VARIABILITY STUDY OF ISOLATES OF *TRICHODERMA* SPECIES

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ABSTRACT

Biological control of the pathogen has gained importance as a component of Integrated Disease Management for sustainable agriculture, as it is long-lasting and eco-friendly. The potential value of genus Trichoderma as a bioagent was also reported. He observed Trichoderma lignorum (Tode) Harz as a parasite with the hyphae coiling around and killing Rhizoctonia solani Kuhn. Seven isolates of Trichoderma harzianum, five of Trichoderma viride, four of Trichoderma koningii, three of Trichoderma aureoviride and two of Trichoderma pseudokoningii were gained. The results of morphological characterization of isolates, which accomplished on the basis of colony growth on PDA after 72 hours, colony colour, odour from colony, change of media colour, conidial shape and colour and phialides shape. The colony growth of isolates of Trichoderma harzianum (48-55 mm), Trichoderma koningii (44-56 mm), Trichoderma pseudokoningii (51-55 mm) and Trichoderma viride (37-46 mm) was comparatively rapid than isolates of Trichoderma aureoviride (25-30 mm) on potato dextrose agar medium. Shape of the conidia was globose, sub-globose, ellipsoidal or obovoid and conidia were of some shade of green or greenish yellow. The shape of phialides was typically flask shaped in T. harzianum, cylindrical in T. viride, flask shaped in T. koningii, typically cylindrical in T. pseudokoningii and horn shaped in T. aureoviride. There was no distinct variation observed within isolates of same species.

KEY WORDS: *Isolation, Trichoderma species, variability,*

INTRODUCTION

Most of the plant pathogens which cause important diseases in cereals, oilseeds, pulses, vegetables and fruits crops *etc.*, are seed borne and soil borne in nature. Repeated and monoculture of a crop in the same piece of land resulted in heavy incidence of soil borne disease due to build up of a high inoculums of the pathogen. Chemical control is one of the classical methods to disease management and the disease is effectively controlled by seed dressing, foliar sprays as well as soil application of various fungicides (Dev and Mary, 1986). Unfortunately the indiscriminate uses of potentially hazardous fungicides possess a serious threat to the environment. The buildup of resistance by the pathogens and the non target effect of fungicides on beneficial organisms, such as nitrogen fixers, residential antagonists and mycorrhizal fungi are the other disadvantages of the application of fungicides (Papavizas, 1985). In order to tackle these problems, integrated disease management programmes amalgamating safe, effective and compatible strategies are essential. Biological control of the pathogen has gained importance as a component of Integrated Disease Management for sustainable agriculture, as it is long-lasting and eco-friendly (Mukhopadhyay, 1987). The

potential value of genus *Trichoderma* as a bioagent was first reported by Weindling in 1932. He observed *Trichoderma lignorum* (Tode) Harz as a parasite with the hyphae coiling around and killing *Rhizoctonia solani* Kuhn. He also reported that *Sclerotium rolfsii* Sacc. and the species of *Pythium*, *Phytophthora* and *Rhizopus* were susceptible to mycoparasitism by *Trichoderma viride*. Nine species *viz.*, *T. harzianum* Rifai; *T. longibrachiatum* Rifai; *T. aureoviride*; *T. pseudokoningii* Rifai; *T. piluliferum* Webster and Rifai; *T. polysporum* (Link ex Pers) Rifai ,*T. hamatum* (Bon) Bain. *T. koningii* and *T. viride* Pers. Gray reported as a biocontrol agent (Rifai, 1969). Later on, two new species *viz.*, *T. citrinoviride* Bissett and *T. atroviride*, Bissett were also recorded (Bissett, 1984).

MATERIALS AND METHODS

1. Collection of Soil and Plant Samples

Fifty soil and roots samples were collected from established castor field plots of different locations of Patan and Banaskantha districts, where the castor is commonly cultivated (Table 1). The soil types of Banaskantha is loamy sand (49.17 % coarse sand, 38.3 % fine sand, 8.52 % silt and 4.01 % clay), whereas Patan soil is sandy loam type (39.0 % coarse sand, 50.3 % fine sand, 7.7 % silt and 3.0 % clay).

Healthy plant of castor (*Ricinus communis* L.) of 60 to 75 days growth were carefully uprooted along with adhering soil and was carried to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizosphere fungi. Soil sample of each plot was used for analysis of organic carbon content, concentration of hydrogen ion (pH) and electric conductivity (EC) studies.

2. Isolation of Isolates of Trichoderma spp.

The isolation of *Trichoderma* species were made by soil dilution plate technique (Johnson and Curl, 1972). From each soil sample, 10 g of closely associated rhizosphere/rhizoplane soil was mixed thoroughly with 90 ml sterile distilled water to prepare stock solution and serially diluted up to 10^{-5} (Harris and Sommers, 1968). One ml of suspension from the soil dilutions were plated on solidified *Trichoderma* selective medium (TSM) and gently shaken to spread evenly. These Petriplates were incubated at $28^{o} \pm 1^{o}$C temperature for one week with periodic observation for the development of colonies of *Trichoderma* species. The early growing colonies of different morphology were examined critically, picked-up and transferred to potato dextrose agar slants. Finally the cultures were purified and maintained on PDA slants at low temperature (5^{o}C) in refrigerator in the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, for further activities.

2.1 Identification

The isolates were identified with the help of their microscopic structure and compared with taxonomic keys of *Trichoderma* species (Rifai, 1969 and Cook and Baker, 1983). For further confirmation, all the isolates were identified at Maharana Pratap University of Agriculture and Technology, Udaipur (Rajasthan).

2.2 Variability study

To study the variability of isolates of *Trichoderma* species, the 5 mm discs of each isolate inoculated aseptically in centre of 90 mm petridishes containing 20 ml of potato dextrose agar (PDA) medium. Inoculated plates were incubated at $28^{o} \pm 1^{o}$C for 10 days. The observations regarding various colony characters were made after 48 h of incubation and subsequently recorded at 24 h intervals up to 10 days. The radial growth of each isolate also measured at the interval of 12 h after inoculation up to 3 days.

RESULTS AND DISCUSSION

Isolation of Trichoderma spp. Isolates from Rhizosphere

On the basis of early growing colonies of different morphology, twenty one isolates of *Trichoderma* spp. were obtained on *Trichoderma* Selective Medium (TSM) from the rhizosphere of castor plant by soil dilution plate technique (10^{-5} dilution) after incubation period of one week at $28^{o} \pm 1^{o}$C. Out of 20 soil samples collected from ten villages of Patan district, 8 isolates of *Trichoderma* species were obtained, whereas 13 isolates were gained from 30 soil samples from seven villages of Banaskantha district (Table 2). Over all, 21 isolates obtained from 50 soil samples. The results are in accordance with the methodology adopted by Sivan and Chet (1989), D'souza *et al.* (2001), Vyas and Mathur (2002), Kavitha *et al.* (2004), Sangle and Bambawale (2004) and Kapil and Kapoor (2005).

The results regarding number of isolates obtained from the Patan and Banaskantha districts (Table 2) reflected that isolates recovered per cent was 40.0 and 43.3 of Patan and Banaskantha districts, respectively, as their soil was sandy loam soil. Khan and Sinha (2005) reported that soil type might be one of the important factors in determining the activities of *Trichoderma*. They observed the low activities of bioagent in sandy loam soil probably due to low water retaining capacity of the soil in comparison to clay loam or silt clay loam soil.

Identification of isolates

Various species of *Trichoderma* were identified with the help of their morphology and microscopic structure. Out of twenty one isolates, seven isolates of *Trichoderma harzianum,* five of *Trichoderma viride,* four of *Trichoderma koningii,* three of *Trichoderma aureoviride* and two of *Trichoderma pseudokoningii* were gained (Table 3).

All the cultures were also identified at Maharana Pratap University of Agriculture and Technology, Udaipur (Rajasthan) for further confirmation. Thereafter, isolates were designated as per respective species (Table 4).

Variability study

To study the variability of isolates of *Trichoderma* species, the 5 mm discs of each isolate inoculated aseptically in centre of 90 mm petridishes containing 20 ml of potato dextrose agar (PDA) medium. Inoculated plates were incubated at $28^{o} \pm 1^{o}$C for 10 days. The observations regarding various colony characters were made after 48 h of incubation and subsequently recorded at 24 h intervals up to 10 days. The radial growth of each isolate also measured at the interval of 12 h after inoculation up to 3 days.

Variability study of isolates of Trichoderma species

As is usually the case species of *Trichoderma* have defined on the basis of their morphology. Morphological characters used in species recognition in *Trichoderma* have been outlined by Rifai (1969) and Bissett (1984). The results of morphological characterization of isolates, which accomplished on the basis of colony growth on PDA after 72 hours, colony colour, odour from colony, change of media colour, conidial shape and colour and phialides shape are presented in Table 5. The colony growth of isolates of *Trichoderma harzianum* (48-55 mm),*Trichoderma koningii* (44-56 mm), *Trichoderma pseudokoningii* (51-55 mm) and *Trichoderma viride* (37-46 mm) was comparatively rapid than isolates of *Trichoderma aureoviride* (25-30 mm) on potato dextrose agar medium. The change of media colour was only observed in isolates of *T. harzianum*. Samuels (1996) reported that characteristics of colonies grown on agar media are subtle. Most *Trichoderma* species grow rapidly, when compared to media such as potato dextrose or oatmeal agar where conidial production is generally much more profuse. However, diffusable pigment forms more reliably on potato dextrose agar than cornmeal agar with dextrose or oatmeal agar, produced distinctive bright yellow-green pigment in the reverse on solid media.

Colony colour was varies from whitish green to dull dark green. Some cultures of *Trichoderma* develop sweet coconut odours, but such odour was not detected here from any cultures of isolates. The coconut odour produced by some strains of *T. viride* and *T. harzianum* has been attributed to an antifungal unsaturated pyrone, 6-pentyl-α-pyrone (Claydon *et al.,* 1987).

Shape of the conidia was globose, sub-globose, ellipsoidal or obovoid and conidia were of some shade of green or greenish yellow. The shape of phialides was typically flask shaped in *T. harzianum*, cylindrical in *T. viride* flask shaped in *T. koningii*, typically cylindrical in *T. pseudokoningii* and horn shaped in *T. aureoviride*. There was no distinct variation observed within isolates of same species. Morphological characters were continually variable and that there is no way of knowing the degree of variation tolerable within an individual species (Samuels, 1996).

CONCLUSION

Seven isolates of *Trichoderma harzianum,* five of *Trichoderma viride,* four of *Trichoderma koningii,* three of *Trichoderma aureoviride* and two of Trichoderma *pseudokoningii* were gained. The results of morphological characterization of isolates, which accomplished on the basis of colony growth on PDA after 72 hours, colony colour, odour from colony, change of media colour, conidial shape and colour and phialides shape. The colony growth of isolates of *Trichoderma harzianum* (48-55 mm), *Trichoderma koningii* (44-56 mm), *Trichoderma pseudokoningii* (51-55 mm) and *Trichoderma viride* (37-46 mm) was comparatively rapid than isolates of *Trichoderma aureoviride* (25-30 mm) on potato dextrose agar medium. Shape of the conidia was globose, sub-globose, ellipsoidal or obovoid and conidia were of some shade of green or greenish yellow. The shape of phialides was typically flask shaped in *T. harzianum*, cylindrical in *T. viride* flask shaped in *T. koningii*, typically cylindrical in *T. pseudokoningii* and horn shaped in *T. aureoviride*. There was no distinct variation observed within isolates of same species.

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Table 1 : Samples' details (Name of village and castor hybrids grown)

Sr. No.	Name of Village	Castor Hybrids/ Varieties	Sr. No.	Name of Village	Castor Hybrids/ Varieties
[A] Patan District :					
1.	Anavada	GCH 2	11.	Paldi	GCH 4
2.	Anavada	GCH 2	12.	Paldi	GCH 2
3.	Anavada	GCH 4	13.	Patan	GCH 4
4.	Brahmanvada	GCH 4	14.	Patan	GCH 4
5.	Brahmanvada	GCH 2	15.	Rajpur	GCH 5
6.	Charup	GCH 5	16.	Rajpur	GCH 4
7.	Charup	GCH 4	17.	Vadu	GCH 2
8.	Khado	GCH 4	18.	Vadu	GCH 4
9.	Matarvadi	GCH 4	19.	Vagdod	GCH 5
10.	Matarvadi	GCH 4	20.	Vagdod	GCH 4
[B] Banaskantha District :					
1.	Chandisar	Sagar	16.	Sardarkrushinagar	GCH 5
2.	Chandisar	Vijay	17.	Sardarkrushinagar	GCH 4
3.	Dantiwada Colony	GCH 5	18.	Sardarkrushinagar	GCH 4
4.	Dantiwada Colony	Sagar	19.	Sardarkrushinagar	GCH 5
5.	Dantiwada Colony	GCH 4	20.	Sardarkrushinagar	GCH 4
6.	Deesa	Vijay	21.	Sardarkrushinagar	GCH 4
7.	Deesa	Dantiwada	22.	Sardarkrushinagar	GCH 4
8.	Ganeshpura	GCH 4	23.	Sardarkrushinagar	GCH 5
9.	Rasana	Vijay	24.	Sardarkrushinagar	GCH 6
10.	Sardarkrushinagar	GCH 4	25.	Sardarkrushinagar	JI 17
11.	Sardarkrushinagar	GCH 4	26.	Sardarkrushinagar	GCH 6
12.	Sardarkrushinagar	GCH 5	27.	Sardarkrushinagar	GCH 5
13.	Sardarkrushinagar	GCH 4	28.	Sardarkrushinagar	GCH 4
14.	Sardarkrushinagar	GCH 5	29.	Vaghrol	GCH 5
15.	Sardarkrushinagar	GCH 5	30.	Vaghrol	GCH 4

Table 2 : District wise number of isolates obtained

Sr. No.	Name of District	Number of Samples Collected	Number of Isolates Obtained	Isolates Obtained (%)
1.	Patan	20	08	40.00
2.	Banaskantha	30	13	43.33
Total		50	21	42.00

Table 3 : Details of number of isolates obtained

Sr. No.	Name of District	No. of Samples Collected	Total Isolates Obtained	Isolates				
				Th	Tv	Tk	Ta	Tpk
1.	Patan	20	8	3	1	2	1	1
2.	Banaskantha	30	13	4	4	2	2	1
Total		50	21	7	5	4	3	2

Table 4 : Designation of isolates of *Trichoderma* species

Sr. No.	Place From Isolate Obtained	Isolates of	Isolate Designated As
1.	Patan	*T. harzianum*	Th_1
2.	Anavada	*T. harzianum*	Th_2
3.	Charup	*T. harzianum*	Th_3
4.	Sardarkrushinagar	*T. harzianum*	Th_4, Th_5
5.	Chandisar	*T. harzianum*	Th_6
6.	Deesa	*T. harzianum*	Th_7
7.	Khado	*T. viride*	Tv_1
8.	Sardarkrushinagar	*T. viride*	Tv_2
9.	Ganeshpura	*T. viride*	Tv_3
10.	Chandisar	*T. viride*	Tv_4
11.	Deesa	*T. viride*	Tv_5
12.	Matarvadi	*T. koningii*	Tk_1
13.	Sardarkrushinagar	*T. koningii*	Tk_2
14.	Rajpur	*T. koningii*	Tk_3
15.	Vaghrol	*T. koningii*	Tk_4
16.	Patan	*T. aureoviride*	Ta_1
17.	Chandisar	*T. aureoviride*	Ta_2
18.	Ganeshpura	*T. aureoviride*	Ta_3
19.	Brahmanvada	*T. pseudokoningii*	Tpk_1
20.	Sardarkrushinagar	*T. pseudokoningii*	Tpk_2

Table 5 : Characterization of *Trichoderma* spp. Isolates

Isolates	Characters						
	Colony Growth on PDA After 72 hrs (mm)	**Colony Colour**	**Odour from Colony**	**Change of Media Colour**	**Conidia**	**Conidial Colour**	**Shape of Phialides**
T. harzianum							
Th_1	53-55	Whitish green to dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Yellow green to dark green	Typically flask shaped
Th_2	51-53	Green to dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Yellow green to dark green	Typically flask shaped
Th_3	48-55	Green to dark green	Not detected	Whitish yellow pigmentation	Globose to Sub-Globose	Deep green	Typically flask shaped
Th_4	50-54	Whitish green to dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Dark green	Typically flask shaped
Th_5	51-57	Dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Dark green	Typically flask shaped
Th_6	54-57	Dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Dark green	Typically flask shaped
Th_7	51-55	Dark green	Not detected	Yellow pigment	Globose to Sub-Globose	Yellow green to dark green	Typically flask shaped
T. viride							
Tv_1	38-42	Dark bluish green	No odour	Absence of discolouration	Ellipsoidal to Sub-Globose	Deep green	Cylindrical
Tv_2	37-45	Dark bluish green	No odour noted	Absence of discolouration	Ellipsoidal to Sub-Globose	Deep green	Cylindrical
Tv_3	40-42	Dark bluish green	No odour noted	Absence of discolouration	Sub-Globose	Deep green	Cylindrical
Tv_4	39-45	Dark green	No noted	Absence of discolouration	Ellipsoidal to Sub-Globose	Deep green	Cylindrical
Tv_5	40-46	Dark bluish green	No noted	Absence of discolouration	Ellipsoidal to Sub-Globose	Deep green	Cylindrical

T. koningii							
Tk_1	48-52	Dull to dark green	Not detected	No colour change	Oblong to Ellipsoidal	Green	Flask shaped
Tk_2	45-50	Dark green	Not detected	No colour change	Oblong to Ellipsoidal	Green	Flask shaped
Tk_3	44-48	Dark green	Not detected	No colour change	Oblong to Ellipsoidal	Green	Flask shaped
Tk_4	47-50	Dark green	Not detected	No colour change	Oblong to Ellipsoidal	Green	Flask shaped
Ta_1	28-30	Yellowish green	No noted	Absence of discolouration	Obovoid	Dull grass green	Horn shaped
Ta_2	25-29	Yellowish green	No noted	Absence of discolouration	Obovoid	Dull grass green	Horn shaped
Ta_3	27-30	Yellowish green	No noted	Absence of discolouration	Obovoid	Dull grass green	Horn shaped
T. pseudokoningii							
Tpk_1	51-53	Whitish to pale yellowish	Odour indistinct	Pale yellow	Cylindrically oblong	Green dilute	Typically cylindrical
Tpk_2	52-55	Whitish to pale yellowish	Odour indistinct	Pale yellow	Cylindrically oblong	Green dilute	Typically cylindrical

[MS received: January 15, 2013] *[MS accepted: March 06, 2013]*