Vegetative compatibility and strain improvement of some Egyptian *Trichoderma* isolates

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Abstract: Vegetative compatibility among 13 isolates of *Trichoderma* representing seven species were evaluated *in vitro* in order to provide information on the use of multiple of *Trichoderma* as biological control agents. The study indicated high degree of vegetative incompatability. The incompatabible interactions represented 80% of the total number of interactions and characterized by zone of inhibition, overgrowth, intermingling, demarcation lines and ridges of conidia which recorded the following occurrence 27, 21,19.15, and 15% respectively. Concerning to compatible interactions which represented 20%, their incidence between self pairings was more frequently (14%) than between non-self pairings (6%). *Trichoderma* isolates showed non-self compatible interactions were utilized to induce strain improvement through the formation of somatic hybrids by co-culturing. Somatic recombination trial indicated that all the tested fusants were morphologically similar to one of parental species, Variable changes in the mycelial growth, sporulation and pigmentation were observed as well as in biological activity, but no significant increase in the activity was accomplished after fusion. The hybrid being generally less active than their parental species.

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1.Introduction

The genus *Trichoderma* comprises a great number of fungal species that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. *Trichoderma* species exert biocontrol effect against some fungal phytopathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, promoting plant growth and induction of plant defensive mechanisms or directly by mechanisms such as mycoparasitism and antibiosis **(Benítez *et al*., 2004)**.

Strain characterization and identification at the species level is an important factor in monitoring of microorganisms and considered as the first step in utilizing the full potential of microorganisms in specific applications. In fact, this is especially important in case of *Trichoderma* species which are widely used in the biocontrol of plant-pathogens.

Malik and Vilgalys (1999) demonstrated that vegetative compatability groups may differ from one another at one, some, or all of the vegetative incompatibility loci that are dispersed throughout the genome and responsible for the vegetative compatability group phenotype. Two isolates are incompatible if they have different alleles at one or more vegetative incompatibility loci. Differences in population structure and history potentially can lead to large differences in the relationship between vegetative compatibility and genetic uniqueness, even within a single species.

Reaves and Crawford (1994) studied the colony interactions among 15 isolates representing seven species of *Trichoderma*. Interactions characterized by zones of inhibition, demarcation lines, ridges of conidia, overgrowth, intermingling, anastomosis, and hyphal coiling were recorded among the isolates. **Gómez *et al.* (1997)** carried out direct confrontation assays of ten isolates of *T. harzianum* previously classified into groups by electrophoresis and analysis of randomly amplified polymorphic DNAs. Direct confrontation assays using isolates of the same group showed compatible interactions. Whereas, the same experiment carried out with isolates of different groups showed an incompatible interaction characterized by an area of cell damage. Microscopic observation of the compatible interactions showed hyphal fusions between the isolates, similar to those described for vegetative compatible groups in other fungi.

Hyphal anastomosis is a prerequisite for the establishment and development of heterokaryons. It consists of fusion between vegetatively compatible hyphae, translocation of one or more nuclei into fused cells, and compatible heterokaryotic state. Therefore, anastomosis is the mechanism of somatic cell fusion and exchange of genetic material in fungi **(Anderson, 1982 and Rayner and Boddy, 1988)**. Heterokaryons were obtained by hyphal anastomosis in *Trichoderma pseudokoningii* using strains with morphologic and double auxotrophic genetic markers. Stable haploid recombinants were detected in monosporic colonies

derived from heterokaryons **(Bagagli *et al*., 1995)**. Furthermore, **Barcellos and Pizzirani-Kleiner (2003)** conducted crossing experiments *via* hyphal anastomosis between two strains of *Trichoderma pseudokoningii* to characterize the somatic recombination process in this specie. Four crossings were made and sixty-eight recombinant colonies were analyzed. Fifty-eight heterokaryotic colonies were stable after four generations and the remainders were unstable, reverting to one of the parentals.

Aim of the work

In this study we aimed to use vegetative compatibility technique to assess self and non-self recognition ability of the different isolates which is very important factor which may control the application of *Trichoderma* in combined formulation forms in future. In addition, the possibility of strain improvement was estimated by subjecting the non-self compatible isolates for somatic recombination.

2.Material and Methods

Tested fungi

Thirteen *Trichoderma* isolates listed in Table (1) and four phytopathogens namely *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kühn, *Fusarium oxysporum* Schlect. f. sp. *lycopersici* Sacc. and *Pyricularia oryzae* Cav. were used in the present study. These fungi were isolated, purified and identified. Isolated fungi were identified according to the description of **Gilman (1957)**, compendium of soil fungi **(Domsch *et al*., 1980)**, **Barnett (1960)**, **Booth (1977)** and **Singh (1982)**. The identified fungi were kept at 5ºC for further studies.

Vegetative Compatibility

Vegetative compatibility was determined according to the method of **Earnshaw and Boland (1997)**. Agar plugs were cut from the growing edge of a colony of each tested *Trichoderma* isolate and placed on opposite sides of Petri plates (90 mm diameter) containing potato dextrose agar. Cultures were allowed to grow at 25 °C in dark for 5 days, after which colony interactions were recorded. The following interaction types were recorded for *Trichoderma* in somatic confrontation cultures: (1) zone of inhibition—area with no mycelium between approaching colonies **(Haran *et al*., 1993)**; (2) intermingling—mycelia merged between colonies **(Sharland and Rayner, 1986)**; (3) demarcation line—a submerged pigmented line formed within the medium between colonies **(Reaves and Crawford, 1994)**; (4) ridge of conidia—abundant conidia formed where the mycelia of colonies met **(Reaves and Crawford, 1994)**; and (5) overgrowth—one colony overgrew the other and sporulated **(Goldfarb *et al*., 1989)**.

Strain improvement by somatic recombination

Trichoderma isolates showing non-self compatible interactions in the vegetative compatibility test were utilized in the formation of somatic hybrids by co-culturing method **(Rayner and Boddy, 1988)**.

Compatible pairs of *Trichoderma* isolates were grown together as long strip inocula on PDA for 5 days at 25 ºC in darkness. From areas of hyphal mixing, hyphal plugs (7 mm in diameter) were cut and transferred to PDA medium.

The inoculated plates were incubated at 25 ºC in darkness and monitored daily for growth. Recombinant colonies showing sparse sporulation and irregular growing borders with sectors formation were neglected while, colonies showed homogeneity in growth were selected as putative hybrids. The selected hybrids were propagated on PDA for four generations to test their stability. The resulted putative hybrids were subjected to morphological studies (mycelial growth, sporulation and pigmentation) on PDA. The biocontrol activity of the new hybrids was tested against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *lycopersici* and *Pyricularia oryzae* using dual culture technique. Strain improvement by somatic recombination was evaluated by comparing the results of morphological and biocontrol activity studies of each hybrid with that recorded for its parental isolates.

Morphological studies:

Mycelial growth of the investigated *Trichoderma* isolates was evaluated on different types of growth media including potato dextrose agar (PDA), Czapek-Dox agar and glucose-peptone agar medium. Agar plugs (5 mm diameter) of each tested Trichoderma isolate were taken from 7-day-old PDA was used to inoculate Petri dish (90 mm diameter) containing each of the tested growth media. Three replicates for each isolate were prepared for each tested growth medium. Plates were incubated at 25 °C under dark condition for 4 days, after which the colony diameters were measured. Sporulation was studied by inoculating each of the previous tested growth medium with a 5mm disk of each tested Trichoderma isolate. The plates were incubated for 7 days at 25°C under dark conditions. At the end of the incubation periode, 1 cm^2 agar discs were cut from the margin of the colony and transferred to a vail containing 10 ml of sterile distilled water. The suspension was shaked for 5 min, and density of spores/ml was counted by a heamocytometer according to Sharma (1989).Three plates were used for each treatment and the mean number of spores was calculated.

Pigmentation of the colony reverse of each *Trichoderma* isolate was inspected on the previously used media depending on the observations described by **Shalini *et al*. (2006)**. The inoculated plates were

incubated at 25 °C under dark condition for 4 days. Pigmentation was observed and marked daily during the incubation period as following; (a) No color in the medium is marked by (–), (b) Light color is marked by (+), (c) Moderate color is marked by (++) and (d) Dense color is marked by (+++).

Dual cultures interaction

The biological control activity of the parental Trichoderma isolate and the new hybrids and their putative hybirds were tested against phytopathogens; *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kühn, *Fusarium oxysporum* Schlect. f. sp. *lycopersici* Sacc. and *Pyricularia oryzae* Cav. by dual culture method described by **Dennis and Webster (1971b)** to screen out their antagonistic potential. Plates were inoculated with 7 days old culture discs (7 mm in diameter) of Phytopathogenic isolates at the peripheral of the plate surface 10 mm from the edge of the plat. A disc (7 mm diameter) of the putative hybrids and parental *Trichoderma* isolate being tested was placed 10 mm from the edge of the plate and positioned diametrically opposite to the pathogen. PDA plates inoculated with only a 7 mm diameter disc of each phytopathogen 10 mm from the edge of the plates were used as control treatments. Three replicates of each treatment were used. Plates were incubated at 25 °C under dark condition for 5 days. After complete growth of control plates, percentage of reduction in the mycelial growth was calculated according to the following formula adopted by **Ferreira *et al.* (1991)** as follows:

$$R = [(A-B)/A] \times 100$$

Where:

R= Percentage of growth reduction

A= Mycelial growth of the pathogenic fungus

B= Mycelial growth of the pathogenic fungus towards the antagonistic fungus

Statistical analysis

The result of vegetative compatibility studies and strain improvement are presented as mean ± SD (standard deviation) of three readings. The Statistical analyses were carried out using SDS version 6.12.

3. Results and Discussion

1. Taxonomic identification of *Trichoderma* isolates

The data presented in Table (1) illustrate a list of the identified *Trichoderma* spp. which were isolated from different soil samples collected from Delta region of Egypt during the period from February 2006 to January 2007. Thirteen isolates of *Trichoderma* belonging to three taxonomic sections (*Trichoderma*, *Pachybasium* and *Longibrachiatum*) and under seven species were collected and identified. *Trichoderma* isolates were given the following codes T12, T13, T25, T26, T27, T28, T29, T45, T58, T61, T75, T79 and T84.

Table 1. Codes and taxonomic identification of *Trichoderma* isolates.

Isolate code	Taxonomic identification	
	Section	Species
T12	*Trichoderma*	*Trichoderma lignorum* Tode
T13		*Trichoderma koningii* Oud.
T26		
T84		
T27		*Trichoderma viride* Pers.: Fr.
T28		
T61		*Trichoderma harzianum* Rifai.
T75		
T45	*Pachybasium*	*Trichoderma hamatum* Bon.
T58		
T25	*Longibrachiatum*	*Trichoderma ressei* Simmons.
T79		*Trichoderma pseudokoningii* Rifai

2. Vegetative compatibility

All the tested *Trichoderma* isolates were subjected to vegetative compatibility study by pairing the isolates in all possible combinations on PDA medium, Tables (2 and 3). Compatible reactions were recorded for combinations showed no rejection sings. Incompatible reactions were invariably observed as presence of inhibition zone, ridge of conidia, intermingling, demarcation line and overgrowth (Plate1).

Compatible reactions were found to represent 20% of the total number of interactions, while incompatible interactions represented 80%. This high incidence of incompatibility ensures the genetic variability between the paired *Trichoderma* isolates.

The incidence of compatible interactions between self parings occurred more frequently (14%) than between non-self parings (6%), Table (2) Concerning to incompatible interactions, Inhibition zone was the most frequently observed represent 22% of the total number of incompatible interactions followed by overgrowth, intermingling, demarcation line formation and ridge of conidia which recorded occurrence 18, 15, 13 and 11%, respectively (Table3 and plate1).

Data in Table (3) indicate that there are obvious variable differences in the inward linear growth of *Trichoderma* isolates and the diameter of the interaction zone according to the tested isolates were grown in confrontation culture. The highest diameter of inhibition zone (autoinhibition) was (0.6 cm) which was recorded in four combinations; *T. koningii* (T13) against *T. harzianum* (T75), *T. viride* (T27) against *T. koningii* (T84), *T. hamatum* (T29) against *T. harzianum* (T75) and *T. hamatum* (T29) against *T.*

koningii (T84). Also, the least diameter of inhibition zones (0.2 cm) was recorded in four culture combinations including *T. lignorum* (T12) against *T. viride* (T28), *T. lignorum* (T12) against *T. hamatum* (T58), *T. lignorum* (T12) against *T. koningii* (T84) and *T. koningii* (T26) against *T. viride* (T28). The variability in the diameter of the intermingling was more obvious; the broadest zone (2 cm) was recorded between *T. harzianum* (T61) and *T. pesudokoningii* (T79), while 0.3 cm was the least diameter of the intermingling and recorded between *T. koningii* (T13) and *T. harzianum* (T61). In case of the formation of ridge of conidia, the recorded diameters were ranged from 0.3 to 1.7 cm. The highest measurement was recorded between *T. lignorum* (T12) and *T. harzianum* (T75) followed by 1.4 cm which was recorded between *T. hamatum* (T58) and *T. koningii* (T84). The somatic interaction between *T. hamatum* (T29) against *T. harzianum* (T61) gave the least diameter (0.3 cm) recorded for the formation of ridge of conidia (Table3). According to our preliminary observations, the compatible interaction showed low incidence 20%, while 80% of the total number of interactions were observed to include sings of antagonism or incompatibility like presence of inhibition zone, ridge of conidia, intermingling, demarcation line and overgrowth. This is in accordance with an earlier report by **Reaves and Crawford (1994)** who studied colony interactions among 15 isolates of *Trichoderrna in vitro*. Interactions characterized by zones of inhibition, demarcation lines, ridges of conidia, overgrowth, intermingling, anastomosis, and hyphal coiling in self-pairings and intraspecific and interspecific pairings of the seven species were recorded.

Inhibition zone was the most frequently observed interaction and represent 22% of the total number of incompatible interactions followed by overgrowth, intermingling, demarcation line formation and ridge of conidia where their occurrence percentage were 18, 15, 13 and 12%, respectively. Zones of inhibition can be caused by diffusion of toxic metabolite(s) in advance of hyphae **(Dennis and Webster, 1971b)**. The presence of zones of inhibition in such frequency was considered to be as an indication for the probability of autoinhibition. **Reaves and Crawford (1994)** suggested that the presence of inhibition in such pairings could be correlated to strongly antagonistic action to pathogenic fungi in culture. In this context, the occurrence of demarcation line established the ability of the paired species to induce the accumulation of specific colored metabolites. The formation of ridges of conidia may be an indicative sing of a triggered response by each isolate to produce an abundance of conidia when physical contact is made between hyphae of different species of *Trichoderma*, whereas the intermingling interaction may be indicative of anstomosis between isolates **(Reaves and Crawford, 1994)**.

Table 2. Vegetative compatible interactions of the tested *Trichoderma* isolates after five days of incubation on PDA at 25°C.

Tested isolates	Number of isolates	Percentage
Self interactions		
T. lignorum against ***T. lignorum*** (T12) (T12)	13	14
T. koningii against ***T. koningii*** (T13) (T13)		
T. ressei against ***T. ressei*** (T25) (T 25)		
T. koningii against ***T. koningii*** (T26) (T26)		
T. viride against ***T. viride*** (T27) (T27)		
T. viride against ***T. viride*** (T28) (T28)		
T. hamatum against ***T. hamatum*** (T29) (T29)		
T. hamatum against ***T. hamatum*** (T45) (T45)		
T. hamatum against ***T. hamatum*** (T58) (T58)		
T. harzianum against ***T. harzianum*** (T61) (T61)		
T. harzianum against ***T. harzianum*** (T75) (T75)		
T. pseudokoningii against ***T. pseudokoningii*** (T79) (T79)		
T. koningii against ***T. koningii*** (T84) (T84)		
Non-self interactions		
T. ressei against ***T. koningii*** (T25) (T26)	5	6
T. ressei against ***T. viride*** (T25) (T27)		
T. koningii against ***T. viride*** (T26) (T27)		
T. koningii against ***T. hamatum*** (T26) (T45)		
T. viride against ***T. viride*** (T27) (T28)		

In addition, the data recorded for the inward mycelial growth of different isolates in somatic pairing cultures it is most likely that there are obvious variable differences in the inward linear growth of *Trichoderma* isolates according to the confronted isolates. These data were in agreement with the observations reported by **Gómez *et al.* (1997)**. In this context, the measures of the inward linear growth of *Trichoderma* isolates in confrontation pairings may be denotative for the possibility of combination during formulation of different *Trichoderma* isolates.

Considering the aforementioned results, combination of compatible isolates is possible, while the use of isolates showing inhibition sings is not recommended to avoid autoinhibition. With respect to the formation of demarcation line, ridge of conidia and intermingling, the presence of such interactions *in vitro* dose not hinder their practicability, but more studies *in vivo* are required to evaluate the impact of these interactions on biological control activity.

3.Strain improvement by somatic recombination

A trial for *Trichoderma* strain improvement was carried out by co-culturing the non-self compatible isolates of *Trichoderma* on PDA The hyphal mixing and anstomosis were the proposed bases of somatic recombination in this trial. The morphological characters and the biocontrol activity of the selected putative hybrids were studied and compared with the parental strains. All fusants were morphologically similar to one of the parental species. However, the results of the morphological study reveal variable changes in the mycelial growth, sporulation and pigmentation (Table 4).

Mycelial growth of all the resulted putative hybrids completely covered the surface of the cultured plates by the third day except H_b which showed high enhanced growth and covered the plate surface by the second day of incubation. Hybrids designated as H_b and H_d showed enhanced sporulation when compared to the parental species, while H_a and H_c gave lower spore counts. On the other hand, pigmentation was negatively affected by the somatic recombination (Table 4).

A high degree of variability in the biocontrol and the mycoparasitic ability of the fusants was observed but no significant increase in the activity was accomplished after fusion, the hybrids being generally less active than their parental strains or showed inhibition percentages similar to one of its parents. The growth inhibition percentages caused by the selected hybrids on the growth of the tested phytopathogens; *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *lycopersici* and *Pyricularia oryzae* were recorded in Table (5). These results show weak enhancement of the biocontrol activity of the resulted hybrids against *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *lycopersici*, where the best result was recorded for H_d with inhibition (60.78%). In case of *Rhizoctonia solani* and *Pyricularia oryzae* the hybrids achieved lower percentages of growth inhibition as compared to their parental isolates. Strain improvement is one of the most important necessities for the commercial and industrial utilization of the bioagents. Strain improvement by genetic manipulation techniques including transformation, mutation and somatic recombination can enhance their biocontrol activity and expand their spectrum **(Harman and Hayes, 1993)**. These techniques offer the possibility to obtain recombinants with desirable characteristics like better antagonistic ability, wider host range, tolerance to pesticides, survival ability in the environment, rhizosphere-competence, tolerance to adverse environmental conditions, vigorous growth and long shelf-life for improving their potential for plant disease control **(Upadhyay and Rai, 1988 and Harman and Stasz, 1991)**.

In *Trichoderma*, the sexual phase of reproduction has not been recorded. Thus, somatic recombination processes to combine desirable characteristics and to optimize the use of genetic potential of the different isolated *Trichoderma* strains are needed. Somatic recombination through anastomosis **(Furlaneto and Pizzirani-Kleiner, 1992 and Bagagli *et al*., 1995).** In the present study, the somatic recombination has been carried out between vegetative compatible *Trichoderma* isolates. The obtained fusants were generally phenotypically similar to one of the parental isolates and they also showed variable changes in morphological characters such as mycelial growth, sporulation and pigmentation. The similarity of the fusants to one of the parental isolates is consistent with the results of previous researchers. For example, in fusions reported between *Trichoderma virens* and *T. harzianum*, 17 out of 24 stable strains formed colonies similar to those of *T. virens* **(Shin and Cho, 1993)**. Similarly, in an intergeneric fusion between *T. longibrachiatum* and *Phanerochaete chrysosporium*, the fusant obtained was phenotypically similar to the *T. longibrachiatum* parent and quite dissimilar to the *P. chrysosporium* parent **(Nutsubidze *et al*., 1991)**. This fusant was reported to differ from the *T. longibrachiatum* parent in pigment production, sporulation, growth rate, and enzymatic activity **(Nutsubidze *et al*., 1991)**. This is consistent with the differences we observed in our fusants which, while generally phenotypically similar to one parent, differed in characters such as mycelial growth, sporulation and pigment production. Progeny from other protoplast fusions have been reported to differ from the parental strains in characteristics such as pigmentation **(Shin and Cho, 1993 and Kumari and Panda, 1994)**, secondary metabolite production **(Kumari and Panda, 1994)** and nutritional status **(Stasz *et al*., 1989 and Kumari and Panda, 1994)**. The similarity of the recombinant progenies to only one of the two parental strains suggests that a mechanism of somatic recombination, other than parasexuality, including parameiosis, may be occurring. Such mechanism of somatic recombination may be similar that proposed by **Stasz and Harman (1990)**, who suggested a new mechanism based on comparable results obtained from crossings involving

species of *T. harzianum*, *T. hamatum*, *T. koningii* and *T. viride*. According to these authors nuclei of the non-prevalent parent may be degraded in the heterokaryon, and small portions of this genome may be incorporated into the genome of the prevalent parent. **Morton *etal*.,(2012)** studied fungal dual cultures were screened for a combined preparation against nematodes. Combination of *Trichoderma harazianum* and Monacrosporium cionopagum are the best candidates. It was also revealed that *T.harazianum* strains are most capable egg-parasites.

The results of dual culture test for the hybrid strains revealed high degree of variability in their antagonistic activity but no significant increase in the activity was accomplished after fusion. Variability in biocontrol activity similar to what we observed has been reported in protoplast fusants of other *Trichoderma* species **(Pe'er and Chet, 1990)**. Also, **Migheli *et al*. (1994)** used protoplast fusion techniques for the production of new antagonistic strains of *Trichoderma* spp. They selected fast-growing and stable fusants and tested them in biocontrol trials against *P. ultimum* on lettuce seedlings and *B. cinera* on grape bunches in comparison with their parental strains. A high degree of variability in the biocontrol and the mycoparasitic ability of the fusants was observed but the hybrids being generally less active than their parental strains. **Hanson and Howel (2002)** carried an attempt to combine *T. virens* with desirable biocontrol characteristics in protoplast fusions with *T. koningii*, which had good storage qualities. All fusants were morphologically similar to one of the parental species. However, when compared to the morphologically similar *T. koningii* parent, two fusants showed better biocontrol activity against *R. solani* on cotton. In addition, one *T. virens*-like fusant gave significantly less control than the *T. virens* parent.

Table 3. Vegetative incompatible interactions of the tested *Trichoderma* isolates after five days of incubation on PDA at 25°C.

Tested isolates	Inward growth (cm)		Diameter of interaction zone (cm)	Number of isolates	Percentage
Inhibition zone					
T. lignorum against *T. koningii* (T12) (T26)	T12	4.6	0.3	20	22
	T26	4.1			
T. lignorum against *T. viride* (T12) (T27)	T12	4.5	0.3		
	T27	4.2			
T. lignorum against *T. viride* (T12) (T28)	T12	4.4	0.2		
	T28	4.4			
T. lignorum against *T. hamatum* (T12) (T29)	T12	4.9	0.3		
	T29	3.8			
T. lignorum against *T. hamatum* (T12) (T58)	T12	4.5	0.2		
	T58	4.3			
T. lignorum against *T. koningii* (T12) (T84)	T12	4.5	0.2		
	T84	4.3			
T. lignoum against *T. harzianum* (T12) (T61)	T12	5.2	0.3		
	T61	3.5			
T. koningii against *T. hamatum* (T13) (T58)	T13	3.6	0.3		
	T58	5.1			
T. koningii against *T. harzianum* (T13) (T75)	T13	4.5	0.6		
	T75	3.9			
T. ressei against *T. viride* (T25) (T28)	T25	5	0.3		
	T28	3.7			
T. ressei against *T. hamatum* (T25) (T29)	T25	5.3	0.3		
	T29	3.4			
T. ressei against *T. harzianum* (T25) (T61)	T25	5.5	0.4		
	T61	3.1			
T. ressei against *T. koningii* (T25) (T84)	T25	5.3	0.5		
	T84	3.2			
T. koningii against *T. viride* (T26) (T28)	T26	4.4	0.2		
	T28	4.4			
T. koningii against *T. hamatum* (T26) (T29)	T26	4.9	0.3		
	T29	3.8			
T. koningii against *T. koningii* (T26) (T84)	T26	4.2	0.3		
	T84	4.5			
T. viride against *T. hamatum* (T27) (T29)	T27	4.2	0.4		
	T29	4.4			
T. viride against *T. koningii* (T27) (T84)	T27	5.2	0.6		
	T84	3.2			

Table 3-cont.

Tested isolates	Inward growth (cm)		Diameter of interaction zone (cm)	Number of isolates	Percentage
T. hamatum against *T. harzianum* (T29) (T75)	T29	4.3	0.6		
	T75	4.1			
T. hamatum against *T. koningii* (T29) (T84)	T29	4.3	0.6		
	T84	4.1			
Overgrowth					
T. lignorum against *T. pseudokoningii* (T12) (T79)	T12	3.6	0.00	16	18
	T79	5.4			
T. koningii against *T. ressei* (T13) (T25)	T13	4	0.00		
	T25	5			
T. koningii against *T. koningii* (T13) (T26)	T13	4.2	0.00		
	T26	4.8			
T. koningii against T. viride (T13) (T27)	T13	3.8	0.00		
	T27	5.2			
T. koningii against T. viride (T13) (T28)	T13	4.2	0.00		
	T28	4.8			
T. koningii against T. hamatum (T13) (T45)	T13	4	0.00		
	T45	5			
T. ressei against T. hamatum (T25) (T45)	T25	4.9	0.00		
	T45	4.1			
T. ressei against T. harzianum (T25) (T75)	T25	5.8	0.00		
	T75	3.2			
T. ressei against T. pseudokoningii (T25) (T79)	T25	5	0.00		
	T79	4			
T. viride against *T. harzianum* (T27) (T61)	T27	3.5	0.00		
	T61	5.5			
T. viride against *T. pseudokoningii* (T27) (T79)	T27	4.9	0.00		
	T79	4.1			
T. koningii against *T. pseudokoningii* (T26) (T79)	T26	2.8	0.00		
	T79	6.2			
T. viride against T. hamatum (T28) (T45)	T28	3.7	0.00		
	T45	5.3			
T. hamatum against *T. harzianum* (T45) (T75)	T45	5.3	0.00		
	T75	3.7			
T. hamatum against *T. harzianum* (T45) (T61)	T45	6.3	0.00		
	T61	2.7			
T. harzianum against *T. koningii* (T61) (T84)	T61	4	0.00		
	T84	5			

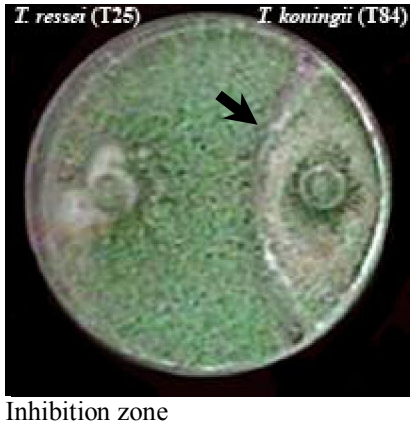
Table 3-cont.

Tested isolates	Inward growth (cm)		Diameter of interaction zone (cm)	Number of isolates	Percentage
Intermingling					
T. lignorum against *T. koningii* (T12) (T13)	T12	3.6	1.2	14	15
	T13	4.2			
T. lignorum against *T. ressei* (T12) (T25)	T12	4	0.4		
	T25	4.6			
T. lignorum against *T. hamatum* (T12) (T45)	T12	4.5	0.5		
	T61	4.5			
T. koningii against *T. harzianum* (T13) (T61)	T13	4.2	0.3		
	T61	4.5			
T. koningii against *T. koningii* (T13) (T84)	T13	4	1.0		
	T84	4			
T. ressei against *T. hamatum* (T25) (T58)	T25	4.1	0.5		
	T58	4.4			
T. viride against *T. harzianum* (T28) (T61)	T28	4.3	0.6		
	T61	4.1			
T. hamatum against *T. koningii* (T45) (T84)	T45	3	0.7		
	T84	5.3			

T. hamatum against *T. pseudokoningii* (T45) (T79)	T45 T79	3.8 4	1.2		
T. harzianum against *T. harzianum* (T61) (T75)	T75 T61	3.8 4.6	0.6		
T. harzianum against *T. koningii* (T75) (T84)	T75 T84	3.9 4.6	0.5		
T. harzianum against *T. pseudokoningii* (T61) (T79)	T61 T79	3.4 4.6	2		
T. hamatum against *T. pseudokoningii* (T58) (T79)	T58 T79	3.7 4.8	0.4		
T. koningii against *T. pseudokoningii* (T84) (T79)	T84 T79	3.6 4.4	1		
Demarcation line					
T. koningii against *T. hamatum* (T26) (T58)	T26 T58	4.5 4.5	0.00	12	13
T. koningii against *T. harzianum* (T26) (T75)	T26 T75	4.9 4.1	0.00		
T. koningii against *T. harzianum* (T26) (T61)	T26 T61	4.9 4.1	0.00		
T. viride against *T. hamatum* (T27) (T45)	T27 T45	5 4	0.00		

Table 3-cont.

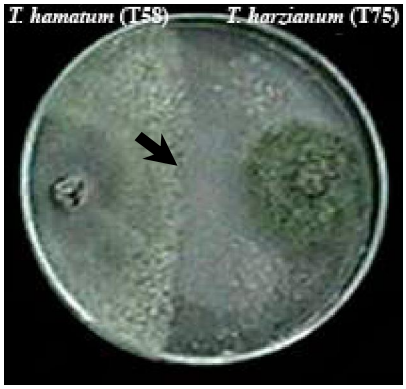
Tested isolates	Inward growth (cm)		Diameter of interaction zone (cm)	Number of isolates	Percentage
T. viride against *T. hamatum* (T27) (T58)	T27 T58	4.1 4.9	0.00		
T. viride against *T. harzianum* (T27) (T75)	T27 T75	5.3 3.7	0.00		
T. viride against *T. hamatum* (T28) (T29)	T28 T29	4.6 4.4	0.00		
T. viride against *T. hamatum* (T28) (T58)	T28 T58	4 5	0.00		
T. viride against *T. koningii* (T28) (T84)	T28 T84	5.1 3.9	0.00		
T. hamatum against *T. hamatum* (T29) (T58)	T29 T58	4.1 4.9	0.00		
T. hamatum against *T. hamatum* (T45) (T58)	T45 T58	5.2 3.8	0.00		
T. hamatum against *T. harzianum* (T58) (T75)	T58 T75	3.9 5.1	0.00		
Ridge of conidia					
T. lignorum against *T. harzianum* (T12) (T75)	T12 T75	3.8 3.5	1.7	11	12
T. koningii against *T. hamatum* (T13) (T29)	T13 T29	3.6 4.8	0.6		
T. koningii against *T. pseudokoningii* (T13) (T79)	T13 T79	3.7 4.4	0.9		
T. viride against *T. harzianum* (T28) (T75)	T28 T75	5.2 2.8	1.0		
T. viride against *T. pseudokoningii* (T28) (T79)	T28 T79	3.5 4.3	1.2		
T. hamatum against *T. hamatum* (T29) (T45)	T29 T45	4.3 3.9	0.8		
T. hamatum against *T. harzianum* (T29) (T61)	T29 T61	3.5 5.2	0.3		
T. hamatum against *T. pseudokoningii* (T29) (T79)	T29 T79	3 5.5	0.5		
T. harzianum against *T. pseudokoningii* (T75) (T79)	T75 T79	3.3 4.7	1.0		
T. hamatum against *T. harzianum* (T58) (T61)	T58 T61	3.7 4.1	1.2		
T. hamatum against *T. koningii* (T58) (T84)	T58 T84	3.4 4.2	1.4		



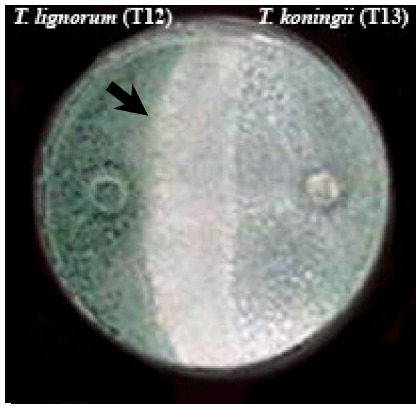
Inhibition zone

Overgrowth

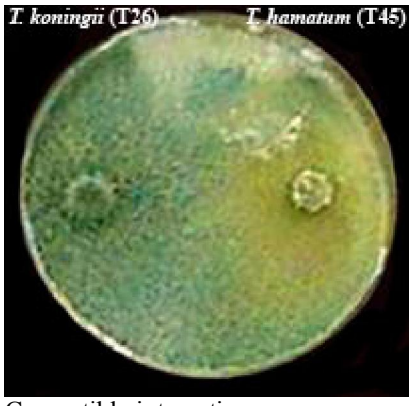
Ridge of conidia



Demarcation line



Intermingling



Compatible interaction

Plate 1. Types of vegetative interaction between *Trichoderma* isolates on PDA after five days of incubation at 25°C.

Table 4. Morphological characters of the parental isolates and their putative hybrids on PDA at 25 ºC.

=Isolate	Mycelial growth (cm)				Sporulation	Pigmentation density			
	1st day	2nd day	3rd day	4th day		1st day	2nd day	3rd day	4th day
Parents (a)									
T. ressei (T25)	1.9±0.164	7.3±0.375	9.0±0.0	9.0±0.0	5±0.346	±	+	+	++
T. koningii (T26)	1.4±0.214	5.4±0.192	9.0±0.0	9.0±0.0	4±0.569	±	+	++	++
Putative hybrid (a)									
H_a Ha_1 Ha_2	1.8±0.1 1.7±0.1 1.5±0.1	7.17±0.25	9.0±0.0	9.0±0.0	2±1.231	±	±	±	±
Parents (b)									
T. ressei (T25)	1.9±0.164	7.3±0.375	9.0±0.0	9.0±0.0	5±0.346	±	+	+	++
T. viride (T27)	2.7±0.033	8.3±0.158	9.0±0.0	9.0±0.0	4±1.049	±	+	+	++

Putative hybrid (b)									
H_b Hb1 Hb2	3.0±0.057 2.8±0.07 2.6±0.1	9.0±0.0	9.0±0.0	9.0±0.0	9±0.125	⁑	⁑	+	+
Parents (c)									
T. koningii (T26)	1.4±0.214	5.4±0.192	9.0±0.0	9.0±0.0	4±0.569	⁑	+	++	++
T. viride (T27)	2.7±0.033	8.3±0.158	9.0±0.0	9.0±0.0	4±1.049	⁑	+	+	++
Putative hybrid (c)									
H_c Hc_1 Hc_2	1.93±0.21 1.72±0.01 1.61±0.02	6.87±0.21	9.0±0.0	9.0±0.0	3±2.5	⁑	+	++	++
Parents (d)									
T. viride (T27)	2.7±0.033	8.3±0.158	9.0±0.0	9.0±0.0	4±1.049	⁑	+	+	++
T. viride (T28)	1.7±0.088	6.6±0.168	9.0±0.0	9.0±0.0	5±0.856	⁑	+	++	+++
Putative hybrid (d)									
H_d Hd_1 Hd_2	1.7±0.1 1.5±0.08 1.4±0.07	4.97±0.321	9.0±0.0	9.0±0.0	15±1.25	⁑	+	+	+

Each numerical value is the mean of three replica ± standard deviation . Each sporulation value is multiplied with 10^7

Table 5. Biocontrol activity of the parental isolates and their putative hybrids in dual culture test against the tested phytopathogens on PDA after five days of incubation at 25 ºC.

Isolate	*Sclerotium rolfsii*		*Rhizoctonia solani*		*Fusarium oxysporum* f. sp. *lycopersici*		*Pyricularia oryzae*	
	Linear growth (cm)	Growth inhibition (%)	Linear growth (cm)	Growth inhibition (%)	Linear growth (cm)	Growth inhibition (%)	Linear growth (cm)	Growth inhibition (%)
Parents (a)								
T. ressei (T25)	3.3±0.15	63.33	3.7±0.651	58.89	2.3±0.115	55.77	2.6±0.451	40.91
T. koningii (T26)	3.8±0.2	57.78	4±0.115	55.55	3±0.666	42.30	2.5±0.115	43.18
Putative hybrid (a)								
H_a	2.9±0.20	67.44	5.1±0.15	43	2.4±0.057	54.44	3.0±0.152	31.34
Parents (b)								
T. ressei (T25)	3.3±0.15	63.33	3.7±0.651	58.89	2.3±0.115	55.77	2.6±0.451	40.91
T. viride (T27)	3.3±0.15	63.33	4.3±0.115	52.22	2.7±0.568	48.08	2.8±0.173	36.36
Putative hybrid (b)								
H_b	3.1±0.26	65.55	4.4±0.057	51.11	2.3±0.15	55.17	2.8±0.208	35.68
Parents (c)								
T. koningii (T26)	3.8±0.2	57.78	4±0.115	55.55	3±0.666	42.30	2.5±0.115	43.18
T. viride (T27)	3.3±0.15	63.33	4.3±0.115	52.22	2.7±0.568	48.08	2.8±0.173	36.36
Putative hybrid (c)								
H_c	3.7±0.32	59.22	4.9±0.15	45.22	2.2±0.2	57.3	2.6±0.208	40.23
Parents (d)								
T. viride (T27)	3.3±0.15	63.33	4.3±0.115	52.22	2.7±0.568	48.08	2.8±0.173	36.36
T. viride (T28)	3.5±0.30	61.11	3.3±0.152	56.67	2.8±0.057	46.15	3.3±0.208	25
Putative hybrid (d)								
H_d	3.5±0.58	60.78	5.3±0.15	40.78	2.7±0.55	60.78	2.9±0.11	34.77

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