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Genetic Relatedness of *Trichoderma* Isolates Antagonistic against *Fusarium oxysporum* f.sp. *dianthi* Inflicting Carnation Wilt

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ABSTRACT. Twenty-eight isolates of *Trichoderma* belonging to four different species were screened *in vitro* for their antagonistic ability against *Fusarium oxysporum* f.sp. *dianthi* causing carnation wilt. Three different levels of antagonism observed in dual plate assay were further confirmed by cell-free culture filtrate experiments. Isolates showing class *I* level of antagonism produced maximum lytic enzymes, chitinases and β-1,3-glucanases. Genetic variability of 25 selected isolates was assessed by random amplified polymorphic DNA technique and the amplified products were correlated for their level of antagonism. Unweighed pair-group method with arithmetical averages cluster analysis revealed prominent inter- and intraspecific genetic variation among the isolates. Based on their genetic relationship, the isolates were mainly distributed into 3 major groups representing *T. atroviride*, *T. pseudokoningii* and *T. harzianum*, with 20–35 % interspecific dissimilarity. However, the polymorphism shown by the isolates did not correlate to their level of antagonism.

Abbreviations

GlcNAc	*N*-acetylglucosamine	PDA	potato dextrose agar
MSB	minimal synthetic broth	PDB	potato dextrose broth
RAPD	random-amplified polymorphic DNA	UPGMA	unweighed pair-group method with arithmetical averages

Fusarium oxysporum f.sp. *dianthi* is an important soil borne pathogen causing substantial yield loss to carnation, leading to deterioration in quality and quantity of the marketable blooms and planting materials. The ability of *Fusarium* spp. to produce highly resistant chlamydospores, volatile inhibitors (Robinson and Garret 1969) and antibiotics like bikaverin (Robinson 1972) makes their management difficult. Although chemical control measures remain the main strategy of disease management, being arduous, uneconomical and not advisable owing to the risk of ground-water pollution, death of non-target beneficial flora and evolution of fungicide-resistant pathogen variants, a variety of alternative approaches have been considered to contain the pathogen. Due to rapid evolution of new virulent forms of the pathogen, genetic plant improvement appears to be less effective and time-consuming.

Use of biological agents, especially *Trichoderma* spp., serves as a potential alternative to chemical control measures and growing pathogen-resistant crop cultivars. These fungi are present in nearly all agricultural soils and in other environments such as decaying wood. They grow tropically toward the hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process, termed mycoparasitism, has been proposed as the major mechanism accounting for their antagonistic activity against the fungal pathogens (Cherif and Benhamou 1990). The extent of mycoparasitism, however, mainly depends on their ability to produce extracellular glycosyl hydrolases (Wells *et al.* 1972; Elad *et al.* 1986; Mukhopadhyay *et al.* 1986), such as chitinases and β-1,3-glucanases that degrade chitin and glucan polymers, which comprise important structural elements in the cell walls of fungal and oomycete organisms (Peberdy 1990). Consistent with their proposed role in mycoparasitism, the action of these purified enzymes on intact fungi can result in cell lysis and death (Lorito *et al.* 1994; Schirmbock *et al.* 1994). These enzymes can degrade not only the immature wall at hyphal apices but also the strong chitin–glucan complexes of mature cell walls and survival structures like chlamydsopores of *Fusarium* spp. and reduce not only the disease symptoms but also the pathogen spread.

Assessment of genetic relatedness among antagonistic *Trichoderma* spp. using fingerprint techniques is important to elucidate their structure and diversity in different crop rhizospheres and to characterize

the biocontrol agents for registration and patenting, recognizing the strains, quality checking during production and ecological characterization (Plimmer 1993; Lemanceau *et al.* 1995). The RAPD procedure involving simultaneous amplification of several anonymous loci in the genome using primers of arbitrary sequence (Williams *et al.* 1990; Welsh and McMclelland 1990) has been used for genetic, taxonomic and ecological studies of several fungi (Crowhurst *et al.* 1995; Fungaro *et al.* 1996; Manulis *et al.* 1993; Zinno *et al.* 1998; Abbasi *et al.* 1999; Paavanem-Huhtala *et al.* 2000). RAPD markers were employed to explore natural genetic variability existing among 14 *Trichoderma* strains and differentiated them as similarity groups. The RAPD profiles of *T. harzianum* strains were analyzed to classify the strains into different groups according to their antagonistic capacity (Gomez *et al.* 1997). In yet another study, 11 strains of *T. viride*, 2 strains of *Hypocrea rufa* and 9 other species of *Trichoderma* were explored for the relationship of RAPD profile to their potential to control *Cryphonectria parasitica* through pairing *in vitro*. The results allowed the construction of a dendrogram wherein the antagonistic strains were gathered in very different groups (Arisan-Atac *et al.* 1995). The suitability of RAPD in selecting *Trichoderma* strains with taxonomic finalities was also reported after analyzing 74 strains. These results were consistent with the morphological, physiological and ecological data of these strains (Fujimori and Okhuda 1994). This paper therefore reports molecular characterization of antagonistic *Trichoderma* spp. selected against *F. oxysporum* f.sp. *dianthi* using RAPD to analyze the genetic variability among the isolates and to corroborate the variability to their level of antagonism, which was ascertained by lytic enzyme production.

MATERIAL AND METHODS

Trichoderma *and* Fusarium *isolates.* Twenty-three isolates of *Trichoderma harzianum*, three isolates of *T. pseudokoningii*, one isolate each of *T. viride*, and *T. atroviride* were obtained from the culture collections of *Floriculture Division* of the Institute (Table I). These species were earlier isolated from rhizosphere soils of various crops cultivated in different agro-climatic zones of Himachal Pradesh (India) and were phenotypically characterized for their identity. Barring GLTR(1)-Kotli (used as control), the rest of the isolates were selected for their antagonistic abilities (*unpublished data*). The target pathogen, *Fusarium oxysporum* f.sp. *dianthi* (PRILL. & DELACR.) W.C. SYNDER & H.N. HANS (MTCC 6659) causing vascular

Table I. Characteristics of *Trichoderma* spp.

Source	Code	*Trichoderma* isolate	Crop rhizosphere
Himachal Pradesh (India)	CATH-Bhatoo	*T. harzianum*	carnation
	CATH-Mandi		*ditto*
	CATH-Mashobra		*ditto*
	GLTH-Bir		gladiolus
	GLTH-Chauntra		*ditto*
	GLTH-Choubin		*ditto*
	GLTH-Cloudland		*ditto*
	GLTH-Darang		*ditto*
	GLTH-IHBT		*ditto*
	GLTR(1)-Kotli		*ditto*
	GLTH(2)-Kotli		*ditto*
	LITH-Sidhbari		lilium
	MATH-Darang		maize
	TETH-Banuri		tea
	TETH-Chauntra		*ditto*
	TETH-Chogun		*ditto*
	TETH-IHBT		*ditto*
	TETH-Khlet		*ditto*
	TETH-Raipur		*ditto*
	TETH-Sungal		*ditto*
	TETV-Darang	*T. viride*	*ditto*
Tamil Nadu (India)	BGTA-Tn	*T. atroviride*	black gram
	BGTK-Tn	*T. pseudokoningii*	*ditto*
	BRTH-Tn	*T. harzianum*	brinjal
	GGTK-Tn	*T. pseudokoningii*	green gram
	RGTK-Tn	*ditto*	*ditto*
	TOTH-Tn	*T. harzianum*	tomato

wilt in carnation was isolated from the infected cuttings on PDA medium and its pathogenicity was established under artificially inoculated conditions by standard methods. Single-spore cultures were prepared from each of these isolates for further use.

Selection of antagonistic Trichoderma *spp.* The *Trichoderma* spp. were evaluated for their antifungal activity against the pathogen by dual culture assay on PDA (Dennis and Webster 1971). The time of inoculation of the pathogen was decided, based on its growth rate with respect to the antagonist. The linear growth of the pathogen was measured when the control plates showed full growth. The *Trichoderma* spp. were classified for their level of antagonism and the isolate that overgrew the pathogen, covering completely or at least ⅔ of the surface, was considered to be antagonistic (Bell *et al.* 1982).

The promising isolates selected from the dual culture studies were further evaluated by cell-free culture filtrate assays by growing in liquid cultures. Fifty mL of potato dextrose broth was taken in 250-mL conical flasks and sterilized (137 kPa, 20 min). Mycelial discs (∅ 5 mm) were taken from the actively growing young cultures of the isolates grown on PDA and used for inoculating the broth. The flasks were then incubated at room temperature (28 ± 2 °C) for 10 d in shake cultures which were filtered by coarse filtration using Büchner flasks; this filtrate was again filtered through *Millipore* filters (pore size 450 nm) and the inhibiting properties were assayed against the pathogen employing poisoned food technique (Zentmyer 1955) and expressed as inhibition (in %) over the control. The experiments were done entirely at random with 3 replicates.

Screening antagonistic Trichoderma *spp. for lytic enzyme production.* The best performing antagonistic *Trichoderma* spp. from each of the classes were grown separately in MSB (Elad *et al.* 1982) amended with pathogen cell wall (0.5 %) to evaluate their efficiency to produce chitinases and β-1,3-glucanases. GLTR(1)-Kotli belonging to class *V* was used as the control. The fungal cell wall was prepared from the fungal mycelial mat grown on PDB (8 d, 28 ± 2 °C; Ajit *et al.* 2006). The mycelium was then collected by filtration through *Whatman* no. 1 filter paper, washed with sterile water and homogenized in chloroform–methanol (1 : 1). The resulting suspension was filtered and homogenized in acetone. The mycelium was washed with distilled water repeatedly to remove excess acetone and dried at 45 °C. The dried cell-wall material was used for amendment in MSB. Colloidal chitin was prepared from crab shell chitin (Berger and Reynolds 1958).

Chitinase. The production was evaluated by colorimetry (Boller and Mauch 1988). The reaction mixture consisted of 0.5 mL of 1 mol/L sodium acetate buffer (pH 4.0), 0.4 mL supernatant from culture filtrate of isolates grown separately in MSB and 0.1 mL colloidal chitin (10 mg) in triplicates. After incubating (1 h), the resulting chitin oligomers were treated with 2 mL 4-dimethylaminobenzaldehyde (37 °C, 20 min) and the absorbance A_{585} was measured; GlcNAc served as the standard. The enzyme activity was expressed as pkat of GlcNAc per mL of culture filtrate.

β-1,3-Glucanase. The reaction mixture contained 500 μL of 5 % (*M*/*V*) laminarin (*Hi-Media*) in 50 mmol/L acetate buffer (pH 4.8) and 200 μL supernatant from culture filtrate. The mixture was incubated (45 °C, ½ h) and the reducing sugar produced was determined using 3,5-dinitrosalicylic acid (El-Katany *et al.* 2001). The amount of reducing sugars released was calculated from standard curves recorded for glucose, enzyme activity being expressed as nkat of glucose released per mL of culture filtrate.

DNA isolation and PCR amplification. Five-d-old submerged-culture mycelium was used for DNA isolation. For submerged culture, well-isolated colonies of *Trichoderma* spp. were inoculated separately in 50 mL of PDB (potato 200 g and dextrose 20 g in 1 L water). The inoculated flasks were incubated on a rotary shaker (3 Hz, 28 ± 2 °C). The mycelium was filtered out on a sterile *Whatman* no. 1 filter paper and washed thrice with sterile distilled water to remove traces of the medium. The fungal mass was air-dried to remove excess of moisture, frozen immediately in liquid nitrogen and lyophilized. The lyophilized mats were ground with a mortar and pestle in liquid nitrogen and stored immediately at –80 °C.

Extraction and amplification of DNA. Genomic DNA was isolated from each *Trichoderma* isolate (Raeder and Broda 1985). One-hundred primers from the kits OPA, OPAA, OPB, OPG and OPH, each consisting of 20 random decamer primers (*Operon Technologies*, USA), were tested. The PCR reagents were supplied by *Bangalore Genei* (India). Amplifications were carried out in 25 μL reaction volume consisting of: 25 μL buffer, 2 μL 25 mmol/L $MgCl_2$, 0.2 μL 20 mmol/L dNTPs, 0.33 μL 3 U *Taq* DNA polymerase, 2 μL 20 pmol/L primer, 25 ng template DNA, and 17.72 μL H_2O in a *Biorad* (USA) thermal cycler using the PCR conditions (94 °C, 1 min – denaturation; 33 °C, 1 min – annealing; 72 °C, 2 min – extension). The total number of cycles was 40, with the final extension of 72 °C for 15 min. The reaction control consisted of all components, except the genomic DNA. The amplified products (25 μL) were size separated on 1.4 % agarose gel containing 0.5 μg/mL ethidium bromide and photographed with the gel documentation system (Alpha Imager 2200). A 500-bp DNA ladder (*Bangalore Genei*, India) was used as molecular size markers.

The PCR-RAPD analysis was repeated at least thrice and the fingerprints were compared. The RAPD bands, which appeared consistently, were evaluated.

Data analysis. The NTSYS.PC (Numerical Taxonomy System Applied Biostatistics; *Setauket*, USA) computer program was employed. The data (presence or absence of band) were introduced in the form of a binary matrix and a pair-wise similarity matrix was constructed using the DICE coefficient (SD) (Sneath and Sokal 1973). SD values were obtained by the double number of shared bands between two patterns divided by the sum of all the bands in the same pattern (value 1 indicates patterns for two individuals and value 0 indicates completely different patterns). The UPGMA grouping of the SD values was generated using the NTSYS program.

RESULTS AND DISCUSSION

In biological control, classification and segregation of antagonistic strains from each other are essential to identify and deploy the highly efficient strains for disease management. Studies on the antagonistic pattern of the *Trichoderma* isolates against *Fusarium* sp. in dual culture indicated that all the isolates except CATH-Bhatoo, GLTH-Darang, CATH-Mashobra, GLTH-Chauntra and GLTR(1)-Kotli were antagonistic, exhibiting class *1* and *2* levels of antagonism. In cell-free culture filtrate assays, the antagonists grouped under class *1* level exhibited maximum inhibition over the control (>75 %) with class *4* isolates showing the least inhibition (<25 %) (Table II).

Table II. Classification of *Trichoderma* isolates screened for antagonism against *F. oxysporum* f.sp. *dianthi*

Antagonism class[a]	Isolates			Inhibition over control, %[b]
1	BGTA-Tn	GLTH-Bir	TETH-Chauntra	>75
	BGTK-Tn	GLTH-Choubin	TETH-Khlet	
	BRTH-Tn	GLTH-Cloudland	TETH-Raipur	
	CATH-Darang	MATH-Darang	TETV-Darang	
	CATH-Mandi	RGTK-Tn	TOTH-Tn	
	GGTK-Tn	TETH-Banuri		
2	GLTH(2)-Kotli	LITH-Sidhbari	TETH-IHBT	50–75
	GLTH-IHBT	TETH-Chogun	TETH-Sungal	
3	CATH-Bhatoo	GLTH-Darang		25–50
4	CATH-Mashobra	GLTH-Chauntra		<25
5	GLTR(1)-Kotli			0

[a]Based on Bell *et al.* (1982):
1 *Trichoderma* grew, overlapped the *Fusarium* colony and covered the whole media surface;
2 *Trichoderma* grew and covered ⅔ of the media surface;
3 *Trichoderma* and *Fusarium* sp. colonized each ½ of the media surface and did not have dominance;
4 *Fusarium* grew and covered ⅔ of the media surface;
5 *Fusarium* grew, overlapped the *Trichoderma* colony and covered the whole media surface.
[b]Cell-free culture filtrate assay.

The levels of antagonism were further ascertained by evaluating them for the production of lytic enzymes, chitinases and β-1,3-glucanases. Significant variation was observed between the isolates exhibiting different levels of antagonism, with class *1* isolates producing maximum chitinase (305–412 pkat/mL) and β-1,3-glucanase (3.78–4.58 nkat/mL of culture filtrate) (Table III). Since the isolates CATH-Mashobra and GLTH-Chauntra showed <25 % mycelial growth inhibition of the pathogen through their culture filtrates, they were not included for further analysis. Antifungal activity of *Trichoderma* spp. against *Fusarium* spp. has been reported by Olive and Bean (1999).

In RAPD, of 100 random primers screened for amplification of DNA of 25 isolates, the following 11 primers, *viz.*

OPA 2 (5′-TGC CGA GCT G)
OPA 5 (5′-AGG GGT CTT G)
OPA 11 (5′-CAA TCG CCG T)
OPA 20 (5′-GTT GCG ATC C)
OPAA 11 (5′-ACC CGA CCT G)
OPAA 19 (5′-TGA GGC GTG T)
OPB 8 (5′-GTC CAC ACG G)
OPG 6 (5′-GTG CCT AAC C)
OPG 18 (5′-GGC TCA TGT G)
OPH 1 (5′-GGT CGG AGA A)
OPH 15 (5′-AAT GGC GCA G)

Table III. Lytic enzyme production by antagonistic *Trichoderma* spp.

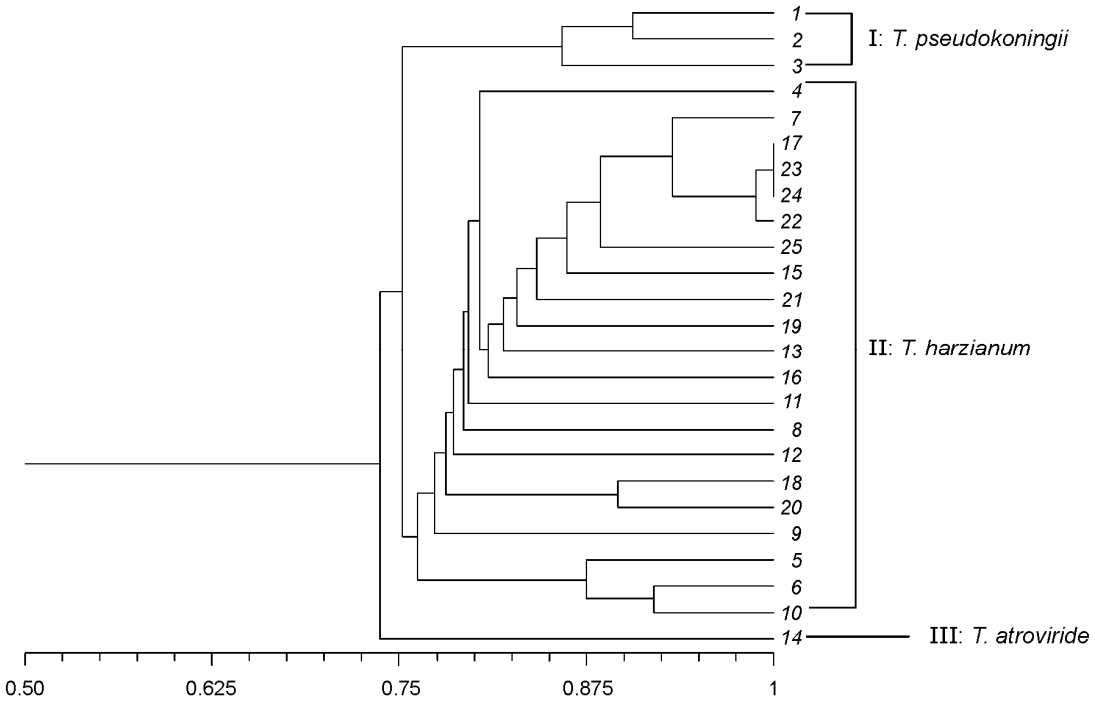
Isolate	Antagonism class	Chitinase[a] pkat of GlcNAc/mL[b]	β-1,3-Glucanase[a] nkat of Glc/mL[b]
GGTK-Tn	*I*	363	4.58
CATH-Mandi	*I*	412	4.27
BGTK-Tn	*I*	312	4.08
BGTA-Tn	*I*	332	3.95
GLTH-Bir	*I*	305	3.78
TETH-IHBT	*II*	257	3.09
GLTH-IHBT	*II*	198	2.70
TETH-Chogun	*II*	228	2.65
GLTH-Darang	*III*	140	1.70
CATV-Mashobra	*IV*	0	0
GLTR(1)-Kotli	*V*[c]	0	0

[a]Mean of triplicates; CD (0.05 %): chitinases 1.5317, β-1,3-glucanases 1.5317.
[b]Of culture filtrate. [c]Control.

generated highly discriminative and reproducible RAPD profiles with consistent fragment patterns in all isolates and distinguished the species from each other. These primers produced 1092 bands showing 100 % polymorphism with OPA 5 followed by OPA 2 generating maximum (153 and 141) polymorphic bands. A total number of 106 unique bands were identified, of which the highest and lowest numbers of 13 and 5 were generated by OPA 5 and/or OPA 20 and OPB 8, respectively. For RAPD profiles of the *Trichoderma* isolates analyzed with primer OPA 5 *see* Fig. 1. The non-selected primers mostly generated non-reproducible banding patterns, which may be probably due to the critical or suboptimal reagent concentration or reaction conditions or perhaps the rare existence of the target amplification in the genome.

The typing efficiency of RAPD could be improved by the parallel use of several primers (Olive and Bean 1999). The typeability of the RAPD technique was observed to be 100 % in generating reproducible and discriminative profiles of *Dermatophilus congolensis* (Larrasa *et al.* 2004).

Assessment of genetic relationships by UPGMA analyses based on DICE similarity index (SD) illustrated prominent inter- and intraspecific genetic variation among the isolates, as they were mainly divided into three major clusters representing three different species. Cluster I represented *T. pseudokoningii*, cluster II represented *T. harzianum* and cluster III represented *T. atroviride.* The graphic phenogram (Fig. 2) distantly placed BGTA-Tn (*T. atroviride)* representing cluster III from the rest of the isolates. In cluster I, the isolate BGTK-Tn (*T. pseudokoningii*) obtained from black gram (*Vigna mungo*) rhizosphere divided itself into a sepa-



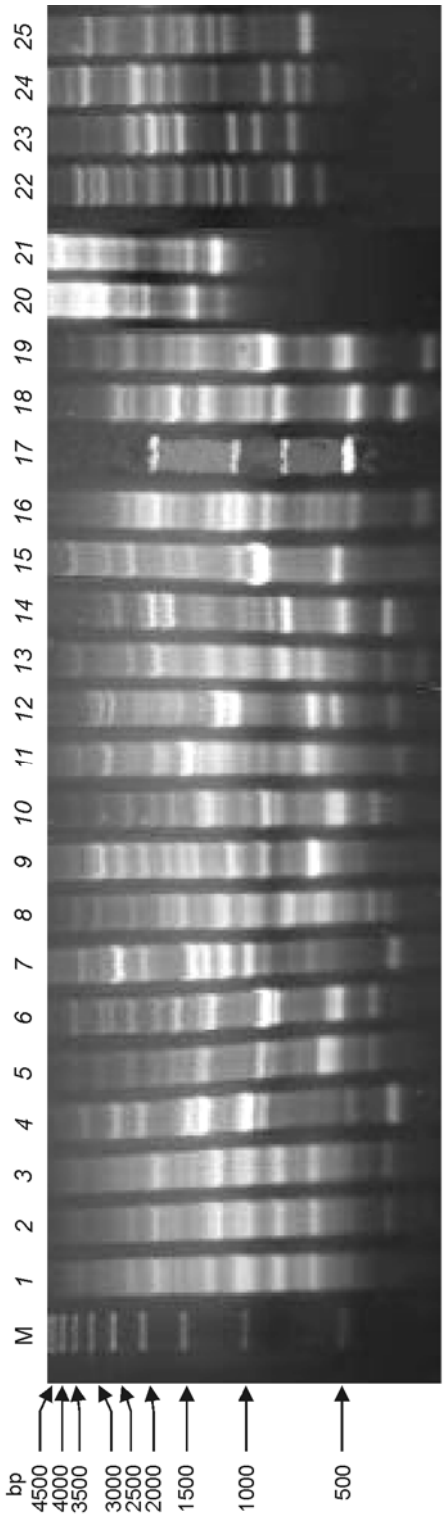


Fig. 1. Representative RAPD profile of *Trichoderma* isolates with one of the primers (OPA 5)

M	molecular-size markers	9	CATH-Mandi
1	BGTK-Tn	10	GLTH-Darang
2	GGTK-Tn	11	GLTH-Choubin
3	RGTK-Tn	12	LITH-Sidhbari
4	GLTH-IHBT	13	MATH-Darang
5	GLTH-Bir	14	BGTA-Tn
6	TETH-Khlet	15	TOTH-Tn
7	TETV-Darang	16	BRTH-Tn
8	TETH-Chogan	17	TETH-IHBT
18	GLTH-Cloudland		
19	CATH-Bhatoo		
20	TETH-Chauntra		
21	TETH-Sungal		
22	TETH-Raipur		
23	GLTH(2)-Kotli		
24	CATH-Darang		
25	TETH-Banuri		

Fig. 2. UPGMA dendrogram based on the DICE similarity index (SD) illustrating the genetic relationships of antagonistic *Trichoderma* isolates

1	BGTK-Tn	8	TETH-Chogan	14	BGTA-Tn	20	TETH-Chauntra
2	GGTK-Tn	9	CATH-Mandi	15	TOTH-Tn	21	TETH-Sungall
3	RGTK-Tn	10	GLTH-Darang	16	BRTH-Tn	22	TETH-Raipur
4	GLTH-IHBT	11	GLTH-Choubin	17	TETH-IHBT	23	GLTH(2)-Kotli
5	GLTH-Bir	12	LITH-Sidhbari	18	GLTH-Cloudland	24	CATH-Darang
6	TETH-Khlet	13	MATH-Darang	19	CATH-Bhatoo	25	TETH-Banuri
7	TETV-Darang						

Table IV. RAPD similarity matrix of *Trichoderma* isolates

BGTK-Tn	1									
GGTK-Tn	0.91	1								
RGTK-Tn	0.86	0.85	1							
GLTH-IHBT	0.69	0.72	0.75	1						
GLTH-Bir	0.65	0.71	0.71	0.69	1					
TETH-Khlet	0.68	0.72	0.73	0.73	0.90	1				
TETV-Darang	0.73	0.79	0.81	0.84	0.76	0.79	1			
TETH-Chogan	0.76	0.79	0.77	0.75	0.70	0.72	0.80	1		
CATH-Mandi	0.70	0.73	0.74	0.73	0.72	0.73	0.81	0.76	1	
GLTH-Darang	0.71	0.75	0.76	0.75	0.85	0.92	0.80	0.75	0.74	1
GLTH-Choubin	0.72	0.75	0.76	0.76	0.72	0.74	0.80	0.75	0.73	0.77
LITH-Sidhbari	0.72	0.74	0.76	0.75	0.73	0.74	0.79	0.79	0.75	0.75
MATH-Darang	0.71	0.74	0.77	0.76	0.74	0.77	0.80	0.77	0.72	0.77
BGTA-Tn	0.70	0.72	0.71	0.70	0.71	0.72	0.75	0.71	0.68	0.74
TOTH-Tn	0.72	0.77	0.77	0.77	0.74	0.76	0.84	0.77	0.77	0.78
BRTH-Tn	0.69	0.72	0.74	0.77	0.74	0.75	0.81	0.76	0.75	0.76
TETH-IHBT	0.79	0.85	0.87	0.85	0.81	0.83	0.94	0.85	0.85	0.85
GLTH-Loudland	0.68	0.72	0.72	0.74	0.71	0.71	0.79	0.73	0.74	0.74
CATH-Bhatoo	0.69	0.73	0.72	0.76	0.73	0.74	0.83	0.76	0.76	0.74
TETH-Chauntra	0.66	0.70	0.73	0.76	0.71	0.73	0.80	0.75	0.76	0.75
TETH-Sungal	0.69	0.73	0.75	0.77	0.73	0.76	0.83	0.74	0.74	0.76
TETH-Raipur	0.78	0.84	0.86	0.84	0.80	0.82	0.92	0.84	0.84	0.84
GLTH(2)-Kotli	0.79	0.85	0.87	0.85	0.81	0.83	0.94	0.85	0.85	0.85
CATH-Darang	0.79	0.85	0.87	0.85	0.81	0.83	0.94	0.85	0.85	0.85
TETH Banuri	0.77	0.80	0.83	0.78	0.74	0.77	0.85	0.78	0.75	0.77

rate entity with GGTK-Tn and RGTK-TN (both *T. pseudokoningii*) representing green gram (*Vigna radiata*) and red gram (*Cajanus cajan*) rhizospheres, respectively. BGTK-Tn shared 91 and 86 % similarity with GGTK-Tn and RGTK-Tn (Table IV). RGTK-Tn was closer to the subclusters containing *T. harzianum* isolates (71–87 % similarity) and shared maximum similarity (87 %) with TETH-IHBT and minimum similarity (71 %) with GLTH-Bir (both *T. harzianum*). In the second group (representing *T. harzianum* isolates), 8 subclusters were observed with less genetic variation (65–100 %) between them. Among the isolates, TETH-IHBT shared 100 % similarity with GLTH(2)-Kotli and CATH-Darang. Similarly, GLTH-Bir shared maximum similarity with TETH-Khlet (90 %) and GLTH-Darang (85 %). Arisan-Atac *et al.* (1995) and Goes *et al.* (2002) reported the type ability of antagonistic *Trichoderma* spp. by RAPD.

Analysis of interspecific genetic variation between the groups indicated that 20–32 % dissimilarity existed between cluster III (*T. atroviridae*) and cluster II (*T. harzianum*); 30 % dissimilarity between cluster III and cluster I (*T. pseudokoningii*) and 21–35 % dissimilarity between cluster I (*T. pseudokoningii*) and cluster II.

The variability of the antagonistic isolates did not corroborate the levels of antagonism exhibited by them. *T. pseudokoningii* isolates, *viz.* BGTK-Tn, GGTK-Tn and RGTK-Tn and the *T. atroviridae* isolate BGTA-Tn were grouped together with *T. harzianum* isolates in class *I* level. It appears that the RAPD profiles divided the isolates into separate clusters based on their genetic relationship rather than their mode of action. This is evident from the fact that certain isolates such as GLTH-Darang and CATH-Bhatoo, though exhibiting class *3* level of antagonism, were grouped together in cluster II along with other antagonistic *T. harzianum* isolates of class *1* and *2* levels. Furthermore, the grouping of isolates of different rhizospheres indicated the possibility of non-influence of host exudates.

Genotyping of certain antifungal compounds producing fluorescent pseudomonads indicated that organisms having similar properties might share some genetic relationship (Santos 1992). In contrast, no such relationship existed in the case of *Trichoderm*a spp. Probably, the environmental conditions other than the host from where the antagonistic isolates were obtained might have influenced their genetic make-up and diversity to group in different clusters, which are to be studied in detail with a fairly larger number of samples. Similar type of dissimilarity had been reported (Williams *et al.* 1990) in the case of *Trichoderma* spp. antagonistic against *Rhizoctonia solani*. No relationship existed between the polymorphism shown by the *R. solani* isolates and their hardness. Fujimori and Okhuda (1994), Zimand *et al.* (1994) and Harmosa *et al.* (2001) have also reported the existence of genetic diversity among *Trichoderma* aggregates.

The foregoing studies conclude that the RAPD banding patterns of *Trichoderma* spp. did not duplicate the level of antagonism exhibited *in vitro* against the pathogen. Nevertheless, their possible role in depicting polymorphisms and grouping the isolates according to their genetic relationship could not be negated.

1														
0.78	1													
0.79	0.79	1												
0.73	0.75	0.79	1											
0.79	0.79	0.79	0.76	1										
0.74	0.75	0.76	0.71	0.78	1									
0.85	0.84	0.86	0.80	0.88	0.86	1								
0.71	0.75	0.73	0.74	0.77	0.78	0.83	1							
0.75	0.72	0.76	0.72	0.80	0.79	0.86	0.82	1						
0.72	0.75	0.76	0.72	0.78	0.76	0.84	0.90	0.81	1					
0.75	0.75	0.78	0.70	0.78	0.76	0.87	0.75	0.78	0.78	1				
0.84	0.83	0.86	0.79	0.87	0.85	0.99	0.82	0.85	0.84	0.86	1			
0.85	0.84	0.86	0.80	0.88	0.86	1.00	0.83	0.86	0.84	0.87	0.99	1		
0.85	0.84	0.86	0.80	0.88	0.86	1.00	0.83	0.86	0.84	0.87	0.99	1.00	1	
0.79	0.76	0.80	0.73	0.81	0.78	0.90	0.75	0.79	0.76	0.82	0.88	0.90	0.90	1

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