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GENETIC DIVERSITY OF *Trichoderma* sp. OBTAINED FROM TOMATO RHIZOSPHERE USING RAPD

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Abstract: Ten isolates of *Trichoderma* sp. *viz*., *T. viride* (five isolate), *Trichoderma* sp. (two isolates) and *T. harzianum* (three isolates) obtained from the rhizosphere soil of tomato fields of south Gujarat region were studied using RAPD. The genetic diversity among ten isolates of *Trichoderma* sp. were analyzed with five random RAPD primers. The percentage of polymorphism ranged from 60.00% to 83.33%. Analysis of dendrogram revealed that similarity coefficient ranged from 0.51 to 0.89. RAPD profiles showed genetic diversity among the isolates with the formation of two clusters. First cluster involved five isolates of *T. viride* and two isolates of *Trichoderma* sp. while second cluster involves three isolates of *T. harzianum.* This result indicated the identification patterns of *Trichoderma* isolates.
Keywords: *Trichoderma viride, Trichoderma harzianum,* RAPD, Genetic diversity.

Introduction

Antagonistic microorganisms, such as *Trichoderma,* reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion [1]. In addition, the release of biocontrol agents into the environment monitor their presence or absence in soil. Therefore, monitoring population dynamics in soil is of much importance. Previous methods employed to identify strains of *Trichoderma* sp. in soil samples have included the use of dilution plates on selective media. However, this method does not distinguish between indigenous strains and artificially introduced ones [2]. The *Trichoderma* isolates were differentiated by mycelial growth rate and colony appearance, as well as microscopic morphological features, including phialides and phialospores. These can also be distinguished by RAPD-PCR, restriction fragment length polymorphisms in mitochondrial DNA and ribosomal DNA and sequence analysis of ribosomal DNA [7]. Molecular characterization of the potential bio-control agents using Randomly Amplified Ploymorphic DNA (RAPD) and Internal Transcribe Spacer-Polymerase Chain Reaction (ITS-PCR), helps to determine the diversity and identification. In

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the present study, genetic variability in ten isolates of *Trichoderma* sp. from different eco systems were evaluated with five different RAPD markers.

Materials and Methods

Isolation of *Trichoderma*:

The soil samples were collected randomly from the rhizosphere soil of tomato crop of the college farm as well as farmer's fields of south Gujarat region with the help of soil sampler (Table-1) by employing serial dilution method [5].Pure cultures of the *Trichoderma* isolates were maintained on PDA slants and incubated at 28±2°C for 6 days under controlled temperature.

Characterization of *Trichoderma* sp. using RAPD

Identification of *Trichoderma* isolates through RAPD, a PCR (Polymerase Chain Reaction) based molecular marker system was performed as detailed below:

Genomic DNA extraction:

Pure cultures of the *Trichoderma* isolates were maintained on PDA slants and incubated at 28±2°C for 6 days under controlled temperature. Mycelia were aseptically transferred to flasks of potato-dextrose broth (PDB, Hi-Media) and incubated for 5 days at 28±2°C without shaking. The mycelia were filtered from the liquid medium and total DNA was extracted [4].

RAPD- primers:

Total twenty five (25) random decamer primers belonging to OPA, OPB, OPE and OPH series obtained from Integrated DNA Technology (IDT) were screened and out of that only five were found to be having complementary sequence present in the set of genome studied from the OPA and OPE series (Table-2).

RAPD analysis:

The genomic DNA was subjected to RAPD analysis. Polymerase Chain Reaction (PCR) was performed in a 200 µl thin walled sterilized PCR tube containing a 25 µl reaction mixture. The PCR reaction mixture consisted of 2.5 µl of 10X Taq Buffer A with1.5 mM $MgCl_2$, 0.5 µl of *Taq*DNA polymerase (3U/ µl), 1 µl of dNTP's (0.2 mM each), 1 µl of primer (10 pmol/ µl) (Supplied by Biogene,USA), 50 ng DNA (1 µl) and 17.5 µl sterilized deionized water. The PCR tubes containing the reaction mixture were tapped gently and spun briefly at 10,000 rpm. The PCR tube containing 25 µl of reaction mixture was placed in the Biorad thermal cycler along with a control (without genomic DNA). The DNA amplification was carried out in thermal cycler with following PCR programme, initial denaturation: 95°C for 5 minutes, denaturation: 95°C for 45 seconds, primer annealing: 38°C for 30 seconds, extension: 72°C

for 45 seconds, repetition of step 2 to 4 for 35 times, final extension: 72 °C for 10 minutes, Hold: at 4° C.

All the amplified PCR products were resolved by electrophoresis on 1.4% agarose for 3 h in 1 X TBE buffer at 60 V, stained with ethidium bromide and photographed using the Gel Documentation System (Gene Genius Syngene, U.K.).

Statistical analysis of RAPD data:

Statistical analysis for the RAPD data from all the polymorphic primers was scored by visual observation. The faint bands were not scored as they were not reproducible. The presence of an amplified band (amplicon) in each position was recorded as 1 and the absence as 0. Based on the presence and absence data Jaccard's similarity index was calculated [8]. Similarity coefficients were used for cluster analysis, performed using the UPGMA cluster analysis protocol (unweighted pair-group methods with arithmetic mean) sub programme of NTSYSpc version 2.2 (Numerical Taxonomy and Multivariate Analysis System) [6] and dendogram was constructed by UPGMA (unweighted pair group method with arithmetic averages).

Results

Ten isolates were obtained using PDA medium from rhizosphere soil of tomato. Among them five isolates were identified as *T. viride*, three isolates were of *T. harzianum* and two isolates as *Trichoderma* sp. (Table -1). Genetic diversity among ten isolates of *Trichoderma* sp. *viz*., *T. viride* (five isolate), *Trichoderma* sp. (two isolates) and *T. harzianum* (three isolates) were analyzed by six random primers OPA-1, OPA-9, OPA-10, OPE-1 and OPE-9 to generate reproducible polymorphism (Table-2). All amplified products with the primers had shown polymorphic and distinguishable banding patterns which indicate the genetic diversity of *Trichoderma* isolates. Total 27 reproducible and scorable bands were generated, out of which 19 were found to be polymorphic and 8 were monomorphic bands. The highest numbers of bands (7) were obtained with primer OPA-1, while the lowest numbers of bands (4) were obtained with primer OPE-5. Different primers showed a variation in their ability to detect polymorphism. The percentage of polymorphism ranged from 60.00% to 83.33%. Primer OPA-9 revealed the highest polymorphism (83.33%) while primer OPA-10 and OPE-1 exhibited the lowest polymorphism (60.00%) (Table-2). For each primer, RAPD fragments were scored on the basis of presence and absence of amplified product.

The Jaccard's similarity index was estimated among the ten isolates of *Trichoderma* sp. (Table-3). The similarity index among the fungus studied ranged from 0.51 to 0.89 indicating

presence of moderate genetic similarity between set of genomes. The two strains of *T. harzianum* had a similarity coefficient of 0.89 which were found more similar with each other. The Jaccard's similarity coefficient matrix was further used to obtain a dendrogram (Fig. 2). Based on the results obtained all the ten isolates can be grouped into two main clusters. One cluster representing five isolates of *T. viride* and two isolates of *Trichoderma* sp., and other *T. harzianum*. Again first cluster is sub grouped into two clusters, one as *T. viride* and other as *Trichoderma* sp. *T. viride* sub cluster is again sub divided in two clusters. First sub cluster with two isolates *i.e.,* TVK and TVM, while other sub cluster with three isolates *i.e.* TVRN, TVM-2 and TVKN. Second cluster of *T. harzianum* contains three isolates of *T. harzianum, i.e.,* THRN, THP and THO. Similarity between *Trichoderma* isolates were observed and reason might be due to these species were isolated from the same host.

Discussion

The present investigation is in conformation [9], who used RAPD technique to distinguish sub groups within 23 strains of *T. harzianum* and 19 strains of *T. viride.* [3] used five different RAPD primers *i.e.* OPA-1, OPA-2, OPA-3, OPA-4 and OPA-9 for checking the variability among six *Trichoderma* spp. *viz., T. virens, T. pseudokoningii, T. hamatum, T. harzianum, T. viride* and *T. koningii* and revealed intra and inter-specific variability amongst *Trichoderma* isolates examined.

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Table 1: Identification of different isolates of *Trichoderma* sp. from different areas of south Gujarat

Sr. No	Locations	Code no.	Isolated identified	ITCC No.
1	Kotha	TVK	*Trichoderma viride*	8414.11
2	Maroli	TVM-1	*T. viride*	8415.11
3	RHRS, Navsari	TVRN	*T. viride*	8416.11
4	Maroli	TVM-2	*T. viride*	8417.11
5	KVK, Navsari	TVKN	*T. viride*	8418.11
6	Kamrej	TK	*Trichoderma* sp.	8419.11
7	Mandvi	TM	*Trichoderma* sp.	8420.11
8	RHRS, Navsari	THRN	*T. harzianum*	8421.11
9	Olpad	THO	*T. harzianum*	8603.11
10	Puna gam	THP	*T. harzianum*	8604.11

Table 2: Details of amplification with different RAPD primers with different isolates of *Trichoderma* sp.

Primer	No. of monomorphic band	No. of polymorphic band	Total no. of bands	Percentage of polymorphism
OPA-1	2	5	7	71.42
OPA-9	1	5	6	83.33
OPA-10	2	3	5	60.00
OPE-1	2	3	5	60.00
OPE-5	1	3	4	75.00
	8	**19**	**27**	**70.37**

Table 3: Jaccard's similarity coefficient among different accessions based on the RAPD data

Accessions	TVK	TVM-1	TVRN	TVM-2	TVKN	TK	TM	THRN	THO	THP
TVK	1.000									
TVM-1	0.737	1.000								
TVRN	0.550	0.684	1.000							
TVM-2	0.632	0.600	0.667	1.000						
TVKN	0.632	0.600	0.765	0.875	1.000					
TK	0.600	0.650	0.550	0.476	0.476	1.000				
TM	0.476	0.524	0.500	0.500	0.579	0.722	1.000			
THRN	0.417	0.522	0.571	0.571	0.571	0.478	0.500	1.000		
THO	0.454	0.571	0.632	0.476	0.550	0.529	0.476	0.889	1.000	
THP	0.500	0.545	0.454	0.454	0.454	0.500	0.524	0.750	0.737	1.000

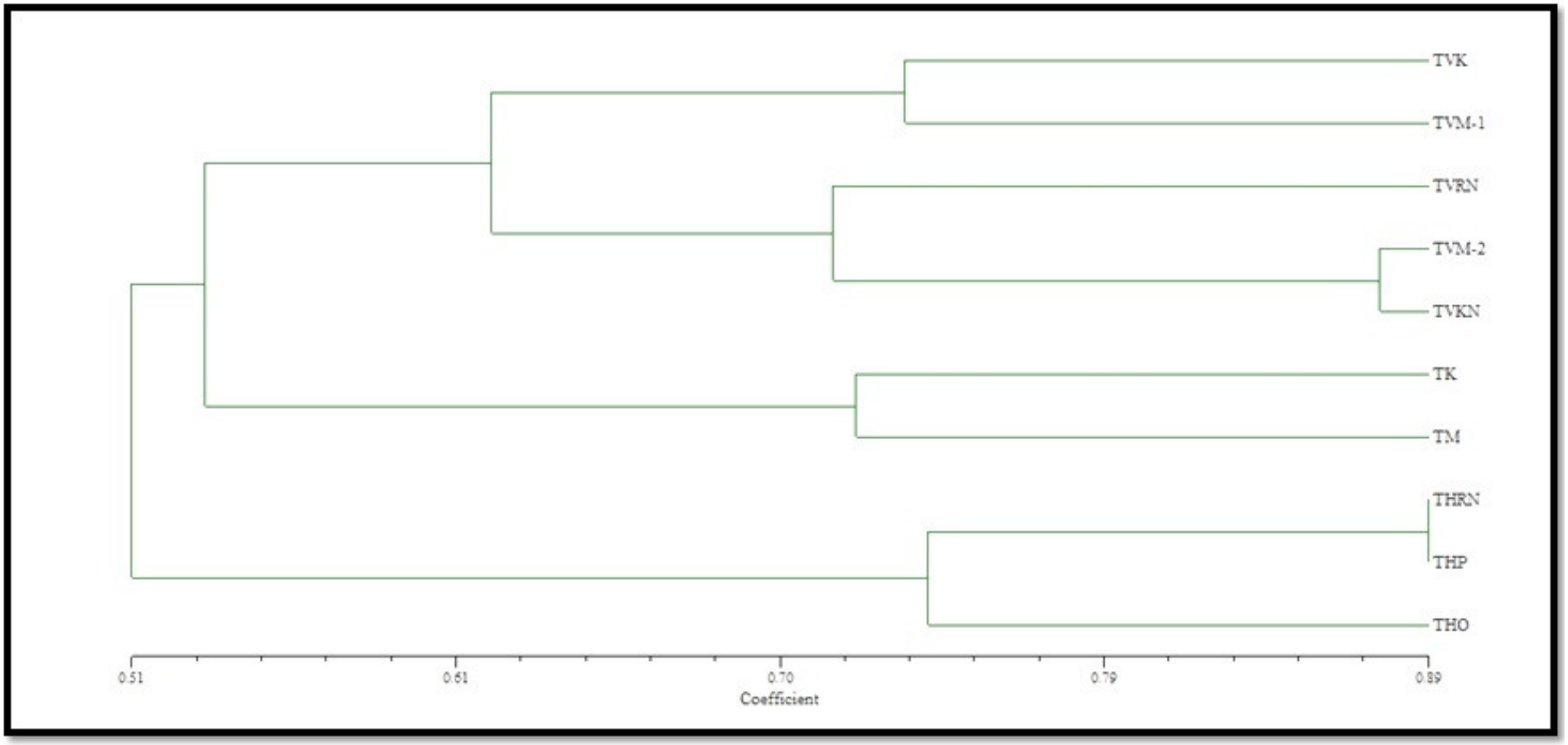

Fig. 2: UPGMA based dendrogram of Jaccard's Similarity Index for RAPD data generated for *Trichoderma* sp. isolates

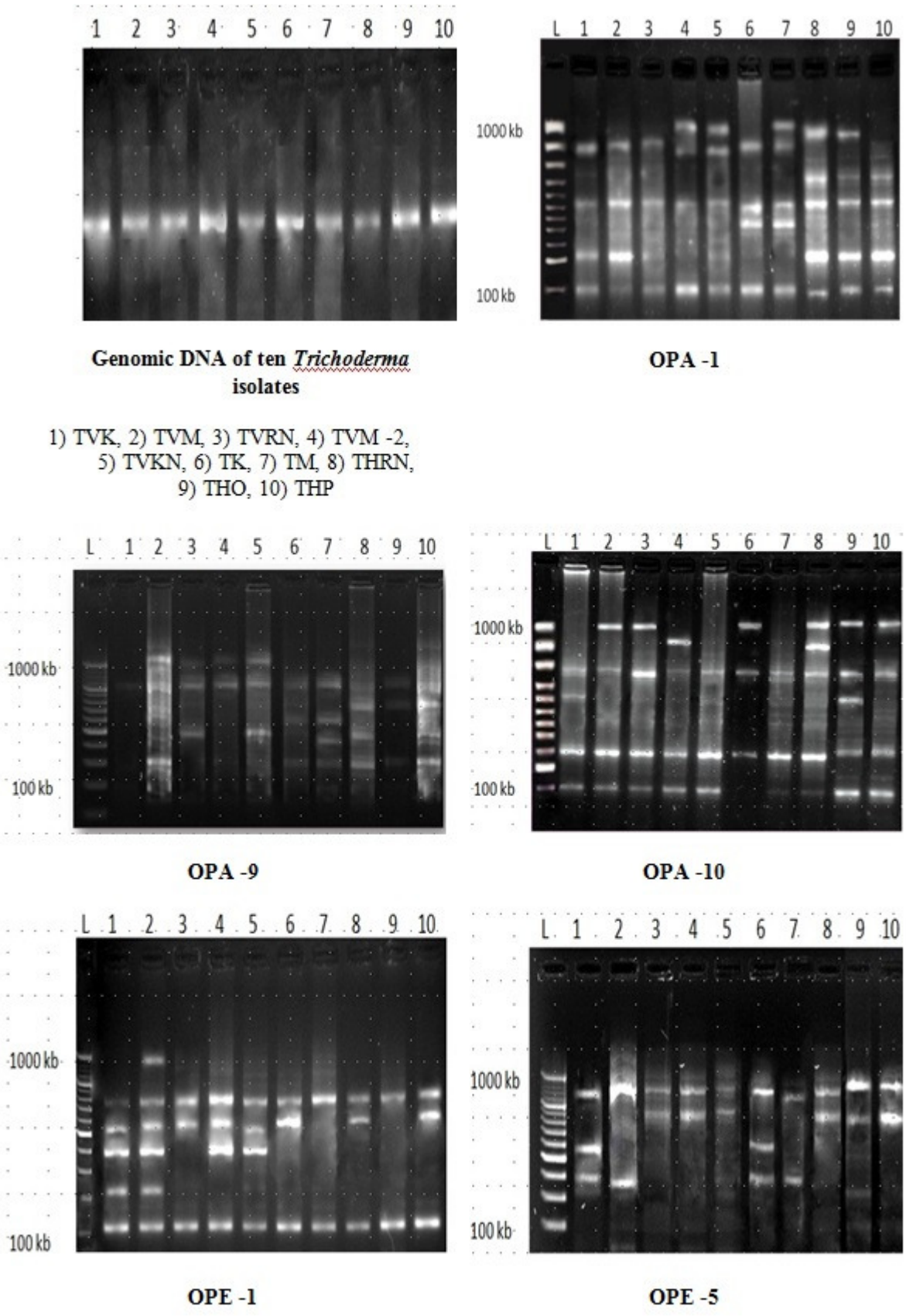

Fig. 1: Genetic diversity of ten isolates of *Trichoderma* sp. using RAPD