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In order to utilize the full potential of *Trichoderma* species in specific applications, precise identification and characterization was vital. The morphological study was based on different parameters like colony growth, colony colour, and pigmentation. Molecular techniques are important analytical tool to characterize genetic variability and diagnosis of microbial population. Molecular variability among the isolates of *T. longibrachiatum* was studied by using Random Amplified Polymorphic DNA (RAPD). The ITS-1 and ITS-4 universal primer were successfully used to amplify genomic DNA. Twenty (20) RAPD primers of OPA series were tested, of which 15 primers produced 113 scorable bands among them 110 bands were polymorphic and level of polymorphism was up to 97.34 %. Three primers namely OPA-8, OPA-9 and OPB-19 each showed one monomorphic band. Similarity coefficient ranged from 0.21 to 0.55, indicating the significant diversity among isolates. On the basis of dendrogram the tested culture were divided into 6 clusters, the cluster A consist TL-1 (Akola), TL-2 (Amravati) and TL-3 (Bhandara). Cluster B consist only one isolate i.e. TL-4 (Buldhana). Cluster C consists of TL-5 (Chandrapur). Cluster D consist of TL-6 (Gadchiroli). TL-7 (Gondia) and TL-8 (Washim) isolate are in cluster E and TL-9 (Yeotmal) isolate was in separate cluster F. It indicates that there is genetic diversity among the isolates of *T. longibrachiatum* isolated from different districts of Vidarbha region.

Key words: *T. longibrachiatum,* morphological, molecular variation, RAPD markers

INTRODUCTION

Identification based on morphological characters consent a relatively simple method for classification of *Trichoderma* as genus, but the species perceptions are complex to construct and there is considerable confusion over the application of specific names. Pioneer workers in research on *Trichoderma* like have observed certain cultural characters that could be used for identification and description of the species. In order to utilize the full potential of *Trichoderma* species in specific applications, precise identification and characterization of these fungi is vital . The *Trichoderma* isolates are differentiated by mycelial growth rate and colony appearance, as well as microscopic morphological features, including phialides and phialospores . Srivastava *et al*. (2012) have collected many isolates of *Trichoderma atroviride* obtained from rhizospheric soils from different parts of Uttar Pradesh which have brought attention due to their highly antagonistic activity. Shahid *et al*. (2013) have studied seven different strains of *Trichoderma which were* isolated from wilt infected leguminous crops of an Indian state and tested their antagonistic activity against *Fusarium* (soil borne pathogen) which is expressed as a zone of inhibition in the culture plates.

Molecular characterization of potential biocontrol agents using Random Amplified Polymorphic DNA (RAPD) and Internal Transcribe Spacer-Polymerase Chain Reaction (ITS-PCR) helps to determine the diversity and identification. The advantages of the RAPDs are the requirement for small amount of DNA (5-20 ng), single short (9 to 10 bp) primers of arbitrary sequence, and the rapidity to screen for polymorphisms, the efficiency to generate a large number of markers for genomic mapping and the potential automation of the technique.

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Gupta *et al.* (2010) have studied RAPD-PCR analysis of seven *Trichoderma* species isolates and their *in vitro* antagonism against wilt pathogens of *Psidium guajava* L. viz. *Fusarium oxysporum* f. sp*. psidii* (*F. o. f.* sp. *psidii*) and *Fusarium solani*. Out of 10 RAPD oligonucleotides (OPA 1–OPA 10) tested, seven markers OPA 1, 3, 5, 7, 8, 9 and 10 efficiently differentiate the isolates of *Trichoderma* and show reproducible banding patterns. A total of 248 bands are obtained from these markers along with a 61.84% per cent similarity among the seven isolates of *Trichoderma*. Kumar *et al*.(2011) also have observed twelve isolates belonging to *Trichoderma harzianum* and *Trichoderma viride* and assess for their mycoparasitic effect on phytopathogens, *Pythium aphanidermatum* and *Sclerotinia sclerotiorum.*

The research aimed at determining morphological characteristic of potential fungal antagonists and identification of the antagonists is conducted, Further *Trichoderma* spp. are difficult to distinguish morphologically, so molecular methods including DNA sequencing and genealogical concordance phylogenetic species recognition using several unliked genes are needed to give accurate identification of *Trichoderma* spp*. (*Gherbawy *et al.* 2014). Gurumurthy *et al.* (2013) also have studied molecular characterization of the promising bio-control agents adopting Random Amplified Polymorphic DNA (RAPD) analysis which helps to determine the diversity and identification. In this endevour attempts have been made to study the morphological and colony characteristic of *Trichoderma longibrachiatum* and to determine the molecular diversity among isolates of *Trichoderma longibrachiatum* by using RAPD markers.

MATERIALS AND METHODS

Several types of glass wares viz., glass Petriplates, conical flasks, test tubes, beakers, glass rod, coverslips, slides, micropipettes, polypropylene centrifuge tube, measuring cylinder Ocular and stage micrometer were used. Standard laboratory equipment's used for different experiments were Autoclave, Hot Air Oven, Laminar Air Flow, Digital weighing balance (Wensar HBT 516), BOD incubator, Research microscope, Centrifuge (Eppendorf 5418), PCR (Eppendorf), Gel electrophoresis unit(Genexy, Scie-Plas), Microwave oven, and Gel Documentation unit, etc.

Morphological identification was done based on cultural (colony and growth rate) characterization and microscopic observation. *Trichoderma longibrachiatum* isolates were sub-cultured from slants to PDA Petriplates and incubated at 28ºC for 24-48 hr. After 2 days when the colonies were visibly growing, but before conidial production, a 5 mm-diameter mycelia disc were cut from actively growing edge of the colony and inoculated at the center on all freshly prepared potato dextrose agar (PDA). Nine isolates of *Trichoderma longibrachiatum* were screened for molecular variability using Random Amplified Polymorphic DNA (RAPD). Genomic DNA was isolated from the nine selected isolates by the cetyldimethylethyl ammonium bromide (CTAB) method with some modifications. Replications were maintained in all cases.

RESULTS AND DISCUSSION

Morphological characters of Trichoderma isolates

Trichoderma isolates maintained on the PDA medium showed variation. The morphological studies were based on different parameters like colony growth, colony colour, and pigmentation and the data are given in Table 1, (Fig. 1 and 2).

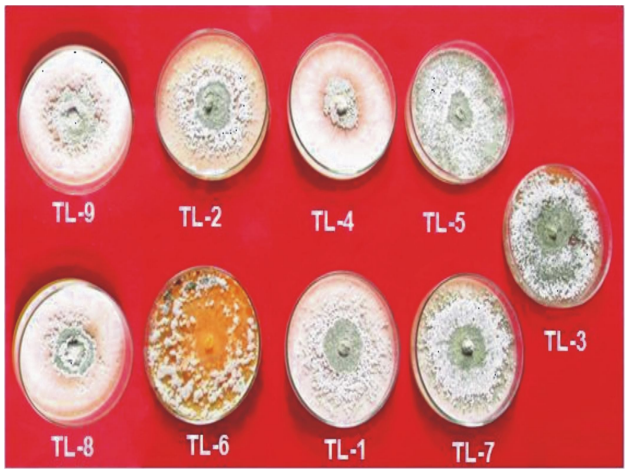

Fig. 1 : Morphological colonies of *Tricnoderma longibrachiatum*

Molecular variation among the isolates of Trichoderma longibrachiatum by using RAPD Markers

Among 20 primers screened 15 primers produced reproducible and scorable bands with high degree of polymorphism. Out of these 15 primers, 12

Table 1 : Morphological characterization of *Trichoderma longibrachiatum* isolates

Isolate name	Colony growth (mm) on 7th DAI	Colony colour	Pigmentation	Phialides	Conidia		Sporulation
					Shape	Size(L × B)	
TL-1 (Akola)	88.30	Whitish to light green	Creamy white	Elongated and cylindrical	Oval	1.96-2.87 x 0.95-1.50 µm.	++++
TL-2 (Amravati)	88.50	Light to dark green	Pale yellow	Lageniform or bottle shaped	Sub globose to oval	1.93-2.04 x 1.03-1.23 µm	++
TL-3 (Bhandara)	88.33	Light olive green with yellow tinge	Colorless to yellowish	Long cylindrical	Sub globose	1.95- 2.03 x 0.95-1.11 µm	+
TL-4 (Buldhana)	80.30	Cottony white	Colorless to creamy white	Cylindrical or slightly inflated	Globose	2.02 - 2.42 x 2.01 - 2.32 µm.	+++
TL-5 (Chandrapur)	90.00	Light green to dark green	Pale yellowish green	Ampuli form	Globose to slightly oval	2.09-2.99 x 2.08-2.29 µm	++++
TL-6 (Gadchiroli)	80.00	Whitish to light green	Dark yellow to dirty yellow	Cylindrical shape	Roundish	1.75- 2.20 x1.74-2.20 µm,	++++
TL-7 (Gondia)	90.00	Light olive green	Whitish to pale green	Lageniform	Sub-cylindrical	2.29 - 3.06 x 1.83 – 2.05 µm	+++ ++++
TL-8 (Washim)	86.35	Whitish to pale greenish	Creamy whitish	Cylindrical or slightly inflated	Oval	1.90 – 2.86 x 1.35 –2.02 µm	
TL-9 (Yeotmal)	90.00	Cottony white	Pale yellowish	Lagenini form	Oval	2.91- 3.10 x 1.86-2.59 µm	+++

L= Length , B = Breadth
+ : Discrete sporulation ++ : Moderate sporulation+++ : Maximum sporulation ++++ : Good and Compact sporulation

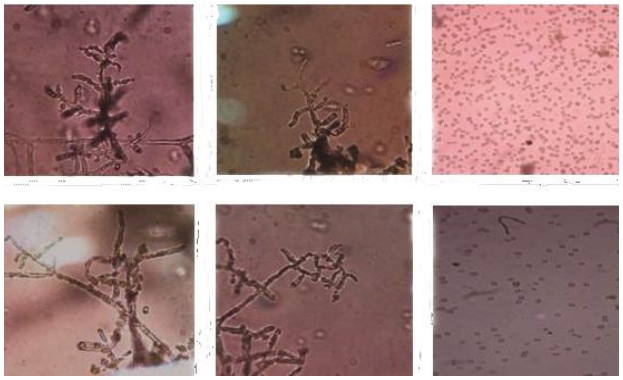

Fig. 2 : Microscopic photographs of *Trichoderma longibrachiatum*

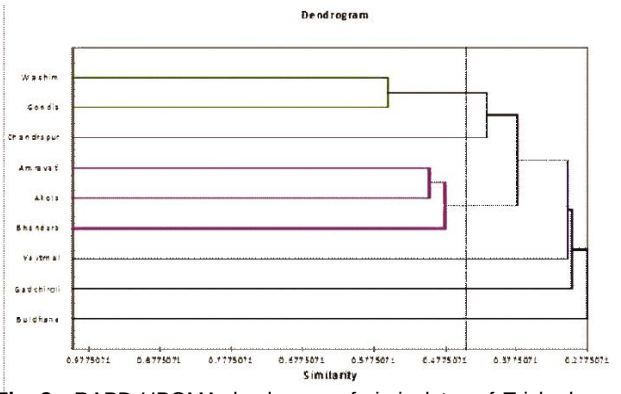

Fig. 3 : RAPD UPGMA dendogram of six isolates of *Trichoderma viride* on Jaccard's similarity

primers showed all polymorphic bands, whereas remaining 3 primers namely OPA-8, OPA-9 and OPA-19 each showed one monomorphic band. A total 113 amplicons were obtained with the 15 primers. Out of 113 bands, 110 were found to be polymorphic and the level of polymorphism was 97.34 per cent (Table 2).

Morphological variation like growth, colony colour, pigmentation, conidial size and shape and phialides shape were recorded among the nine isolates of *T. longibrachiatum*. The colony colour was from whitish green to olive green, creamy to dirty yellow pigmentation was observed. Conidia globuse to

Table 2 : Per cent polymorphism shown by RAPD primers

Primers	Total amplicons	Polymorphic amplicons	Monomorphic amplicons	% Polymorphism
OPA-1	05	05	0	100
OPA-2	09	09	0	100
OPA-3	10	10	0	100
OPA-4	12	12	0	100
OPA-5	06	06	0	100
OPA-8	08	07	1	87.5
OPA-9	07	06	1	85.71
OPA-10	03	03	0	100
OPA-12	07	07	0	100
OPA-15	08	08	0	100
OPA-18	10	10	0	100
OPB-19	03	02	1	66.66
OPB-5	15	15	0	100
OPB-17	04	04	0	100
OPB-20	06	06	0	100
Total	113	110	3	97.34

sub globuse, size of conidia was in the range of 1.75 to 2.91µm length and 0.95 to 2.59 breadths. Phialides shape was lageniform to cylindrical. The spore count was least to moderate. Morphological variation within the *T. longibrachiatum* isolates collected from different places. Twenty RAPD primers were used for the molecular diversity analysis among nine *Trichoderma longibrachiatum.* Among 20 primers screened 15 primers produced reproducible and scorable bands with high degree of polymorphism. Out of these 15 primers, 12 primers showed all polymorphic bands, whereas remaining 3 primers namely OPA-8, OPA-9 and OPA-19 each showed one monomorphic band.

A total 113 amplicons were obtained with the 15 primers. Out of 113 bands, 110 were found to be polymorphic and the level of polymorphism was 97.34 per cent. Similarity coefficient for RAPD marker ranged from 0.21 to 0.55, indicating the significant diversity among isolates. The maximum base pair band was 6500bp and the minimum base pair band produced was 200bp.

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