Genetic Analysis of *Trichoderma* Isolates in Relation to Antagonism Against *Rhizoctonia solani*

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SUMMARY

The present study was carried out in Genetics Department, faculty of Agriculture, Fayoum University, Egypt, during the period from 1999 to 2005. The study included eight *Trichoderma* isolates, to achieve the following objectives:

1) To isolate a collection of *Trichoderma* isolates different in their antagonistic potential against *Rhizoctoni solani.*
2) To explore the taxonomic relationships among *Trichoderma* isolates based on the species level.
3) To analyse the genetic variability of *Trichoderma* isolates using the biochemical marker (proteins), randomly amplified polymorphic DNA (RAPD) molecular marker and their antagonistic potential against *R. solani.*

The obtained results were summarized as the follows:

1- Morphological characterization of *Trichoderma* isolates:

Six isolates of *Trichoderma* spp. previously isolated from the rhizospheres of different plants growing in Fayoum Governorate, and two reference strains (*T. harzianum* and *T. koningii*) obtained from Faculty of Agriculture, Ain Shams University. Four *Trichoderma* isolates were characterized a morphologically as *T. harzianum*, one as *T. koningii* and one as *T. viride.*

2- Biocontrol of bean root rot using *Trichoderma* isolates:

The pathogenicity test showed that *Rhizoctonia solani* was responsible for root rot and to some extent to damping off disease. The percentage of diseased plants gradually increased by increasing the

inoculum density up to 7% (V/V). No significant differences between inoculum 5% and inoculum 7 %. All fungal *Trichoderma* isolates inhibited the growth of *Rhizoctonia solani* which caused the root rot disease on bean and varied in their antagonistic effect.

Under greenhouse conditions, infestation of the soil with culture or filtrate of *Trichoderma* isolates greatly reduced the percentage of infected plants of bean root rot. The isolate *T. harzianum* (FE_1) was the most effective in this aspect. Bean seed bed treated with *Trichoderma* isolates grown on sorghum/sand medium decreased the percentage of pathogenic fungal infections than by fungal filtrate treatment. The isolate *T. harzianum* (FE_1) showed the highest effect on controlling the pathogenic fungi.

This investigation was planned to study plates and carbohydrate tube antagonistic studies under laboratory conditions. The isolate *T. harzianum* (FE_1) was the most effective in this aspect.

Protein marker:

The molecular weight of protien banding patterns of the *Trichoderma* isolates ranged between 114.411 to 12.614 kDa. There were many clear differences between different *Trichoderma* isotates. The monomorphic of one band with molecular weights (MW) 30.187 kDa and 30 polymorphic bands were detected. These results revealed 96.77 % polymorphism for proteins

The genetic relationships among the eight *Trichoderma* isolates were demonstrated by dendrogram tree. The highest similarity was found between *Trichoderma* isolates, *T. harzianum* (FE_4) and *T. harzianum* (FE_2) (82.4%), whereas the lowest similarity was found between *Trichoderma* isolates, *T. viride* (FE_6) and *T. harzianum* (FE_1) (29.6%). The dendrogram was divided into two clusters. The first cluster containd *Trichoderma*

isolates, *T. viride* (FE_6), while the second cluster containd the rest of the antagonistic fungi. The cluster was divided into two subclusters. The frist subcluster included *Trichoderma* isolates *T. harzianum* (I_7). The second subcluster was divided into two sub subclusters.

RAPD marker:

The total numbers of RAPD-PCR fragments were 161 detected by 7 RAPD primers in the eight *Trichoderma* isolates. The results revealed that the differentiation between the identified isolates was carried out on the basis of DNA sequence. The total number of amplicons detected by the five successful RAPD primers (OPA-2, OPA-3, OPA-4, OPA-6 and OPA-7) was 46 including 39 polymorphic amplicons. This represents a level of polymorphism of 84.78 %.

The strongest relationship was scored between *T. harzianum* (FE_3) and *T. harzianum* (FE_2) isolates with the similarity of 75%, while the lowest one was scored between *T. viride* (FE_6) and *T. harzianum* (FE_4) isolats with the similarity of 34.3 %. The dendrogram was divided into two clusters. The first cluster included *T. koningii* (FE_5) and *T. viride* (FE_6) isolates, while the second cluster was divided into two subclusters. The first subcluster included *T. harzianum* (FE_1) and *T. harzianum* (I_7) isolates. The second subcluster was divided into two sub-subcluesters. The first sub subcluster included *T. harzianum* (FE_4) and *T. koningii* (I_8) isolates, and the second sub- subcluster included *T. harzianum* (FE_3) and *T. harzianum* (FE_2) isolates. Cluster analysis of RAPD marker separated *T. koningii* isolate (FE_5) and *T. viride* (FE_6) in one cluster, while the other six isolates fall in a second cluster.

Identification of *Trichoderma* Isolates by Unique Biochemical and

Molecular Markers:

Unique markers obtained by different markers (protein and RAPD) were used in the present study to characterize the eight *Trichoderma* isolates. The total specific markers generated by biochemical analysis were 5 markers. A number of 4 bands were scored as negative markers, while one was scored as positive marker. The protein marker was successful in identifying 4 out of the eight *Trichoderma* isolates with 5 isolate specific unique markers. The RAPD assay (using 5 random primers) identified 6 out of the eight *Trichoderma* isolates with 19 isolate specific unique markers. The *T. harzianum* (FE_1) isolate was characterized by the highest number of positive unique markers (5), while the isolate *T. harzianum* (FE_3)was characterized by two positive unique markers. Each of the isolates, *T. harzianum* (FE_4), *T. koningii* (FE_5), *T. viride* (FE_6) and *T. koningii* (I_8), was characterized by three positive unique markers. On the other hand, FE_2 and I_7 isolates revealed no specific markers. The least number of RAPD-PCR markers was detected for primers OPA-6 (one marker out of 4 amplified bands), while the largest number of RAPD-PCR markers was detected for primer OPA-4 (7 markers out of 14 bands).

The protein marker was successful in identifying 4 out of the eight *Trichoderma* isolates with 5 isolate specific unique markers while, RAPD assay (using 5 random primers) identified 6 out of the eight isolates with 19 isolate specific unique markers.