Based Markers. *Mol. Biotechnol*. **49**: 65-76.

Goulao, L. and Oliveira, C. M. 2001. Molecular characterisation of cultivars of apple (Malus × domestica Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica.* **122(1)**: 81-89.

Gupta, P. K., Balyan, H. S., Sharma, P. C. Ramesh, B. 1996. Microsatellites in Plants: A new class of molecular markers. *Curr. Sci.*, **70**(1): 45-54.

Kaplan, D. R. 2001. The Science of Plant Morphology: Definition, History, and Role in Modern Biology. *Am. J. Bot.*, **88**(10): 1711-1741.

Kress, W. J., Prince, L. M. and Williams, K. J. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *Am. J. Bot.*, **89**: 1682-1696.

Larsen, K. and Smith, R. M. 1978. A new species of *Curcuma* from Thailand. Notes Royal Botanical Garden. Edinburgh **30**, 269-272.

Lefort, F., Brachet, S., Frascaria-Lacoste, N., Edwards, K. J. and Douglas, G. C. 1999. Identification and charatcterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae). *Mol. Ecol.*, **8**:1075-1092.

Litt, M. and Luty, J. A. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of dinucleotide repeat within the cardiac muscle action gene. *Am. J. Hum. Genet.*, **44**: 397-401.

Manimekalai, R., Nagarajan, P. and Kumaran, P. M. 2007. Comparison of effectiveness of RAPD, ISSR and SSR markers for analysis of coconut (*Cocos nucifera* L.) germplasm accessions. *J. Trop, Agric.*, **18**: 217-226.

Powell, W., Machray, G. C. and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. *Tren. Plant Sci.*, **1**: 215-222.

Prasanth, N., Bhavani, N. L. and Yugander, A. 2015. DNA Isolation and PCR Amplification of Turmeric Varieties from Telangana State. *Int. J. Curr. Microbiol. App. Sci.*, **4(5):** 485-490.

Purseglove, J. W. 1968. Tropical crops: Monocotyledons, Longman, London.

Purseglove, J. W., Brown, E. G., Green, C. L. and Robin, S. R. J. 1981. Turmeric. In: Spices, Vol. 11. New York, Longman. pp 532-580.

Rafalski, J. A., Vogel, J. M., Morgante, M., Powell, W., Andre, C. and Tingey, S. V. 1996. Generating and using DNA markers in plants. In: Non-mammalian Genome Analysis. A Practical Guide (Birren, B. and Lai, E., eds.). Academic Press, San Diego, pp 75-134.

Ravindran, P. N., Nirmal Babu, K., Shiva, K. N. 2007. Botany and crop improvement of turmeric. In: Ravindran PN, Nirmal Babu K, Sivaraman K (Eds.), Turmeric The Genus *Curcuma*, CRC press, Boca Raton. pp 15-70.

Sabu, M. 1991. A taxonomic and phylogenetic study of South Indian Zingiberaceae. Ph.D. Thesis, University of Calicut, Kerala, India. p 322.

Saha, K., Sinha, R. K., Basak, S. and Sinha, S. 2016. ISSR Fingerprinting to Ascertain the Genetic Relationship of *Curcuma* sp. of Tripura. *Am. J. Plant Sci.*, **7:** 259-266.

Santapau, H. 1952. On a common species of *Curcuma* of Bombay and Salsette Islands. *J. Bombay Nat. Hist. Soc.* **51:** 135-139.

Skornickova, J., Reshe, T. and Sabu, M. 2007. Other economically important *Curcuma* species. In: Ravindran, P.N., Nirmal Babu, K. and Sivaraman, K. (Eds.), Turmeric The Genus *Curcuma*, CRC press, Boca Raton. pp 451-467.

Syamkumar, S. and Sasikumar, B. 2007. Molecular marker based genetic diversity analysis of *Curcuma* species from India. *Scientia Horticulturae*. **112**: 235-241.

Syamkumar, S. 2008. Molecular, biochemical and morphological characterization of selected *Curcuma* accessions. Ph. D. Thesis, Indian Institute of Spices Research, University of Calicut, Kerala, India.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* **17**(16): 6463-6471.

Wunsch, A. and Hormaza, J. I. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA. *Euphytica*, **125**: 59-67.

Yang, G. P., Maroof, M., Xu, C., Zhang, Q. and Biyashev, R. 1994. Comparative analysis of microsatellite DNA polymorphism inland races and cultivars of rice. *Mol. Gen. Gen.*, **245**: 187-194.