International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 10, 2017

Original Article

GENETIC DIVERSITY IN ACCESSIONS OF INDIAN TURMERIC (*CURCUMA LONGA* L.) USING RAPD MARKERS

KAMRAN ASHRAF[1,2,3], ALTAF AHMAD[4], SYED ADNAN ALI SHAH[1,2], MOHD MUJEEB*[3]

[1]Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia, [2]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia, [3]Bioactive Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard Univ, New Delhi-62, India, [4]Department of Botany, Aligarh Muslim University Aligarh, Aligarh, Uttar Pradesh, India
Email: pharmaresearch9@gmail.com

Received: 27 Mar 2017 Revised and Accepted: 31 Aug 2017

ABSTRACT

Objective: The present investigation was undertaken for identification and assessment of eight accessions of *Curcuma longa* collected from all ecological zones in India by random amplification of polymorphic DNA (RAPD) markers.

Methods: DNA was isolated using modified cetyl trimethyl ammonium bromide (CTAB) method. Polymerase chain reaction (PCR) was performed according to the method based on Williams *et al.* and data analysis was done using Alpha Imager EC software.

Results: Eleven out of twenty primers screened were informative and produced 150 amplification products among which 132 products (88%) were found to be polymorphic. The percentage polymorphism of all 08 accessions ranged from 44.44% to 100%. A total of 150 amplification products were scored with an average frequency of 13.63 bands per primer. Most of the RAPD markers studied showed a different level of genetic polymorphism. The data of 150 RAPD bands were used to generate Jaccard's similarity coefficients and to construct a dendrogram by means of UPGMA.

Conclusion: Results shows that *C. longa* undergoes genetic variation due to a wide range of ecological conditions within distribution area of its population in India. This investigation as an understanding of the level and partitioning of genetic variation within the accessions and would provide an important input into determining efficient management strategies and will help to breeders for turmeric improvement program.

Keywords: *Curcuma longa*, RAPD, CTAB, UPGMA, India

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i10.18715

INTRODUCTION

Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae comprises more than 80 species of rhizomatous perennial herbs and has extensive occurrence in the tropics of Asia Africa and Australia [1]. India is the largest producer, consumer and exporter of turmeric in the world and contains highest diversity (40 species) of *curcuma longa* [2]. The World Health Organization has recommended the use of turmeric as spice [3]. Globally *Curcuma* is gaining importance as a budding source of new drug (s) to combat a variety of ailments as the species contain molecules endorsed with anti-fungal properties [4], anti-inflammatory, hepatoprotective, antitumor, antiviral [5], and anticancer activities [6].

There are several molecular-based techniques like RAPD, amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), restriction fragment length polymorphism (RFLP) were used to study the intra and inter specificity diversity among plant species. Syamkumar and Sasikumar [7] have developed genetic fingerprints of 15 *Curcuma* species using RAPD and ISSR markers. Analysis of the genetic of *Zingiber, Curcuma* and *Alpinia* using rice microsatellites as RAPD markers also demonstrated high polymorphism and confirmed the usefulness of these in genetic diversity studies of the Zingiberaceae [8]. Now it is believed that all 80 *curcuma* species may not be true but there could be synonyms among some species as reported earlier [9]. Polymorphism based on DNA is the best approach among molecular genetic fingerprinting for the use of novel characters and races identification. Genetic studies involving molecular markers are used to detect relationship and genetic variability among germplasm. This would help to conserve the genetic resources and get consistent variability [10]. Molecular marker technology is vital in spices and medicinal and aromatic plants for identifying accessions, germplasm organization, identifying market samples, and gene cloning. Random amplified polymorphic DNA (RAPD) is one of most ever used technique among all molecular marker techniques. This is because of its simplicity and low-cost nature, rapid, inexpensive and effective system for studying plant genetic relationships [11, 12].

To our knowledge very few or no previous reports on this method to elucidate the genetic diversity of C. longa in various eco geographical zones like south, north, east and west of India has ever been reported. The objective of this study was to evaluate the presence and pattern of genetic variability and relatedness among different accession of turmeric collected from almost all major ecological zones of India by RAPD markers.

MATERIALS AND METHODS

Plant materials

The crude rhizomes of *C. longa* of 08 samples were collected from eight different provinces (table 1) from the Indian subcontinent. All collected samples Trivandrum, Lucknow, Nashik, Delhi, Erode, Guwahati, Surat and Patna were authenticated by taxonomist Prof. M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. All voucher specimens (BNPL/T-12) were deposited in Molecular Ecology Laboratory, Jamia Hamdard, New Delhi, India.

Isolation of genomic DNA

Genomic DNA of frozen rhizomes samples was isolated by modified cetyltrimethylammonium bromide (CTAB) extraction method [11, 13]. 1g of rhizome sample along with polyvinylpyrrolidone was triturated in liquid nitrogen to a fine powder. 5 ml of 1% CTAB (100 mmol Tris-HCl buffer pH 8.0, 1.4 M NaCl, 20 mmol, EDTA, 1% mercaptoethanol) buffer was added to the homogenate and centrifuged at 3200 × g for about 10 min. Added, 2 vol. 2% CTAB (100 mmol Tris-HCl buffer, 1.4 M NaCl, 20 mmol EDTA) to the collected aqueous phase. This mixture was incubated at 65 °C for about 60 min with intermittent shaking. The suspension was then cooled to room temperature and an equal volume of chloroform and isoamyl alcohol (24:1) was added. The mixture was then centrifuged

at 13,000 × g for 10 min. The aqueous phase was collected, and to it was added 0.6 volume of cold isopropanol and 1/30 volume of sodium acetate (3 M, pH 5.2) and incubated at–20 °C for 1 h. The sample was centrifuged at 13,000 × g for 10 min to obtain DNA pellet. The pellet was washed with 80% ethanol twice, air dried and dissolved in TE buffer (10 mmol Tris buffer, pH 8.0, 1 mmol Na_2EDTA). The isolated DNA was treated with RNase A (10µg/ml) at 37 °C for 30 min. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide.

RAPD amplification

Polymerase chain reaction (PCR) was performed according to the method based on Ashraf *et al.* [11] and Williams *et al.* [12]**.** PCR reaction was carried out in 25µl reaction tubes. Amplification reaction contained PCR buffer (Promega; 20 mmol Tris–HCl (pH 8.4); 50 mmol KCl), 1.5 mmol $MgCl_2$, 300 µM each of deoxynucleotide triphosphate (dNTP), 25 pM decanucleotide primer (Operon technology Inc., USA), 1 unit Taq DNA polymerase (Promega) and 50 ng of template genomic DNA. Amplification was performed in a thermal cycler (Master Cycler, Eppendorf, USA) using the following conditions: an initial denaturation step at 95 °C for 2 min, followed by 40 cycles at 95 °C for 30 sec., annealing at 50 °C for 1 min., and extension at 72 °C for 2 min., finally at 72 °C for 5 min for RAPD amplification. Amplification products were separated alongside a molecular weight marker (100 bp plus ladder, M/S Bangalore Genei and Bio-Basic. Inc.) by 1.2% agarose gel electrophoresis in 1×TAE (Tris-acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Doc System (UVitech, USA) and the amplification product sizes were evaluated using the software Quantity one (Bio-Rad, USA).

Data analysis

Data analysis was done using Alpha Imager EC software. For each accession, each fragment/band that was amplified was treated as a unit character. Amplified agarose gel pictures were compared with each other, by considering the presence of bands represented by (1) and absence represented by (0). The molecular size of the amplification product was calculated with 100bp plus molecular size weight marker (Bangalore Genei and Bio-Basic. Inc.). All the amplified profiles were analyzed together to form a binary data matrix. The commercial software package NTSYS-PC version 2.0 [14] was used to develop similarity matrix. These data were used to construct dendrogram for cluster analysis based on unweighted pair group method with arithmatic means (UPGMA).

RESULTS

Isolation of DNA

Genetic variability studies in *C. longa* collected from different geographical regions of India have been carried out using RAPD markers. DNA was isolated by modified CTAB method [11, 13]**.** The purity of DNA determined from the ratio of optical density of 260/280 ratio ranged from 1.80 to 1.92 for all the samples indicates the purity of DNA in all samples.

Genetic diversity analysis

The present study offers an optimization of primer screening for evaluation of genetic relationship among eight accessions of *C. longa* through RAPD analysis. Out of twenty decamer, random primers used for 08 accessions 09 primers did not produce any amplification at all in initial screening while 11 primers showed an amplified polymorphic pattern. These primers then used for RAPD analysis for 08 accessions. The selected primers generated distinctive products in the range of 0.1 to 3.0 Kb. The maximum and a minimum number of bands were produced by the primers OPA-17(20), OPA-11(09), respectively (table 2). A total number of 150 amplified fragments were scored across eight accessions of turmeric for the selected primers and was used to estimate genetic relationships among themselves. Out of 150 fragments obtained, 132 fragments (88%) were polymorphic. A total of 150 amplification products were scored with an average frequency of 13.63 bands per primer. The 11 polymorphic primers exhibited variation with % monomorphism. The pattern of RAPD produced by the primer OPAA-17 is shown in fig. 1. Pair-wise genetic similarities ranged from 0.23 to 0.41 in all the accessions (table 3).

DISCUSSION

RAPD marker was used to carried out genetic diversity studies in *C. longa* collected from different geographical regions of India. DNA was isolated by modified CTAB method. DNA extraction of turmeric proved difficult due to the presence of secondary metabolites. A modified CTAB method was proved to be fruitful. The modified method included higher incubation temperature (65 °C). Random amplified polymorphic DNA and related techniques require less DNA, but purity is necessary to ensure repeatability and confidence [11-12]. The purity of DNA determined from the ratio of optical density of 260/280 ratio which ranged from 1.80 to 1.92 for the samples indicates the purity of DNA in all samples. Based on the dendrogram (fig. 2), the 08 accessions were grouped into two main clusters (1 and 11). Upper cluster divided into two main subcluster 1A and 11B. Trivandrum and Lucknow shows similarity at 39%. In cluster 1B, Nashik and Delhi shows similarity at 41%. In lower cluster 11B, the similarity between Erode and Patna is found to be 41%. Accession Surat shows somewhat divergence from the main cluster. The high difference in gene diversity among accessions reveals the presence of strong genetic structure between them and thus significant differences exist in the genotypic diversity among themselves. Therefore from clustering results of different accessions suggested that *C. longa* undergoes genetic variation.

Our results are in conformity with those reported by Thaikert and Paisooksantivatana [15] and Angle *et al.* [16] that high genetic variations may exit within species of *C. longa* even though this plant species is clonally propagated. Islam and his coworker [17] also supported our results that high level of genetic diversity occurred within *C. zedoaria* populations. This outcome is supported by Nayak *et al.* [18] who established that main cause of polymorphism could be intraspecific variation among different cultivars. Metabolic diversity and genetic diversity study have been well correlated by Ashraf *et al.* [19-21] in different accessions of ginger and turmeric. Sikha *et al.* [22] examined the genetic diversity among turmeric accessions from 10 different agro-climatic regions comprising 5 cultivars and 55 accessions by using random amplified polymorphism DNA (RAPD) and inter-simple sequence repeat (ISSR) in turmeric genotypes and found 62% correlation between the genetic similarity and geographical location. Ragesh *et al.* [23] carried out analysis of genetic diversity of 19 *Curcuma* species using morphological characters and random amplified polymorphic DNA. A dichotomous key and a dendrogram for the species were also developed. Sushma *et al.* [24] carried out an assessment of genetic diversity in indigenous turmeric (*Curcuma longa*) germplasm from India using directed amplification of minisatellite DNA (DAMD) and inter-simple sequence repeats (ISSR) markers and reported 82 % polymorphism across the turmeric genotypes. Paul and his co-researcher [25] reported a wide genetic variation among the different turmeric accessions using RAPD analysis. The result exhibited 80% polymorphism among five samples collected across three different states of northeast India. RAPD and ISSR markers were successfully used to evaluate the genetic fidelity as well as variation of other micro propagated plant species [26-28].

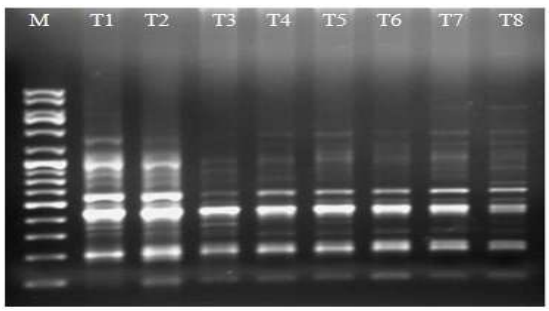

Fig. 1: RAPD amplification pattern of 08 accessions of *C. longa* using primer OPA-17, M-molecular marker (100-3000bps),T1-Trivandrum, T2-Lucknow, T3-Nashik, T4-Delhi, T5-Erode, T6-Guwahati, T7-Surat, T8-Patna

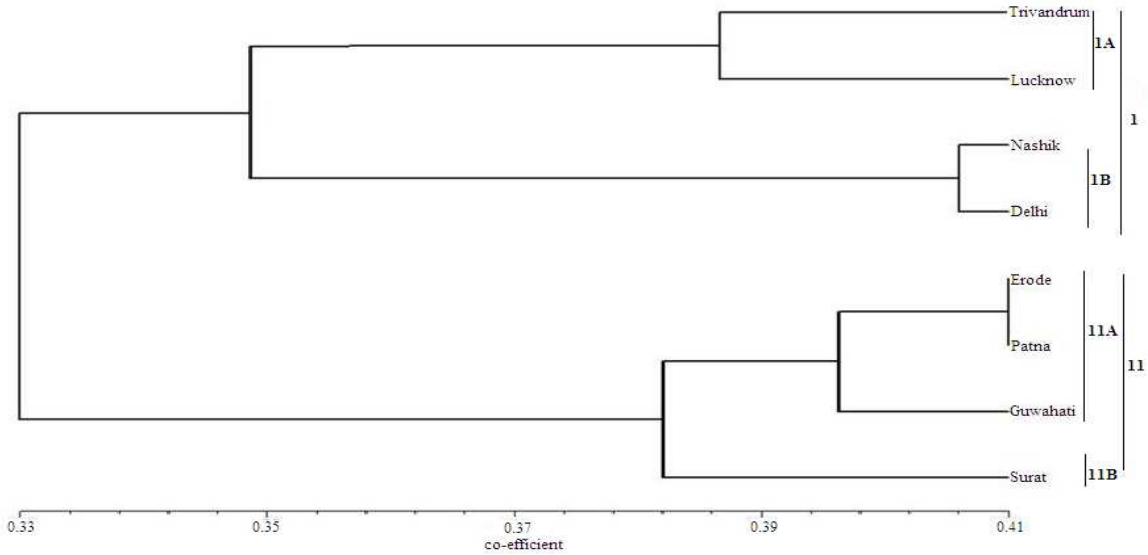

Fig. 2: Dendrogram based on UPGMA (Unweighted pair-group method with arithmetic averages) analysis of genetic similarities estimated among the 08 accessions of *C. longa*by the means of 11 RAPD primers

Table 1: List of different accessions of *C. longa* used in present study

S. No.	Code No.	Cultivation regions (provinces)	Geographical Co-ordinates	Samples type	Source
1	T1	Trivandrum(Kerala)	8 ° 29′ 15″ N, 76 ° 57′ 9″E	Cultivated	Local farmer
2	T2	Lucknow(U. P.)	26 ° 50′ 49.2″ N,80 ° 56′ 49.2″ E	Cultivated	Herbal garden, Integral Univ.
3	T3	Nashik(Maharashtra)	20 ° 0′ 0″ N, 73 ° 46′ 48″ E	Cultivated	Local farmer
4	T4	Delhi (Delhi)	28 ° 36′ 36″ N,77 °13′ 48″E	Cultivated	Herbal garden, Hamdard University
5	T5	Erode (Tamilnadu)	11 ° 21′ 0″ N, 77 ° 44′ 0″ E	Cultivated	Local farmer
6	T6	Guwahati (Assam)	26 ° 11′ 0″ N, 91 ° 44′ 0″ E	Cultivated	Local farmer
7	T7	Surat (Gujrat)	26 ° 11′ 0″ N, 91 ° 44′ 0″ E	Cultivated	Local farmer
8	T8	Patna(Bihar)	25 °36′39.6″N,85 °8′38.4″E	Cultivated	Local farmer

Table 2: Primer sequence and amplified band of 08 accessions of *C. longa*

Primer	Sequence of primer(5'-3')	Size of product amplified(bps)100-3000bps	Total band	No. polymorphic band	% polymorphism
OPAA-01	AGACGGCTCC	345-996	11	11	100.00
OPAA-02	GAGACCAGAC	345-973	12	12	100.00
OPAA-03	TTAGCGCCCC	223-987	13	12	92.30
OPAA-05	GGCTTTAGCC	209-975	14	14	100.00
OPAA-06	TCAAGCTAAC	218-931	14	14	100.00
OPAA-07	CTACGCTCAC	210-955	14	13	92.30
OPAA-08	TCCGCAGTAG	190-938	13	13	100.00
OPAA-11	ACCCGACCTG	210-1500	09	04	44.44
OPAA-12	GGACCTCTTG	280-2100	14	08	57.14
OPAA-14	AACGGGCCAA	190-2029	16	13	81.25
OPAA-17	GAGCCCGACT	100-2200	20	18	90.00

Table 3: Similarity matrix table of 08 samples of *C. longa*

	T1	T2	T3	T4	T5	T6	T7	T8
T1	1.00							
T2	0.39	1.00						
T3	0.36	0.34	1.00					
T4	0.35	0.34	0.41	1.00				
T5	0.28	0.33	0.41	0.38	1.00			
T6	0.33	0.31	0.33	0.36	0.40	1.00		
T7	0.27	0.32	0.34	0.38	0.36	0.39	1.00	
T8	0.31	0.34	0.29	0.23	0.41	0.40	0.39	1.00

T1-Trivandrum, T2-Lucknow, T3-Nashik, T4-Delhi, T5-Erode, T6-Guwahati, T7-Surat, T8-Patna

CONCLUSION

The present findings include the identification and genetic variation within eight accessions of *C. longa.* Analysis of RAPD could be useful to detect genetic diversity of *C. longa* among 08 accessions of Indian subcontinent. From this study, it was understood that each location varied with respect to environmental factors and genetic parameter. The dendrogram shows the distant variation within the accessions. Molecular biology techniques like RAPD markers assume significance and can be used for diversity studies and for future breeding programs of this important genus. The genetic relation through RAPD analysis shows that there is high level of polymorphism among different accessions. The reason could be that the overall genetic diversity of all populations of *C. longa* was high possibly due to a wide range of ecological conditions within the distribution area of its populations in India. This investigation as an understanding of the level and partitioning of genetic variation within the varieties would provide an important input into determining efficient management strategies.

ACKNOWLEDGMENT

Kamran Ashraf is thankful to the University Grant Commision Govt. Of India for providing research fellowship (SRF). The authors are highly thankful to Universiti Teknologi MARA for the financial support under the reference number (600-IRMI/MyRA 5/3/ LESTARI (079/2017) and MOHE, FRGS (Reference No. FRGS/1/2015/SG05/UiTM/02/6).

AUTHORS CONTRIBUTION

First author has carried out the research. Second third and fourth authors have provided study conception, design of work and drafting of manuscript and critical revision.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Purseglove JW, Brown EG, Green CL. Robbins SR. Spices Vol. I-Longman, London. New York; 1981.
2. Ashraf K, Mujeeb M, Ahmad A, Amir M, Mallick MN, Sharma D. Validated HPTLC analysis method for quantification of variability in content of curcumin in *Curcuma longa* L (turmeric) collected from different geographical region of India. Asian Pac J Trop Biomed 2012;2:584-8.
3. Vavilova NI. Turmeric a new spice and medicinal plant. Skogo-Instituta Rastenievodstva-Imeni 1990;4:77-9.
4. Afaq F, Adhami VM, Ahmad N. Botanical antioxidants for chemoprevention of photo carcinogenesis. Front Biosci 2002;7:784-92.
5. Ammon HP, Wahl M. Pharmacology of *Curcuma longa*. Planta Med 1991;57:1-7.
6. Polasa K, Raghuram TC, Krishna TP. Turmeric (*Curcuma longa*L.) induced reduction in urinary mutagens. Food Chem Toxicol 1991;29:699-706.
7. Syamkumar S, Sasikumar B. Molecular marker based genetic diversity analysis of *Curcuma* species from India. Sci Hort 2007;112:235-41.
8. Jatoi SA, Kikuchi A, San-San YI, Naig KW, Yamanaka S, Watanabe JA, *et al.* Use of rice SSR markers as RAPD markers for genetic diversity analysis in Zingiberaceae. Breed Sci 2006;56:107-11.
9. Sasikumar B. Genetic resources of *Curcuma*: diversity, characterization and utilization. Plant Genet Resour 2005;3:230-51.
10. Jan HU, Rabbani MA, Shinwari ZK. Assessment of genetic diversity of indigenous turmeric (*Curcuma longa* L.) germplasm from Pakistan using RAPD markers. J Med Plants Res 2011;5:823-30.
11. Ashraf K, Ahmad A, Choudhary A, Mujeeb M, Ahmad S, Amir M, *et al.* Genetic diversity analysis in *Zingiber Officinale* roscoe by RAPD collected from subcontinent of India. Saudi J Biol Sci 2014;21:159-65.
12. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Aci Res 1990;18:6531-5.
13. DoyleJJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990;12:13–5.
14. Rohlf FJ. NTSYS-PC–numerical taxonomy and multivariate analysis system. Version 2.0, Exter Software, Setauket, New York, USA; 1988.
15. Thaikert R, Paisooksantivatana Y. Variation of total curcuminoids content, antioxidant activity and genetic diversity in turmeric (*Curcuma longa* L.) collections. Kasetsart J 2009;43:507–18.
16. Angle GR, Makeshkumar T, Mohan C, Vimala B, Nambisan B. RAPD genetic diversity analysis of starchy *Curcuma* species using markers. Plant Biochem Biotech 2008;17:971-7811.
17. Islam MA, Meister A, Schubert V, Kloppstech K, Esch E. Genetic diversity and cytogenetic analysis in *Curcuma zedoaria* (Christm) roscoe from Bangladesh. Genet Resour Crop Evol 2005;54:149-56.
18. Nayak S, Naik PK, Acharya LK, Pattnaik AK. Detection and evalution of genetic variation in 17 promising cultivars of turmeric (*Curcuma longa* L.) using nuclear DNA content and RAPD markers. Cytologia 2006;71:49-75.
19. Ashraf K, Shah SAS, Mujeeb M. Determination of 10-gingerol in Indian ginger by validated HPTLC method of samples collected across subcontinent of India. Int J Pharm Pharm Sci 2016;8:190-3.
20. Ashraf K, Mujeeb M, Ahmad A, Ahmad S, Ahmad N, Amir M. Determination of gingerols in ginger by ultra-high performance liquid chromatography-tandem mass spectrometry. Anal Lett 2014;47:2120-8.
21. Ashraf K, Mujeeb M, Ahmad A, Ahmad N, Amir M. Determination of curcuminoids in *Curcuma longa* linn. by UPLC/Q-TOF–MS: an application in turmeric cultivation. J Chrom Sci 2015;53:1346–52.
22. Singh S, Panda MK, Nayak S. Evaluation of genetic diversity in turmeric (*Curcuma longa* L.) using RAPD and ISSR markers. Indust Crops Pdts 2012;37:284–91.
23. Kumar R, Kumar S, Yusuf. Morphological characters and random amplified polymorphic DNA based genetic diversity analysis of *Curcum*a species (Zingiberaceae) from India. Int J Plant Animal and Environ Sci 2016;6:4.
24. Verma S, Singh S, Sharma S, Tewari SK, Roy RK, Goel AK, *et al.* Assessment of genetic diversity in indigenous turmeric (*Curcuma longa*) germplasm from India using molecular markers. Physiol Mol Biol Plants 2015;21:233–42.
25. Paul R, Bhau BS, Zaman K, Sharma HK. RAPD analysis of DNA isolated from turmeric rhizomes collected from Northeast India. Adv Genet Eng 2016;5:146.
26. Leory XJ, Leon K, Hily JM, Chaumeli P, Branchard M. Detection of *in vitro* culture-induced instability through intersimple sequence repeat analysis. Theor Appl Genet 2001;102:885–91.
27. Devarumath R, Nandy S, Rani V, Marimuthu S, Muraleedharan N, Raina S. RAPD ISSR and RFLP fingerprints as useful marker to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type), *C. assamica* ssp. Assamica (Assam-India type). Plant Cell Rep 2002;21:166.
28. Mohanty S, Parida R, Sandeep S, Sahoo S, Nayak S. Evaluation of drug yelding potential of micropropagated *Curcuma aromatic*. Int J Pharm Pharm Sci 2014;7:71-6.

How to cite this article

- Kamran Ashraf[1,2,3], Altaf Ahmad[4], Syed Adnan Ali Shah[1,2], Mohd Mujeeb. Genetic diversity in accessionsof Indian turmeric (*Curcuma longa* L.) using rapd markers. Int J Pharm Pharm Sci 2017;9(10):288-291.