International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

GENETIC DIVERSITY OF TURMERIC (CURCUMA LONGA) ACCESSIONS OF MANIPUR

Sushma Phurailatpam, Swati Sen-Mandi* and G.J. Sharma**

Department of Life Sciences, Manipur University, Imphal-795003, India

* Department of Botany, Bose Institute, Kolkata-700009, India

ABSTRACT

Randomly amplified polymorphic DNA (RAPD) fingerprints of seven turmeric accessions were analyzed by polymerase chain reaction of genomic DNA using ten random decamer primers. Out of the ten primers screened, five primers generated fiftynine polymorphic and reproducible bands. The RAPD fragments were scored for presence or absence to calculate Jaccard's similarity index. Clustering based on similarity index was done following unweighted pair group with arithmetic mean method (UPGMA) and a dendrogram was constructed.

Key words: Curcuma longa, curcumin, anti-oxidant, RAPD, genetic diversity

INTRODUCTION

Curcuma consists of approximately seventy species (Purseglove, 1974) distributed in India, south-east Asian countries and north Australia. Curcuma longa L. is the most common and important plant among the species of Curcuma reported and studied till date. It is effectively used in herbal remedies since times immemorial. In Indian system of traditional medicine (ISTM), the rhizomes have been used alone or in combination with other herbs for various remedies.

The rhizomes contain three major curcuminoid compounds, i.e., curcumin (77%), demethoxycurcumin (17%) and bismethoxycurcumin (3%). Curcumin is the active principle which is a yellow-orange compound insoluble in water but soluble in organic solvents. Curcumin is known for its anti-oxidant, anti-inflamatory (Ammon et al., 1993) and anti-tumorigenic properties (Chen et al., 1996). It is also reported as a gastro-protectant (Lee et al., 2003), an anti-ulcer (Sinha et al., 1975), anti-spasmodic (Itthipanichpong et al., 2003) and anti-flatulent agent. It exhibits anti-cataract (Suryanarayana et al., 2003), anti-diabetic (Narayanasamy et al., 2002), anti-coagulent activities and is active against many bacteria (Shankar & Murthy, 1979) and protozoans such as Entamoeba and Leishmania (Koide et al., 2002). It has anti-viral activity as it inhibits the HIV integrase enzyme (Mazumdar et al., 1995). It shows anti-fertility effects in rat and inhibits human sperm motility in vitro (Rithaporn et al., 2001).

This important multidisciplinary usage plant has many cultivars, among them seven cultivars collected from various areas of Manipur have been selected. They are Churachandpur, Ethai-Moirangpurel, Meitei-yaingang, Shilot and Tamenglong cultivars. G.L.Puram and Lakadong cultivars are not used for comparision of the results. The cultivars show variation in the morphology of rhizome size, shape and colour when cut, and also in their leaves. A study of the molecular profile to obtain DNA fingerprints in order to establish the genetic diversity seems a worthwhile endeavour as knowledge of genetic variability is important for any breeding programme. It also provides the basis for the development of desirable genotypes. The DNA fingerprinting pattern would also help in monitoring the genetic relatedness among the cultivars of a species (Zhang, 1996). Randomly amplified polymorphic DNA markers have often been used for studying genetic diversity within plant germplasm collections and have been proposed as efficient tools for identification of DNA markers associated with important traits (Hittalmani et al., 1995) . In view of this, an attempt has been made to use RAPD studies to establish DNA fingerprints that could be co-related with some important morphological and biochemical parameters.

Materials and Methods

Plant material

From a number of field trips conducted, fresh turmeric rhizomes were collected and planted in the Experimental Field , Manipur University and in earthern pots at Bose Institute, Kolkata. The young , tender and unbruised leaves were collected in the early hours of the day for DNA extraction, and fresh two year-old rhizomes were taken for screening of anti-oxidant and curcumin contents.

Experimental

DNA extraction and amplification:

DNA was extracted from approximately 200mg of young, tender and unbruised leaf by using a modified technique of Greene et al. (1994). Ten decamer oligonucleotides (Operon Technolgies) were used for PCR amplification. PCR conditions including the concentration of template DNA, PCR buffer, primer, dNTPs, $MgCl_2$ and Taq polymerase were optimized to generate RAPD profiles represented by highly intense and sharp bands with a clear gel background.

The 25µl reaction mixture contained 30ng template DNA, 100µM of each dNTPs (Boehringer), 33ng primer (Operon Technologies), 1unit Taq DNA polymerase (Gibco BRL) and 1xPCR buffer (10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM $MgCl_2$). The mixture amplification was gently mixed. Amplification was performed in a Gene Amp® System 9700 (Perkin Elmer). Amplification program has initial denaturation of 3 mins at 94ºC, 45 cycles of 1 min at 94ºC, 1 min at 36ºC, 2 mins at 72ºC with final elongation of 5 mins at 72ºC. After amplification, 5µl of 10X loading dye was mixed to the whole amount of PCR products and loaded to a 1.2% agarose gel containing ethidium bromide. The gel was run in 1X TBE buffer (Sambrook et al., 1989), then viewed under ultraviolet light and was photographed. Molecular weights were estimated by using a 1Kb DNA ladder (Gibco BRL) for OPAC-19 and pUC18/Sau3AI – pUC18/TaqI Digest for all the primer. RAPD generated bands were scored 1 for presence and 0 for absence. These RAPD data, generated with five primers were used to compile a binary matrix for cluster analysis using SPSS version 7.5. Genetic similarity among cultivars was calculated according to Jaccard's similarity coefficients. The similarity coefficients were then used to construct a dendrogram using the unweighted pair-group method with arithmetical average (UPGMA) method.

RESULTS

The data of Plant size, rootstock and leaves (Table 1) have shown that Lakadong has size. This was followed by Shilot, G.L.Puram, Ethai-Moirangpurel, Tamenglong, Churachandpur cultivars and was found to be lowest in Meitei-yaingang cultivar.

Lakadong has reddish rhizome outside and it has bright golden yellow colour inside when cut. Shilot, GLPuram, Ethai-Moirangpurel and Tamenglong has Brownish rhizome outside while Churachandpur and Meitei yaingang has whitish brown colour. The rhizome of Shilot also show bright golden yellow colour inside when cut but Lakadong colour is darker. G.L.Puram , Ethai-Moirangpurel, Tamenglong and Churachandpur rhizome has dark yellow colour inside but the degree of darkness differ (table 1). Meitei-yainang rhizome show light yellow colour inside when cut. The datas suggested that Lakadong and Shilot cultivars which were golden-yellow in colour may have higher curcumin and total anti-oxidant potential whereas Meitei-yaingang which has light-yellow colour may have lowest curcumin content and total anti-oxidant potential.

RAPD analysis has revealed that out of 10 primers with GC contents ranging from 60-70% used to study the genetic diversity in the seven cultivars of C. longa, five showed satisfactory banding in the gel. Identical gel pattern was obtained in repeated experiments (on different populations from each cultivar) using the same primer on the genome of each cultivar established the genetic homogeneity of populations within each cultivar. A total of sixty

seven bands were scored out of which fifty-nine bands showed polymorphism (Table 2). The calculated average percentage of polymorphism per primer pair was 89%. DNA bands on the fingerprint were scored as (+1) for presence and as (+0) for absence and estimates of similarities between genotypes were determined based on the probability that an amplified fragment from one accesion will also be present in another (Nei et al., 1979). A representative RAPD gel pattern for analysis of genome using OPAB-06, OPAC-14, OPAC-19, OPAG-18 and OPAH-02 primers on all the cultivars presented in Figs. 1 gave a holistic estimate of polymorphism of RAPD bands.

To determine the level of relatedness among these genotypes, a similarity matrix was constructed with RAPD as a phenetic marker assuming that the presence or absence of a discrete character in two or more genotypes results from the same genetic changes (Scrotch et al., 1992). Simple matching co-efficient was used for cluster analysis by UPGMA and a dendrogram was constructed (Fig. 2) that gives the degree of genetic relationship between the different turmeric cultivars studied. The dendrogram revealed the overall similarity between the plants studied ranges between 60% and 96%. The highest similarities were observed between Lakadong and G.L.Puram cultivars. The above cluster is more genetically related with Shilot followed by Meitei-yaingang, Tamenglong , Churachandpur and Ethai-Moirangpurel respectively.

Such data provide the basis for indicating feasibility of genetic cross breeding.

The dendrogram revealed the overall similarity between the plants studied ranges between 60% and 96%. The highest similarity were observed between Lakadong and G.L.Puram cultivars . The above cluster is more genetically related with Shilot followed by Meitei-yaingang, Tamenglong , Churachandpur and Ethai-Moirangpurel respectively.

DISCUSSION

The high level of polymorphism of bands observed suggested the suitability of using the RAPD method for developing specific DNA markers. It is pertinent to mention here that in the present study, reproducible banding pattern for each cultivar was obtained even in different populations. RAPD analysis has also been used to ascertain variations between germplasm collections and also for assessing fidelity of micropropagated plants (Parani et al., 1997) as well as for studying genetic variation in a wide cultivar of species in natural populations (Williams et al., 1990). Lakadong and Shilot cultivars which have golden-yellow colour also reveal darker RAPD bands at 900 bp using OPAC-19 primer. Meitei-yaingang cultivar which is light-yellow has no such band and other cultivars which have intermediate colour have lighter bands at 900 bp using the same primer. . Therefore, these results suggest the possibility of developing a molecular marker for antioxidant character of curcumin using the primer OPAC-19.

ACKNOWLEDGEMENT

One of the authors (Sushma Phurailatpam) is thankful to the Council of Scientific and Industrial Research, New Delhi for providing a Junior Research Fellowship.

REFERENCES

1. Ammon, H.P.T., H. Safayhi, T. Mack and J. Sabieraj, 1993. Mechanism of anti-inflammatory actions of curcumin and boswellic acids. Ethnopharmacol. 38 (2/3), 113-119.
2. Chen, X, Y. Li, G. Liu and B.He, 1997 A preliminary study of the effect of external qigong on lymphoma. Anticancer Res. **17**: 2555-64.
3. Greene B., R. Walko and S. Hake, 1994. Mutator insertions in an intron of the maize knotted 1 gene result in dominant suppressible mutations. Genetics **138**: 1275-1285
4. Gupta, P.K. and R.K. Varshney, 1999. Molecular markers for genetic fidelity during micropropagation and germplasm conservation. Curr.Sci. **76**: 1308-1310.
5. Hittalmani,S., M.R. Foolad, T.V. Mew, R.L. Rodriguez and N. Huang, 1995. Development of PCR-based marker to identify rice blast resistance gene, Pi-2(t) in a segregating population. Theor. Appl. Genet. **91**:9-10.
6. Itthipanichpong, C., Ruangrugrin, W. Kensre and A. Sawasdepanich, 2003. Antispasmodic effects of curcuminoids on isolated guinea pig ileum and rat uterus. J. Med. Assoc. Thai.
7. Koide, T., M. Nose, Y. Ogihara, Y. Yabu and N. Ohta, 2002. Leishmanicidal effects of curcumin in vitro. Biol. Pharm. Bull. 25 (1): 131-133.
8. Lee, C.J., J.H. Lee, J.H., Seok, G.M. Hur, Y.C. Park, I.C. Seol and Y.H. Kim, 2003. Effects of baicalein, berberine, curcumin and hesperidin on mucin release from airway goblet cells. Planta Med. 69(6):523-6.
9. Mazumdar, A., K. Raghavan, J. Weinstein, K.W. Kohn and Y. Pommier, 1995. Inhibition of human immunodeficiency virus type 1 integrase by curcumin. Biochem. Pharmacol. **49** : 1165 – 1170.
10. Narayannasamy, A., N. Namasivayam and K. Radha, 2002. Effect of turmeric on the enzymes of glucose metabolism in diabetic rats. J. Herbs, Spices Medicinal plants **10** : 75-84.
11. Nei, M. and W.H. Li, 1979. Mathematical model for studying genetical variation in terms of restriction endonuclease. Proc. Natl. Acad. Sci. USA, **74**: 5267-5273.
12. Parani, M., A. Ananda, and A. Parida, 1997. Application of RAPD fingerprinting in selection of micropropagated plants of Piper longum for conservation. Curr. Sci. **73**: 81-83.
13. Purseglove, J.W. 1974. Tropical Crops Monocotyledons. London: Longman.
14. Reibeiro, A.B., D.H.S.Silva, V. de Silva and B.V. de Silva , 2002. Antioxidant flavonoid glycosides from Nectandra grandiflora (Lauraceace) Eclet Quim 27. Special Sao.Paulo ISSN.0100-4670.versao.impressia.
15. Rithaporn, T., M. Rajasekaran and M. Monga, 2001. Curcumin, a natural herb with anti-HIV activity, inhibits human sperm motility. J. Androl. **22** (suppl): P1/2-138.
16. Sambrook, J., E.F Fritsch. and P. Maniatis, 1989. Molecular cloning: a laboratory manual 1, 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
17. Shankar,T.B. and V. S. Murthy, 1979. Indian J.Exp. Biol. **17**: 1363.
18. Sinha, M., B.P. Mukerjee, B. Mukerjee, S. Sikdar and S.P. Dasgupta, 1975. Study of the mechanism of action of curcumin: an anti-ulcer agent. Indian J. Pharmacol. **7**: 98-99.

19. Scrotch, B. P., J. Tibang and J. Nienhuis, 1992. Analysis of genetic relationship using the RAPD marker data. In, Proceedings of the Symposium on the Application of RAPD Technology in Plant Breeding, Minneapolis, Crop Science Society America, 26-30
20. Suryanarayana, K., K. Krishnamurthy and G.B. Reddy, 2003. Effect of curcumin on galactose-induced cataractogenesis in rats. Mol. Vis. **9**: 223-230.
21. Willams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531- 6535.
22. Zhang, J., M.B. McDonald and M.S. Patricia, 1996. Random amplified polymorphic DNA (RAPDs) from seed of differing soyabean and maize genotypes. Seed Sci. Technol. **24**: 513-522

Varieties	Plant size	Rootstock	Rhizome colour (outside)	Rhizome colour (inside)	Leaves
Lakadong	Large (++++++)	Large (++++++)	Reddish	Bright golden yellow (++++++)	Larger and broader (++++++)
Shilot	Large (+++++)	Large (+++++)	Brownish	Bright golden yellow (+++++)	Leaves larger (+++++)
GL Puram	Medium (++++)	Medium (++++)	Brownish	Yellow inside (++++)	Medium (++++)
Ethai Moirangpurel	Medium (+++)	Medium (+++)	Brownish	Yellow inside (+++)	Medium (+++)
Tamenglong	Medium (++)	Medium (++)	Brownish	Yellow inside (++)	Medium (++)
Churachandpur	Medium (+)	Medium (+)	Whitish Brown	Yellow inside (+)	Smaller (+)
Meitei yaingang	Smaller	Small	Whitish Brown	Light yellow inside	Smaller and narrower

Table 1: Morphological data of turmeric cultivars

Primer	Sequence of primers 5'→3'	No. of amplified bands	No. of polymorphic bands	Polymorphic bands (%)
OPAA-01	AGACGGCTCC	10	7	70
OPAA-14	AACGGGCCAA	13	9	69.2
OPAB-06	GTGGCTTGGA	11	10	90.9
OPAB-17	TCGCATCCAG	12	11	91.7
OPAB-18	GGGCTAGTCA	12	10	83.3
OPAC-14	GTCGGTTGTC	15	13	86.7
OPAC-19	AGTCCGCCTG	16	16	100
OPAG-18	GTCGGCATAC	12	9	75
OPAH-02	CACTTCCGCT	13	11	84.6
OPAH-12	TCCAACGGCT	11	7	63.6
Total		125	103	82.4

Table 2: Sequences of five random primers with the number of scorable amplified and polymorphic band and their percentages

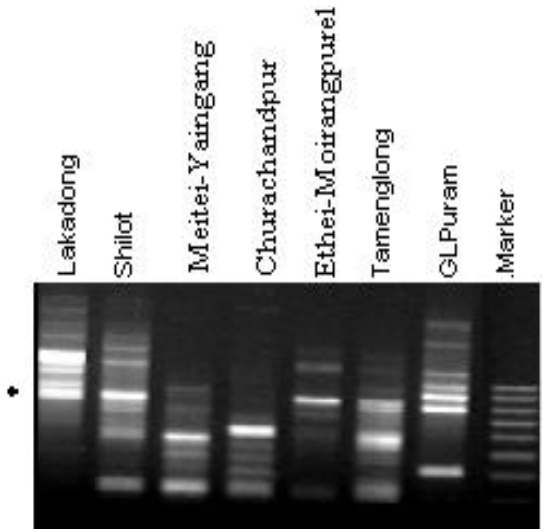

Fig 1:-Gel electrophoresis of PCR products by using primer "AGTCCGCCTG"

Turmeric cultivars	*Lakadong*	*Shilot*	*G.L.Puram*	*Ethai-Moirangpurel*	*Tamenglong*	*Churachandpur*	*Meitei-yaingang*
Lakadong		.340	.422	.404	.383	.233	.186
Shilot	.340		.523	.500	.511	.366	.286
G.L.Puram	.422	.523		.429	.380	.390	.310
Ethai-Moirangpurel	.404	.500	.429		.511	.341	.295
Tamenglong	.383	.511	.380	.511		.450	.302
Churachandpur	.233	.366	.390	.341	.450		.419
Meitei-yaingang	.186	.286	.310	.295	.302	.419	

Table 3: Similarity matrix based on the Jaccard's similarity index in Turmeric cultivars

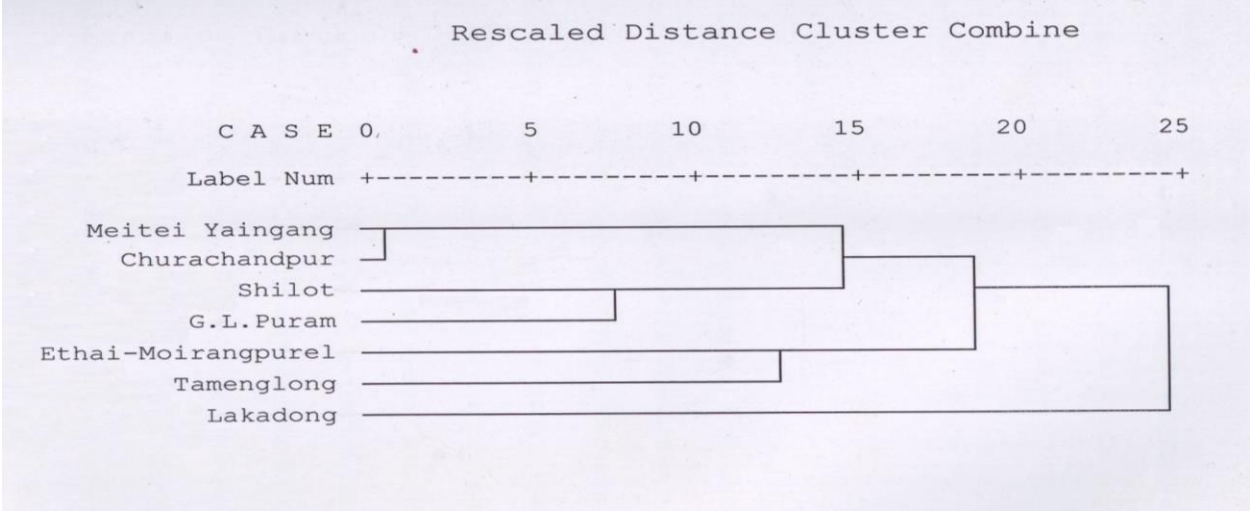

Fig 2:- Dendrogram using Average Linkage (Between Groups)

1- Lakadong, 2- Shilot, 3- G.L.Puram, 4- Ethai-Moirangpurel, 5- Tamenglong, 6- Churachandpur, 7- Meitei-yaingang