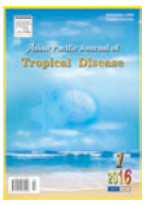
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Original article doi: 10.1016/S2222-1808(15)60987-2

Curcumin profiling and genetic diversity of different accessions of *Curcuma longa* L.

Neelam Arya[1], Om Prakash[1*], Sundip Kumar[2], Vivekanand[1], Anil Kumar Pant[1]

[1]*Department of Chemistry, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, India*

[2]*Department of Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, India*

ARTICLE INFO

Article history:
Received 21 Sep 2015
Received in revised form 10 Oct 2015
Accepted 30 Oct 2015
Available online 12 Dec 2015

Keywords:
Curcumin
Genetic diversity
Random amplification polymorphism DNA
Primer
Turmeric

ABSTRACT

Objective: To investigate the genetic diversity and variation in active compound of turmeric rhizomes collected from different niches of Uttarakhand.
Methods: Genetic diversity and relationship of *Curcuma longa* accessions was evaluated by random amplification polymorphism DNA analysis and curcumin profiling was evaluated by high performance liquid chromatography method.
Results: The curcumin contents in 20 accessions of turmeric rhizomes were found to be in the range of 0.90% to 3.26%. All accessions were separated into six groups (92% genetic similarity) by using 10 decamer oligonucleotide primers for the amplification of genomic DNA.
Conclusions: The results indicated the possibility of selecting high quality clones for large scale production.

1. Introduction

Curcumin, a main coloring agent of turmeric rhizome, was commonly known for its medicinal as well as traditional values. Curcumin, demethoxycurcumin and bisdemethoxycurcumin, collectively known as curcuminoids, refer to group of phenolic substances which account for 60%–80%, 15%–30% and 2%–6% respectively[1]. Curcuminoids have been reported to possess anti-inflammatory, antidiabetic, hepatoprotective, antitumor, anti-atherogenic, antipsoriatic, immunostimulatory, anti-cancer, antibacterial, antiviral activity as well as used in gastrointestinal and respiratory disorders[2]. Curcuminoids exhibit antioxidant activity as well as free-radical scavenging properties[3] and also contribute to the incorporation of the healing process of dermal wound[4] and the prevention of Alzheimer's disease[5,6].

Curcumin is a yellow-orange powder that is insoluble in water and ether but soluble in ethanol, dimethylsulfoxide and acetone. It is stable at acidic pH but unstable at neutral and basic pH and degraded to ferulic acid (hydroxycinnamic acid) and feruloylmethane. Curcumin exists in enolic and β-diketonic forms and its radical scavenging ability is due to its enolic form[7,8]. Curcuminoids are estimated by several methods *viz.* spectrophotometric, thin layer chromatography and column chromatography. However, high performance liquid chromatography (HPLC) method is sensitive, precise and accurate for the determination of individual curcuminoids in the extract of turmeric rhizomes[9]. Therefore, in this study, we used HPLC method to estimate the curcumin content.

The wide geographical and climatic distribution in Western Himalayan region was indicative of the fact of the existence of a tremendous genetic diversity in *Curcuma longa* L. (*C. longa*) which needs to be identified and catalogued. The evaluation of *C. longa* genetic diversity and relationship is still insufficiently carried out in Uttarakhand Region. As this species has high ecological, economical and medicinal value, an accurate assessment of genetic diversity and relatedness will be helpful for efficient management of this species. Therefore, this study was concerned with the evaluation of the genetic diversity and relationship of *C. longa* accessions by

*Corresponding author: Om Prakah, Department of Chemistry, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, India.
Tel: +919411159551
E-mail: oporgchem@gmail.com

random amplification polymorphism DNA (RAPD) analysis and curcumin profiling collected from selected areas of Uttarakhand, India.

2. Materials and methods

Rhizomes of *C. longa* were collected from twenty different altitudes *viz.* hill and Tarai areas of Uttarakhand State (Garhwal and Kumaun Region) in India in the month of October–November (Table 1). Identification of plants was done by plant taxonomist, Department of Biological Sciences of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The solvents and chemicals used were of laboratory and analytical grade and were obtained from E. Merck, Molychem and Himedia Company in India.

Table 1
Collection site and curcumin content in twenty accessions of turmeric rhizome.

Genotype code	Place	District	Altitude (m)	Curcumin (%)
G1	Harsil	Uttarkashi	2620	1.51 ± 0.30^{e}
G2	Munsiyari	Pithoragarh	2298	1.54 ± 0.16^{e}
G3	Champawat	Champawat	1615	2.43 ± 0.30^{j}
G4	Chakrata	Dehradun	2270	0.96 ± 0.36^{b}
G5	Lohaghat	Champawat	1745	2.49 ± 0.05^{j}
G6	Kausani	Almora	1890	1.11 ± 0.08^{c}
G7	Khatima	Udham Singh Nagar	198	0.76 ± 0.51^{a}
G8	Joshimath	Chamoli	2100	1.61 ± 0.26^{f}
G9	Chamoli	Chamoli	1755	3.26 ± 0.40^{o}
G10	Binsar	Almora	2412	1.99 ± 0.15^{h,i}
G11	Nanakmatta	Udham Singh Nagar	298	1.16 ± 0.29^{c}
G12	Srinagar	Pauri Garhwal	560	3.15 ± 0.31^{n}
G13	Takula	Almora	1400	1.96 ± 0.42^{h}
G14	Dholiya pata	Bageshwar	522	1.41 ± 0.28^{d}
G15	Ramgarh	Nainital	1518	2.94 ± 0.10^{m}
G16	Chaukhutia	Almora	1039	1.50 ± 0.20^{e}
G17	Roorkee	Haridwar	268	2.57 ± 0.10^{k}
G18	Kanda	Bageshwar	345	2.02 ± 0.09^{i}
G19	Patanpatni	Champawat	258	2.68 ± 0.23^{l}
G20	Kota (pati)	Champawat	1020	1.72 ± 0.27^{g}

Each value of curcumin content is the mean ± SD of three replicate experiments. Different letters within columns indicate significant difference ($P < 0.01$).

2.1. Assays for curcumin profiling

The curcumin content in all the twenty rhizomes of turmeric was estimated by HPLC. For this analysis, 100 mg/L alcoholic extract of each accession and pure curcumin were prepared in acetonitrile.

HPLC was conducted with a Dionex (UltiMate 3000) model system. The HPLC system consisted of a RP C-18 (250 mm × 4.6 mm) 5 μm column and UV-vis detector. The mobile phase for analysis of curcumin was a gradient of deionized water, acetonitrile and acetic acid (44:54:2, v/v/v). A calibration curve was plotted between peak area and concentration. The curcumin content was estimated by calibration curve and the results were expressed in percentage. Different concentrations *i.e.* 1, 5, 10, 15, 20, 25 mg/L of pure curcumin were used for the preparation of calibration curve. A volume of 20 μL of each sample and standard was injected separately in triplicate. The retention time of the sample and standard was observed 9.1 min in a run time of 12 min.

2.2. DNA profiling using RAPD technique

2.2.1. DNA isolation

Fresh rhizomes of turmeric were used for the isolation of DNA. The genomic DNA was isolated by the cetyltrimethyl ammonium bromide method[10]. PCR amplification was performed in 25 μL reaction volume [0.5 mmol/L dNTPs, 1 IU of *Taq* DNA polymerase, 2.5 μL buffer (containing 2.5 mmol/L $MgCl_2$), 10 pmol of decanucleotide primers, 8 μL of nuclease free water and 25 ng of DNA] using a DNA thermal cycler. Cycling conditions consisted of a 4 min initial denaturation at 94 ℃ followed by 1 min denaturing at 94 ℃, 1 min annealing at 37 ℃ and 1 min extension at 72 ℃ repeated for 35 cycles and 10 min extension at 72 ℃. The amplified products were loaded in a 1.5% agarose gel containing 0.5 μg/mL of EtBr and visualized by a gel documentation system. The bands were scored by using 1 kb DNA ladder marker (Himedia).

2.2.2. Data scoring and analysis

The DNA bands were scored with regard to polymorphic band individual lanes being either present (1) or absent (0). Polymorphic information content (PIC) was used to determine allele diversity at each locus and was calculated according to Roldan-Ruiz *et al*.[11] as: $PIC_i = 2f_i\ (1 - f_i)$, where f_i is the frequency of the amplified allele (band present) and $(1 - f_i)$ is the frequency of null allele (band absent) of marker i. The genetic similarities among the accession were calculated according to Jaccard's coefficient[12]. A phylogenetic tree was constructed using unweighted pair group method with arithmetic means (UPGMA) method[13]. The observed heterozygosity, Nei's average gene diversity[14], fixation index and Shannon's informative index were calculated using Popgene 1.32 software[15].

2.3. Statistical analysis

Total curcumin content was expressed as mean ± SD ($n = 3$). Significance of difference was calculated by Duncan's new multiple range test and results with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Curcumin profiling

The percentage composition of curcumin in different collections

of turmeric had been given in Table 1. The results revealed that the amount of curcumin in alcoholic extracts from different turmeric collections varied from 0.90% to 3.26%. The maximum amount found in the collection was obtained from Chamoli [(32.6 ± 0.126) mg/g] and the minimum amount was obtained from Khatima [(7.6 ± 0.51) mg/g]. Based on the Duncan *post-hoc* analysis by SPSS, the samples can be grouped into 15 groups. No significant difference was observed between samples falling in a single group, depicted with the same superscript (Table 1). The turmeric samples in different groups differed significantly ($P \leqslant 0.01$) from each other with respect to their curcumin content.

3.2. DNA fingerprinting

In order to confirm the genetic integrity, RAPD analysis of turmeric rhizome was carried out. A total of 20 accessions collected from different niches were tested with 10 primers to determine their genetic diversity. Among the 10 decamer oligonucleotides primers used for the amplification of the turmeric genomic DNA, 3 primers performed poorly and produced either faint bands or did not amplify the DNA in some of the samples. Primers varied greatly in their ability to resolve variability among the different samples. Some primers generated several bands, while others generated only a few. A summary of genotypic diversity in all 20 accessions was shown in Table 2.

Table 2
Summary of genetic diversity of 20 accessions of turmeric rhizome using 10 RAPD markers.

Marker	NA	ASR (bp)	Ho	Nei	Fis	I	PIC
OPA02	10	300–1000	0.158	0.172	0.174	0.152	0.390
OPA08	9	120–700	0.427	0.393	0.382	0.846	0.290
OPA09	9	130–800	0.567	0.367	0.311	0.334	0.450
OPC05	10	150–900	0.289	0.432	0.266	0.362	0.530
OPC11	9	140–950	0.187	0.564	0.600	0.238	0.240
OPD08	8	200–220	0.578	0.209	0.125	0.171	0.350
OPD20	6	100–900	0.324	0.154	0.452	0.740	0.770
OPN04	7	100–800	0.564	0.356	0.527	0.137	0.490
OPN06	9	150–900	0.672	0.278	0.591	0.670	0.520
OPN16	7	100–900	0.231	0.421	0.339	0.599	0.300
Mean ± SD	8.4		0.399 ± 0.186	0.335 ± 0.130	0.377	0.425 ± 0.266	0.433

NA: Number of allele; ASR: Allele size range; Ho: Observed heterozygosity; Nei: Expected heterozygosities or gene diversity; Fis: Fixation index; I: Shannon's informative index.

The analysis of polymorphic markers (43.3%) in this study was carried out using tools described in material and methods section. A total of 84 alleles were found, with average alleles of 8.4 per locus varying from seven to ten. Substantial variations in allelic polymorphism were also observed and the size ranged from 100 to 1 000 bp. The PIC value extended from 0.24 (OPC 11) to 0.77 (OPD 20) with the mean of 0.433. Generally, PIC values increased proportionally with increasing heterozygosity at a locus.

The observed heterozygosity for individual loci varied from 0.158 to 0.672 with an average of 0.399 per locus. The expected heterozygosities or gene diversity ranged from 0.154 to 0.564 with an average of 0.335 per loci.

Fixation index, measure of genetic diversity was calculated, which ranged from 0.125 to 0.600 with an average of 0.377. Positive value of fixation index represented excess of observed homozygotes whereas negative value demonstrated extra heterozygotes. The Shannon's informative index of loci varied from 0.137 to 0.846 with the mean of 0.425 per locus. The data of PIC value, observed heterozygosity, expected heterozygosities or gene diversity, fixation index, Shannon's informative index of loci were shown in Table 2.

3.3. Genetic relationship

The genetic similarities among the accession were calculated according to Jaccard's coefficient[12] using NTSYS-pc software package version 2.10d (Table 3). The similarity coefficient values ranged from 0.56 to 0.92. The highest value of similarity coefficient (0.92) was found between the G19 and G5 which was followed by similarity coefficient of 0.91 between accessions G19 and G3.

The UPGMA clustering method based on the expected heterozygosities or gene diversity[13] classified all the 20 turmeric accessions into two major groups (I and II) comprising six clusters (Figure 1). The second major group consisted of two subgroups IIA and IIB with 16 genotypes. The subgroup IIA was further subdivided into four clusters, the first comprising five genotypes (G3, G18, G4, G7 and G11). The second consisted of three accessions (G5, G19 and G20), the third cluster also comprised five accessions (G14, G16, G9, G12, G15), the fourth cluster consisted of two accessions (G6 and G13), and the fifth cluster represented only one accession (G17), which was also included in IIB subgroup. The first group consisted of four accessions which were divided into two subgroups (IA and IB). IA comprised three accessions (G1, G2 and G8) and IB cluster represented only one accession (G10), respectively. Subgroup IA contained G1 and G2 with similarity of 90%. G8 was similar with G1 and G2 with 87%. Subgroups IA and IB included in Group I exhibited the similarity of 74% and showed 56% similarity with the accessions of Group II. The first cluster of Group IIA was further divided into two subcluster Ia and Ib. Subgroup Ia contained accessions G3 and G18 with 90% similarity. Subgroup Ib contained accessions G4 and G7 with 86% similarity. Accession G11 was found similar with G4 and G7 with 85% similarity. Subgroup Ia and Ib included in first cluster exhibited similarity of 78%. The second cluster comprised three accessions (G5, G19 and G20) which was further subdivided into two clusters IIa and IIb. Cluster IIa contained G5 and G19 with the maximum similarity (92%). Genotype G20 was similar with G5 and G19 with 81% similarity. The first cluster and second cluster included in the third cluster with the similarity of 72%. The third cluster was comprised of five accessions (G14, G16, G9, G12 and G15). This third cluster was further subdivided into three

Table 3
Genetic dissimilarity matrix of various genotypes of *C. longa* based on RAPD analysis.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1.00																			
G2	0.90	1.00																		
G3	0.55	0.67	1.00																	
G4	0.57	0.56	0.69	1.00																
G5	0.58	0.57	0.82	0.79	1.00															
G6	0.50	0.59	0.71	0.67	0.59	1.00														
G7	0.54	0.53	0.82	0.86	0.77	0.65	1.00													
G8	0.90	0.82	0.55	0.64	0.54	0.56	0.62	1.00												
G9	0.58	0.57	0.67	0.67	0.69	0.59	0.64	0.54	1.00											
G10	0.73	0.83	0.82	0.67	0.69	0.69	0.64	0.67	0.69	1.00										
G11	0.67	0.64	0.82	0.86	0.77	0.75	0.85	0.75	0.77	0.77	1.00									
G12	0.62	0.60	0.69	0.69	0.71	0.71	0.67	0.57	0.85	0.71	0.79	1.00								
G13	0.31	0.33	0.57	0.59	0.60	0.71	0.56	0.38	0.41	0.41	0.56	0.53	1.00							
G14	0.54	0.50	0.75	0.69	0.71	0.71	0.79	0.62	0.60	0.60	0.79	0.73	0.63	1.00						
G15	0.50	0.50	0.50	0.69	0.60	0.61	0.56	0.57	0.71	0.60	0.67	0.73	0.53	0.63	1.00					
G16	0.38	0.40	0.64	0.60	0.62	0.63	0.69	0.46	0.75	0.50	0.69	0.77	0.53	0.77	0.77	1.00				
G17	0.33	0.41	0.69	0.59	0.50	0.53	0.67	0.40	0.60	0.50	0.56	0.53	0.44	0.63	0.53	0.64	1.00			
G18	0.50	0.50	0.90	0.71	0.76	0.63	0.83	0.58	0.62	0.62	0.83	0.64	0.64	0.77	0.53	0.67	0.64	1.00		
G19	0.54	0.64	0.91	0.73	0.92	0.65	0.71	0.50	0.64	0.77	0.71	0.67	0.56	0.67	0.56	0.57	0.56	0.69	1.00	
G20	0.43	0.53	0.75	0.63	0.77	0.56	0.60	0.40	0.53	0.64	0.60	0.56	0.47	0.56	0.47	0.57	0.47	0.57	0.85	1.00

subclusters of IIIa, IIIb and IIIc. Clusters IIIa and IIIb contained G14 and G16, G9 and G12 with the similarity of 85% and 77%. Cluster IIIc contained single accession and showed similarity with Cluster IIIb at 73%. This cluster included IIIa with 71% similarity. Cluster IV contained two accessions G6 and G13 with 70% similarity. Cluster V contained a single accession G17 and exhibited the minimum similarity of 58% with the cluster included in Group II. Clusters I, II and III exhibited 58% and 61% similarity with Clusters IV and V.

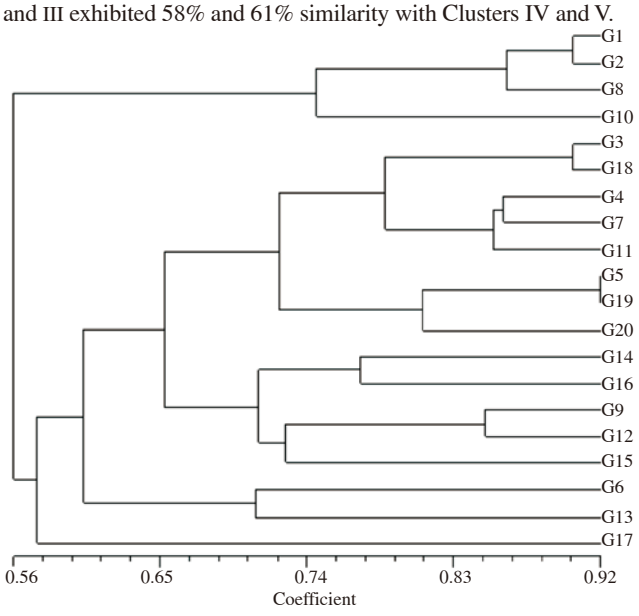


Figure 1. UPGMA cluster analysis showed the relationship (diversity) among turmeric rhizome genotypes using 10 RAPD primers.

4. Discussion

Jayaprakasha *et al*. analysed the curcuminoids profile of the traded varieties like Salem, Erode, and Balasore using HPLC and reported the curcumin percentage in the range of (1.060 ± 0.061)% to (5.650 ± 0.040)% respectively[16] whereas Paramasivam *et al*. described high performance thin layer chromatography method for the determination of curcuminoids of *C. longa* germplasm[17]. The results suggested that two Indian varieties of *C. longa*, *viz.*, Nimbarg and Kalimpong have higher amounts of curcuminoids, 6.18% and 5.37%, respectively. Similarly, Wichitnithad *et al*. reported an isocratic HPLC method for the determination of curcuminoids in commercial turmeric extracts and quantified 2.73 μg/mL for curcumin[1]. Our results showed variation in the quantity of curcumin which might be because of climatic, edaphic and genetic variation.

Genetic similarity coefficient was carefully observed for deducing relationships between the different accessions. The similarity coefficient 0.92 was observed in G5 and G19 which showed that these accessions have the same parents while accession G17 exhibited the minimum similarity coefficient 0.57 resulted in the maximum variation. From the results, we concluded that the sample collected from Lohaghat and Patanpatni had same accessions. Sample collected from Harsil, Munsiyari, Joshimath and Binsar fall in the same cluster in which sample collected from Harsil and Munsiyari have closely related accessions. Sample collected from Kanda and Champawat was also having the closely related accessions. From the dendogram, rhizomes of nearby accessions fall in the same 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. Our finding was supported by result of Jan *et al*.[18], Archana *et al*.[19] and Ashraf *et al*.[20] who reported that the genetic variation occurred among the different cultivars using RAPD markers.

From our study, it was understood that each location varied with respect to environmental factors and genetic parameters. Results showed that the accessions, whose cultivation regions were very close, showed the maximum similarity among them as compared to accessions which were further apart. This outcome is supported by Nayak *et al*. who established that the main cause of polymorphism could be intraspecific variation among different cultivars[21]. The results indicated the possibility of selecting high quality clones for large scale production. These findings will also provide an important contribution in determining resourceful management strategies to breeders for turmeric improvement program.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Dr. D.S.Rawat, Assistant Professor and Plant Taxonomist, Department of Biological Sciences of G. B. Pant University of Agriculture and Technology, Pantnagar for the identification of plant specimens and the first author is also thankful to the University Grants Commission (UGC) for providing financial assistance.

References

[1] Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. *Phytochem Anal* 2009; **20**: 314-9.

[2] Ravindran PN, Babu KN, Sivaraman K. *Turmeric: the genus Curcuma (medicinal and aromatic plants-industrial profiles)*. Boca Raton: CRC Press; 2007.

[3] Kalpravidh RW, Siritanaratkul N, Insain P, Charoensakdi R, Panichkul N, Hatairaktham S, et al. Improvement in oxidative stress and antioxidant parameters in β-thalassemia/Hb E patients treated with curcuminoids. *Clin Biochem* 2010; **43**(4-5): 424-9.

[4] Gopinath D, Ahmed MR, Gomathi K, Chitra K, Sehgal PK, Jayakumar R. Dermal wound healing processes with curcumin incorporated collagen films. *Biomaterials* 2004; **25**: 1911-7.

[5] Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci* 2001; **21**: 8370-7.

[6] Shen L, Ji HF. The pharmacology of curcumin: is it the degradation products? *Trends Mol Med* 2012; **18**(3): 138-44.

[7] Shen L, Ji HF. Low stability remedies the low bioavailability of curcumin. *Trends Mol Med* 2012; **18**: 363-4.

[8] Liang G, Shao L, Wang Y, Zhao C, Chu Y, Xiao J, et al. Exploration and synthesis of curcumin analogues with improved structural stability both *in vitro* and *in vivo* as cytotoxic agents. *Bioorg Med Chem* 2009; **17**: 2623-31.

[9] Mishra A, Dewangan G, Singh WR, Hazra S, Mandal TK. A simple reversed phase high-performance liquid chromatography (RP-HPLC) method for determination of curcumin in aqueous humor of rabbit. *J Adv Pharm Technol Res* 2014; **5**(3): 147-9.

[10] Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci U S A* 1984; **81**: 8014-8.

[11] Roldan-Ruiz I, Van Euwijk FA, Gilliland TJ, Dubreuil P, Dillmann C, Lallemand J, et al. A comparative study of molecular and morphological methods of describing relationships between perennial rye grass (*Lolium perenne* L.) varieties. *Theor Appl Genet* 2001; **103**: 1138-50.

[12] Jaccard P. Nouvelles recherché sur la distribution florale. *Bull Soc Vaud Sci Nat* 1908; **44**: 223-70. French.

[13] Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 1978; **89**: 583-90.

[14] Nei M. Analysis of gene diversity in subdivided population. *Proc Natl Acad Sci U S A* 1973; **70**: 3321-3.

[15] Yeh FC, Boyle TJB. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg J Bot* 1997; **129**: 157-63.

[16] Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Improved HPLC method for the determination of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *J Agric Food Chem* 2002; **50**: 3668-72.

[17] Paramasivam M, Poi R, Banerjee H, Bandyopadhyay A. High-performance thin layer chromatographic method for quantitave determination of curcuminoids in *Curcuma longa* germplasm. *Food Chem* 2009; **113**: 640-4.

[18] 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**(5): 823-30.

[19] Archana CP, Deepu V, Geetha SP, Indira B. RAPD assessment for identification of clonal fidelity of microrhizome induced plants of Turmeric (*Curcuma longa* L.) cultivars. *Int Food Res J* 2013; **20**(6): 3325-8.

[20] Ashraf K, Ahmad A, Chaudhary A, Mujeeb M, Ahmad S, Amir M, et al. Genetic diversity analysis of *Zingiber officinale* Roscoe by RAPD collected from subcontinent of India. *Saudi J Biol Sci* 2014; **21**(2): 159-65.

[21] Nayak S, Naik PK, Acharya LK, Pattnaik AK. Detection and evaluation of genetic variation in 17 promising cultivars of turmeric (*Curcuma longa* L.) using nuclear DNA content and RAPD markers. *Cytologia* 2006; **71**: 49-75.