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Morphological and Molecular Screening of Turmeric (*Curcuma longa* L.) Cultivars for Resistance against Parasitic Nematode, *Meloidogyne incognita*

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Abstract

Turmeric (*Curcuma longa*) is a high value export oriented important commercial crop among the spices. The production was declined due to several biotic and abiotic stresses. Among biotic stresses, root-knot nematode, *Meloidogyne incognita* is a major threat to turmeric cultivation. Seventy cultivars were screened to identify the resistance to root-knot nematode, *Meloidogyne incognita*. The result revealed that cultivars 'Dugirala', 'PTS-31', 'Ansitapani', 'PTS-42', 'PTS-47'noted as fully resistant;'361 Gorakhpur', '328 Sugandham', 'PTS-21' rated as moderately resistant and rest other cultivars were susceptible. The cultivar '328 Sugandham' was moderately resistant to root-knot nematode. This was further confirmed through DNA amplification studies with ISSR markers. The similarity matrix was obtained after multivariate analysis using Nei and Li's coefficient and the matrix value was ranged from 0.35 to 0.89, with a mean value of 0.62. The two cultivars 'Dugirala' and '361 Gorakhpur' with 48% similarity with other 21 cultivars. Both the cultivars were resistance to root knot nematode (RKN) having indexed ranged from 2.0 to 3.0. The five cultivars i.e. 'Tu No.4', 'Tu No.1', 'Erode local', 'TC-4' and ' 'Phulbani Wild' were 78% similarity and susceptible to RKN having index from 4.0 to 5.0. Cultivars 'Dugirala', '328 Sugandham' and 'PTS-47' exhibited resistance to both root knot nematodes. This investigation as an understanding of the level and partitioning of genetic variation within the cultivars with resistant/susceptible to root knot nematode disease would provide an important input into determining efficient management strategies for breeding program.

Keywords: *Meloidogyne incognita*; Turmeric; Screening; Resistance; ISSR marker

Introduction

Turmeric (*Curcuma longa*), an herbaceous plant is native to tropical south East Asia. It is a high value export oriented important commercial crop among spices in India. The tuber crops represent the most important food commodity in many subtropical and tropical countries [1]. The rhizome has 1.8 to 5.4 percent curcumin, the pigment and 2.5 to 7.2 percent of essential oil. It is used as a dye with varied application in drug and cosmetic industries. In India, it is grown in an area of 104,500 ha producing annually 3,28,800 tones. Although, India is leading in its production (75% of world output), the average productivity and quality are not satisfactory for which the export value is reduced dramatically. Annually 18 to 20 crores worth of turmeric are exported. In India, Andhra Pradesh is the leading state followed by Maharashtra, Tamil Nadu, Orissa, Kerala and Bihar. However, the production and productivity of this high value cash crop is declining day by day because of several biotic and abiotic stresses. Among biotic stresses root-knot nematode, *Meloidogyne incognita* is a major threat to turmeric cultivation [2,3]. Nematodes causes' serious yield performance and quality reduction in most of the tuber crops [4-6]. Root-knot nematodes (*Meloidogyne* spp), first identified as a potential threat to yam production [7] (Bridge) and also in sweet potato [8]. This extensive polyphagous species is a sedentary endo-parasitic nematode that induces multinucleated modified transfer cells inside the vascular bundles of roots through a series of physiological and biochemical changes thereby resulting in galling root dysfunction, reduced water flow and photosynthesis [9]. Management of this important phytophagous nematode through conventional tactics has become a difficult task because of limited availability of nematicides in the world market as well as environmental concern. Few reports are available on molecular screening on tomato, cotton, peanut with regard to root knot nematode resistance [10-13]. Molecular markers have now come up as the most desirable tool for detecting and characterizing variation among the resistance and susceptible at the DNA level Among the different molecular markers, inter simple sequence repeats (ISSR) techniques have proven to be a reliable, reproducible, easy to generate, inexpensive and versatile set of markers that relies on repeatable amplification of DNA sequences using single primers. Therefore, the present study was undertaken to identify some resistant turmeric cultivars as an ecofriendly alternative to nematicides based on physical markers and to correlate these findings with molecular investigation through ISSR marker assisted DNA amplification studies.

Materials and Methods

Seventy cultivars of turmeric were collected from Regional Research Technology Transfer Station (RRTTS), Pottangi (Odisha) for screening their resistance against root-knot nematode, *Meloidogyne incognita*. These cultivars were planted in the 8″ diameter surface sterilized earthen pots containing 3 kg steam sterilized soil and kept in the experimental garden of the Department of Nematology, College of Agriculture, Orissa University of Agriculture and Technology,

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Bhubaneswar. One month after the planting freshly hatched second stage juveniles of *M. incognita* were inoculated @ 3000 J2/pot around the root zone of the plant for infection and development. Sixty days after inoculation, the plants in pots were uprooted carefully and roots were evaluated for resistance against *M. incognita* by following 1-5 point scale [14] on the basis of development of galls and egg masses on the root as follow (Table 1).

Data on root-knot indices were subjected to statistical analysis by following analysis of variance through complete randomized block design. Leaf samples of selected resistant, moderately resistant, susceptible and highly susceptible cultivars were collected for DNA extraction and amplification by ISSR marker and these selected cultivars were planted in raised beds (1 m × 3 m) at RRTTS, Pottangi with three replications. Yield was recorded after fully maturation of the cultivars. Data were subjected to statistical analysis through analysis of variance in a randomized block design. The results of the pot culture studies are pertaining to resistance by *M. incognita*, plant growth and yield were correlated to confirm the resistance based on morphological features with that of molecular investigation.

Sl. No.	Cultivar	Root-knot Index	Reaction
V1	Dugirala	2.00	R
V2	Tu. No.4	4.00	S
V3	Erode local	4.00	S
V4	PTS-53	3.50	S
V5	Sudarsan	4.50	HS
V6	PTS-31	2.00	R
V7	CLS-33	5.00	HS
V8	TC-4	3.50	S
V9	Phulbani Wild	4.00	S
V10	361 Gorakhpur	3.00	MR
V11	Ansitapani	2.00	R
V12	Tu. No.1	4.50	HS
V13	PTS-34	4.50	HS
V14	Bataguda	4.00	S
V15	PTS-17	5.00	HS
V16	PTS-8	4.50	HS
V17	PTS-42	2.00	R
V18	Ethamkalam	5.00	HS
V19	328 Sugandham	2.50	MR
V20	PTS-47	2.00	R
V21	PTS-21	2.50	MR
V22	Kasturi Manjari	4.50	HS
V23	PCT-7	3.50	S
V24	Black turmeric	5.00	HS
V25	Chayapusupu-1	5.00	HS
V26	CAS-15	5.00	HS
V27	CAS-51	5.00	HS
V28	CAS-53	5.00	HS
V29	CLS-3	3.50	S
V30	CLS-21	5.00	HS
V31	Florescent	4.00	S
V32	GL-Puram	5.00	HS
V33	Kuchipudi	4.00	S
V34	K. Local	5.00	HS
V35	Lakadong	4.00	S
V36	Mydukur	4.00	S
V37	Mundapadar	5.00	HS
Sl. No.	**Cultivar**	**Root-knot Index**	**Reaction**
V38	NB-60	5.00	HS
V39	NB-6206	5.00	HS
V40	No.38	4.00	S
V41	PCT-9	5.00	HS
V42	PTS-1	4.00	S
V43	PTS-20	4.00	S
V44	PTS-3	5.00	HS
V45	PTS-4	4.00	S
V46	PTS-11	5.00	HS
V47	PTS-12	4.00	S
V48	PTS-13	4.00	S
V49	PTS-27	4.50	S
V50	PTS-30	4.00	S
V51	PTS-33	5.00	HS
V52	PTS-43	4.00	S
V53	PTS-44	5.00	HS
V54	PTS-48	4.00	S
V55	PTS-50	5.00	HS
V56	PTS-51	5.00	HS
V57	PTS-54	4.00	S
V58	PTS-55	5.00	HS
V59	PTS-57	4.00	S
V60	PTS-62	5.00	HS
V61	Rajpuri local	5.00	HS
V62	Rajendra Sonia	5.00	HS
V63	Ranga	5.00	HS
V64	Raikia	5.00	HS
V65	Roma	5.00	HS
V66	Surama	5.00	HS
V67	Tu. No.-6	5.00	HS
V68	VK-9	5.00	HS
V69	VK-154	4.00	S
V70	Wynad local	5.00	HS
	Mean	4.27	
	Sem (0.05)	0.21	
	CD (0.05)	0.58	
	CV	6.85	

R: Resistance, MR: Moderate Resistance, S: Susceptible, HS: Highly Susceptible.

Table 1: Screening of turmeric cultivars against *M. incognita*.

Genomic DNA extraction and quantification

DNA was extracted from fresh leaves using the cetyl-trimethyl ammonium bromide (CTAB) method [15,16]. Approx. 200 mg of fresh leaves were ground to a powder in liquid nitrogen using a mortar and pestle. The powder was transferred to a 50-ml falcon tube with 10 ml of CTAB buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris (tris(hydroxymethyl) amino methane)-HCl, pH 8.0, and 0.2% (v/v) β-mercaptoethanol. The homogenate was incubated at 60°C for 2 h, extracted with an equal volume of chloroform/isoamyl alcohol (24:1, v/v), and centrifuged at 9838 × *g* for 20 min. DNA was precipitated from the aqueous phase by mixing it with unequal volume of isopropanol. After centrifugation at 9838 × *g* for 10 min, the resultant DNA pellet was washed with 70% (v/v) ethanol, air-dried, and re-suspended in TE (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA) buffer. DNA quantifications were performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel at 50 V for 45 min and comparing with a known amount of lambda DNA marker

(Emerk Bioscience, India). The resuspended DNA was then diluted in TE buffer to 5 µg/µl concentration for use in polymerase chain reaction (PCR).

Primer screening

Twenty synthesized inter simple sequence repeat(ISSR) primers (M/S Emerk Bioscience, Bangalore, India) were initially screened to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved, and polymorphic amplified products within the varieties. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

ISSR assay

PCRs with a single primer were carried out in a final volume of 25 µl containing 20 ng template DNA, 100 µM of each deoxyribonucleotide triphosphate, 20 ng of oligonucleotides synthesized primer (M/S Bangalore Genei, Bangalore, India), 1.5 mM $MgCl_2$, 1X Taq buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.001% gelatin), and 0.5 U Taq DNA polymerase (M/S Emerk Bioscience, India). Amplification was performed in a thermal cycler (Peqlab, United Kingdom) programmed for a preliminary 2 min denaturation step at 94°C, followed by 40cycles of denaturation at 94°C for 20s, annealing at required temperature for 30s, extension at 72°C for 1 min, and finally amplification at 72°C for 10 min. Amplification products were separated alongside a molecular weight marker (3.0 Kb plus ladder, M/S Emerk Bioscience, India) by 1.5% (w/v) agarose gel. Electrophoresis in 1X TAE (Tris acetate/EDTA) buffer. The gel was prestained with ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Documentation System (Gel Doc., UVITECH, UK), and the amplification product sizes were evaluated using the software Quantity one (Bio-Rad) (Rohlf).

Data analysis

During data analysis, only reproducible polymorphic bands in amplification reactions were considered as present. Each band was treated as a separate putative locus, and scored as present (1) or absent (0) in each cultivar. The binary data of the ISSR fingerprints were used further for population genetic analyses. The numbers of monomorphic and polymorphic bands were derived from the binary data, and their percentages were calculated

Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity index was estimated using the formula, $S=2\ N_{AB}/N_A+N_B$ [17].

Where, N_{AB} is the number of amplified products common to both A and B.

Results and Discussion

The screening of seventy cultivars against the *M. incognita* on the basis of varying degree of galling in the plant roots as indicated by root-knot indices. None of the seventy tested cultivars reacted highly resistant to *M. incognita* (Table 1). The root-knot indices of all the cultivars ranged between 2.0-5.0. Statistical analysis of data indicated that there were significant differences among the cultivars. 'Dugirala', 'PTS-31', 'Ansitapani', 'PTS-42' and 'PTS-47' with root-knot index 2.0 and resistant to *M. incognita*, which were significantly different from other cultivars. The root knot indices of cultivars '361-Gorakhpur', '328-Sugandham' and 'PTS-21' were 3.0, 2.5 and 2.5 respectively and rated as moderately resistant. Rest other cultivars were susceptible to highly susceptible to root-knot indices ranging between 4.0-5.0. Eapen et al. [18] reported that cultivars like 'Erode', 'Cls. No.4' were rated as highly resistant and 'C 11.320', 'Kattapana', 'Cls. No.21' as a moderate resistant to *M. incognita*. In the present study, the cultivar 'Dugirala' showed resistance to nematode which was conformity with Mani et al. [19]. There were significant differences among the cultivars on the basis of plant growth, yield performance and root-knot indices. 'PTS-21' rated moderately resistant to *M. incognita* has shown highest plant height, leaf length, leaf width and rhizome yield. The rest two moderately resistant cultivars '361-Gorakhpur' and '328-Sugandham' have shown moderate plant growth and rhizome yield. Some of the cultivars resistant to *M. incognita* exhibited moderate to low plant growth, rhizome yield and low root-knot indices. Among these, 'PTS-47' (6.9 Kg/3m^2) was the highest yielder followed by 'PTS-42' (6.46 Kg/3m^2). The cultivars like '361-Gorakhpur' and 'PTS-21'exhibiting moderately resistant and the cultivars like 'Ansitapani' and'PTS-42' exhibiting resistant to *M. incognita*. Similarly, cultivars like 'Erode local', 'PTS-53', 'Sudarsan', 'CLS-33', 'Phulbani Wild', 'PTS-17' and 'Kasturi Manjari' were susceptible to *M. incognita*. Two cultivars 'Dugirala' and 'PTS-47' were found resistant to *M. incognita*.

On the basis of root-knot indices, out of seventy cultivars, 23 cultivars were selected to compare the resistance, moderate resistance, susceptible and highly susceptible on the basis of ISSR markers. The present study offers an optimization of primer screening for evaluation of genetic relationship among twenty three cultivars of *Curcuma longa* through ISSR analysis (Table 2). The cultivar 'PTS-53' was used initially for screening of synthesis primers for amplification by using polymerase chain reactions. The results showed some primers produced relatively more amplification fragments compared to other primers. The reproducibility of the amplification product was tested on DNA from three independent extractions of the cultivars. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. Among the twenty primers tested, only eleven of them produced unambiguous DNA fragments. All the twenty three cultivars of *Curcuma longa* extensively amplified using these eleven ISSR primers (Table 3) and produced 66 fragments ranging from 100bp to 2500bp. The minimum size fragment of 100bp was amplified by the primer USB-835 and the maximum size fragment of 2500bp was amplified by primer USB-807, USB-708, USB-810, and USB-837 and USB-840. Out of 66 fragments, only 50 fragments (75%) were polymorphic. The pattern of ISSR produced by the primers USB-810, USB-841, USB-807 and USB-835 are shown in Figure 1. The genetic variation through molecular markers has been highlighted in a number of medicinal plants [2,20-22]. The present results have shown the narrow variation within some of the cultivars. The similarity matrix was obtained after multivariate analysis using Nei and Li's coefficient and is presented in Table 4. The matrix value was ranged from 0.35 to 0.89, with a mean value of 0.62. The high matrix values indicated that there were distantly related to each other. The similarity matrix obtained in the present study was used to construct a dendrogram with the unweight UPGMA method and resulted in their distant clustering in the dendrogram (Figure 2). The dendrogram shows two major clusters. The first major cluster (A) had only two cultivars 'Dugirala' and '361 Gorakhpur' with 48% similarity with other major cluster (B) having 21 cultivars. Both the cultivars of major cluster -1 were resistance to root knot nematode (RKN) having indexed ranged

Sl. No.	Turmeric cultivars	Plant Ht. (cm)	Leaves/Tiller	Tillers/Plant	Leaf Length (cm)	Leaf Width (cm)	Yield of fresh rhizome (kg/3m^2)	Root-knot Index	Reaction to Root-knot nematode	Reaction to Taphrina leaf blotch
V1	Dugirala	87.8	6.6	3.4	42.4	9.7	2.1	2.00	R	R
V2	Tu. No.4	102.4	6.2	3.2	50.4	11.2	8.6	4.00	S	HS
V3	Erode local	96.4	6.2	3	46.8	10.8	8.5	4.00	S	R
V4	PTS-53	82.8	6.8	3	41	12.5	6.9	3.50	S	R
V5	Sudarsan	76.8	7.2	2.2	36.8	13	2.9	4.50	HS	R
V6	PTS-31	87.2	6.4	3.2	25	10.7	1.8	2.00	R	S
V7	CLS-33	73.4	6	1.6	36.6	11.4	5	5.00	HS	R
V8	TC-4	71.4	6.8	2.4	37.4	11.4	5.4	3.50	S	S
V9	Phulbani Wild	72.8	5.6	3.4	38	8.9	3.8	4.00	S	R
V10	361 Gorakhpur	78.6	6	1.8	37.6	10.9	7.2	3.00	MR	S
V11	Ansitapani	87.6	5.6	3.4	46.2	11.8	5.4	2.00	R	S
V12	Tu. No.1	83	6	2.2	40.2	12.9	6.7	4.50	HS	HS
V13	PTS-34	82.2	5.8	3.2	43.4	12.9	4.6	4.50	HS	HS
V14	Bataguda	84	6	2.8	39.6	12	8.5	4.00	S	S
V15	PTS-17	92.4	6.4	2	44.4	11.4	6.5	5.00	HS	R
V16	PTS-8	96.6	6.2	3.2	51.6	13.4	4.1	4.50	HS	HS
V17	PTS-42	73.8	6.2	2.6	35.2	10.4	6.4	2.00	R	HS
V18	Ethamkalam	80.2	6.8	3	43.6	9.7	2.2	5.00	HS	S
V19	328 Sugandham	92	5.8	2.8	45.2	12.5	5.9	2.50	MR	R
V20	PTS-47	89.8	6.4	2.8	42.6	13.2	6.9	2.00	R	R
V21	PTS-21	121.8	6.6	2.2	60.6	13.8	12.3	2.50	MR	HS
V22	Kasturi Manjari	85.4	5.2	3	43.4	11.8	5	4.50	HS	R
V23	PCT-7	90.8	5.2	3.2	49.6	12.7	4.9	3.50	HS	S
	Sem (0.05)	0.49	0.29	0.10	0.46	0.30	0.15	0.33		
	CD (0.05)	1.45	0.85	0.30	1.35	0.89	0.43	0.96		
	CV	0.99	8.1	6.45	1.88	4.51	4.49	13.08		
	Mean	86.5	6.17	2.76	42.5	11.7	5.72	3.57		

R: Resistance, MR: Moderate Resistance, S: Susceptible, HS: Highly Susceptible

Table 2: Morphological characteristics of 23 cultivars of *C.longa* and reaction of turmeric cultivars to *M. incognita* and *T. maculans*.

Name of Primer	Sequence of the primer	Total No. amplification products	No. of polymorphic products	Size range (Kb)
USB-807	5'-AGAGAGAGAGAGAGAGT-3'	07	07	200-2500
USB-808	5'- AGAGAGAGAGAGAGC-3'	07	06	100-2500
USB-810	5'-GAGAGAGAGAGAGAT -3'	06	05	600-2500
USB-811	5'-GAGAGAGAGAGAGAC-3'	05	04	200-2000
USB-815	5'-CTCTCTCTCTCTCTG-3'	07	05	200-1500
USB-835	5'-AGAGAGAGAGAGAGTC-3'	04	01	500-1500
USB-836	5'-AGAGAGAGAGAGAGT-3'	06	06	200-2000
USB-837	5'-AGAGAGAGAGAGAGCC-3'	07	05	500-2500
USB-840	5'-GAGAGAGAGAGAGACTT-3'	06	03	200-2500
USB-841	5'-GAGAGAGAGAGAGACTC-3'	05	02	500-1500
USB-842	5'-AGAGAGAGAGAGAGCA-3'	06	06	500 -2000

Table 3: Total number of amplified fragments and number of polymorphic bands generated by PCR using selected ISSR primers in 23 cultivars of *Curcuma longa*.

from 2.0 to 3.0. Second major cluster (B) having 21 cultivars and again divided into two minor clusters (B1 and B2). One minor cluster (B1) having five cultivars i.e. 'Tu No.4', 'Tu No.1', 'Erode local', 'TC-4' and 'Phulbani Wild'. Among the five cultivars, two cultivars i.e. 'Tu No.4', 'Tu No.1' were making one group with 78% similarity and susceptible to RKN having index from 4.0 to 5.0. Another cultivar 'Erode local' making one group with 71% similarity with other two cultivars were also susceptible to RKN. Second minor cluster (B2) again subdivided into two sub-minor clusters i.e. C1 and C2. First sub-minor cluster (C1) having 4 cultivars with 63% similarity and all are highly susceptible to RKN. Second sub-minor cluster (C2) having 12 cultivars and making two groups. One group having two cultivars ('Ansitapami' and 'PST-31') with 75% similarity and other group having 10 cultivars with 89% similarity. The cultivars 'PTS-21', '328 Sugandham','PTS-42' and 'PTS-47' were resistance to RKN with root knot indices ranged from 2.0 -2.50. Chu et al. [12] identified RAPD based markers to select for nematode resistance in *Arachis hypogaea*. In another study, Tahery [23] revealed that the identification of ISSR markers associated with root knot nematode resistance of *Hibiscus cannabinus*. He found 13 polymorphic ISSR markers between the resistant and susceptible

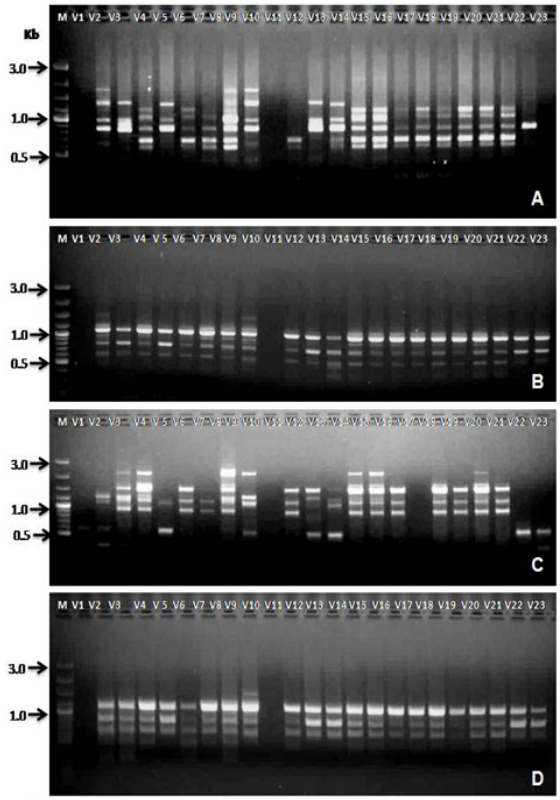

Figure 1: ISSR patterns of 23 varieties of *Curcurma longa* generated by primer USB-810(A), USB-841 (B), USB-807(C) and USB-835(D) MKb molecular weight ladder, V1-V23 assigned as cultivars indicated in Table 2.

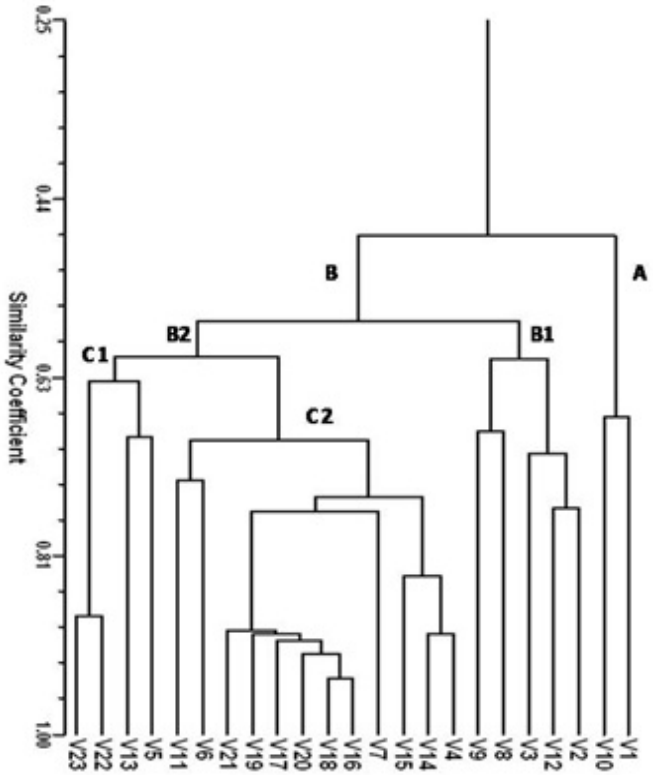

Figure 2: UPGMA dendrogram showing the genetic relationships 23 varieties of *Curcurma longa*. V1-V23 assigned as cultivars indicated in Table 2.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23
V1	1.00																						
V2	0.48	1.00																					
V3	0.43	0.70	1.00																				
V4	0.45	0.52	0.68	1.00																			
V5	0.60	0.65	0.52	0.48	1.00																		
V6	0.56	0.48	0.50	0.78	0.59	1.00																	
V7	0.50	0.50	0.52	0.72	0.63	0.69	1.00																
V8	**0.35**	0.60	0.70	0.81	0.50	0.62	0.57	1.00															
V9	0.40	0.68	0.50	0.59	0.67	0.55	0.50	0.68	1.00														
V10	0.67	0.38	0.33	0.42	0.47	0.53	0.57	0.38	0.44	1.00													
V11	0.44	0.52	0.55	0.67	0.67	0.73	0.67	0.60	0.61	0.50	1.00												
V12	0.56	0.76	0.71	0.59	0.67	0.55	0.50	0.61	0.55	0.37	0.61	1.00											
V13	0.56	0.55	0.50	0.60	0.69	0.65	0.59	0.55	0.63	0.53	0.73	0.72	1.00										
V14	0.40	0.48	0.64	0.94	0.72	0.76	0.76	0.72	0.62	0.44	0.71	0.55	0.63	1.00									
V15	0.44	0.52	0.62	0.83	0.56	0.71	0.75	0.67	0.60	0.50	0.80	0.60	0.71	0.88	1.00								
V16	0.47	0.54	0.57	0.75	0.58	0.72	0.76	0.61	0.55	0.44	0.61	0.55	0.55	0.79	0.68	1.00							
V17	0.53	0.46	0.55	0.74	0.56	0.71	0.75	0.59	0.45	0.50	0.59	0.60	0.61	0.78	0.67	0.88	1.00						
V18	0.50	0.50	0.59	0.79	0.61	0.76	0.81	0.64	0.50	0.47	0.65	0.57	0.58	0.83	0.72	0.94	0.94	1.00					
V19	0.44	0.46	0.55	0.74	0.56	0.71	0.75	0.59	0.45	0.41	0.59	0.52	0.53	0.78	0.67	0.88	0.88	0.94	1.00				
V20	0.47	0.48	0.64	0.84	0.58	0.72	0.76	0.68	0.55	0.44	0.61	0.55	0.55	0.89	0.78	**0.89**	0.88	0.94	0.88	1.00			
V21	0.53	0.52	0.55	0.74	0.65	0.81	0.75	0.59	0.52	0.50	0.69	0.60	0.61	0.78	0.76	0.88	0.88	0.94	0.88	0.88	1.00		
V22	0.63	0.59	0.48	0.57	0.65	0.61	0.65	0.46	0.52	0.50	0.50	0.60	0.61	0.60	0.58	0.68	0.76	0.72	0.67	0.68	0.76	1.00	
V23	0.63	0.67	0.55	0.50	0.65	0.53	0.65	0.40	0.45	0.50	0.50	0.68	0.61	0.52	0.58	0.60	0.67	0.63	0.58	0.60	0.67	0.88	1.00

Table 4: Similarity matrix for Nei and Li's coefficient of a total of twenty three variety of turmeric. V1-V23 assigned as name of the cultivars indicated in Table 2.

parents. The marker analysis showed that the ISSR primers were significantly associated with the root knot nematode gall index. Jenkins et al. [10] identified SSR markers for marker assisted selection of root-knot nematode resistant to cotton. They found that the chromosome 11 and 14 of cotton genotype have been associated with root knot nematode resistance which opening the way for marker assisted selection in applied breeding. This investigation as an understanding of the level and partitioning of genetic variation within the cultivars with resistant/susceptible to root knot nematode disease would provide an important input into determining efficient management strategies [24]. The genetic variability in a gene pool is normally considered as the major resource for turmeric improvement program.

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