



Identification of *Trichoderma* species in South Khorasan Province, Eastern Desert Region of Iran

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Abstract

In this study, a total of 99 isolates of *Trichoderma* species were isolated from soil and living and rotting plant tissues from Birjand, Qaen, Khusf, Sarbisheh, and Sarayan regions in South Khorasan Province during 2012-2014. On the basis of growth rates on different media, macroscopic and microscopic characteristics such as shapes and sizes of conidias, conidiophores, phialides, chlamydospores, and aerial hyphae, *Trichoderma* isolates were classified. ITS regions of 17 isolates were amplified and sequenced using ITS1 and ITS4 primers. Based on morphological characteristics and sequences of ITS regions, the study isolates were classified into four species of *T. harzianum* from the *Trichoderma* section, *T. longibrachiatum* from the *Longibrachiatum* section, and *T. virens* and *T. brevicompactum* from the *Pachybasium* section. *Trichoderma*, *Longibrachiatum*, and *Pachybasium* sections appropriated 74, 3, and 22 isolates, respectively and *T. harzianum* was the most common in all of the habitats. The results showed that the number and frequency of the isolates belonged to *T. harzianum*, *T. virens*, *T. brevicompactum*, and *T. longibrachiatum* species respectively in South Khorasan Province.

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Introduction

Trichoderma is a globally distributed fungus associated with soil and plant roots and debris and known as a common component of different climatic ecosystems (Brotman *et al.*, 2010). Nearly 150 species of *Trichoderma* have been already described. Yet, based on a number of the *Hypocreales* order arrays described by anamorph *Trichoderma* morphology, its actual number is estimated to be more than 200 species (Mukherjee *et al.*, 2013; Lu *et al.*, 2004; Chaverri *et al.*, 2003; Samuels *et al.*, 2002).

The biodiversity of the genus *Trichoderma* has been examined by morphological and molecular methods in different natural ecosystems of Iran. Zafari *et al.* (2004) isolated ten species including *T. citrinoviride*, *T. longibrachiatum*, *T. saturnisporum*, *T. hamatum*, *T. harzianum*, *T. inhamalum*, *T. tomentosum*, *T. virens*, *T. asperellum*, *T. koningii*, *T. ghanense*, *T. spirale*, and *T. atroviride* from different parts of Iran. Isolated *T. brevicompactum*, *T. virens*, and *T. harzianum* from Kurdistan province, a wet and cold province in the West of Iran. Khalili *et al.* (2012) recovered *T. harzianum*, *T. virens* and *T. atroviride* species from rice paddy fields in Golestan and Mazandaran provinces, the major rice growing region in Southern coast of Caspian Sea, in the north of Iran. The South Khorasan province is located in the eastern dry and arid region of Iran with an annual rainfall of less than 100 mm. This province is the major habitat of medicinal plants such as barberry and jujube in Iran. This study aimed to isolate *Trichoderma* species associated with agricultural, horticultural, and medicinal plants from this part of Iran.

Materials and methods

Fungal isolation

Different types of samples such as soil and plant tissues were collected from farms, orchards, meadows, desert plains, decayed plant tissues, plant residues, and tree barks were performed in 11 different geographical parts of South Khorasan Province during 2012-2014. *Trichoderma* strains were isolated from soil by spreading soil extract suspension on modified culture medium containing

NH₄NO₃ (1 gL⁻¹), MgSO₄·7H₂O (0.2 gL⁻¹), KH₂PO₄ (0.9 gL⁻¹), KCl (0.15 gL⁻¹), glucose (3 gL⁻¹), agar (20 gL⁻¹), Rose Bengal (0.15 gL⁻¹), PCNB (0.2 gL⁻¹), chloramphenicol (0.25 gL⁻¹), captan (0.2 gL⁻¹). An alternative isolation involved using the *Agaricus bisporus* baiting method. First, mushrooms were divided into small pieces and were surface-sterilized by 10% sodium hypochlorite solution, added to the bags containing moist soil kept in the dark condition, and isolation was done with modified Elad and Chet (1983) medium after a few days. Also, isolation from plant barks and other tissues were implemented through tissue sterilization and cultured on modified Elad and Chet (1983) culture medium.

Morphological characteristics

The *Trichoderma* isolates were identified based on growth rates, macroscopic features of colonies, and micromorphological characteristics of conidiophores, phialides, conidia, chlamydo-spores, and aerial hyphae according to the Bissett (1984, 1991b, 1991a), Gams and Bissett (1998), Samuels *et al.* (2012) and Kraus *et al.* (2004)

Phylogenetic analysis

DNA was isolated from fresh mycelium using the modified method of Chen *et al.* (1999). ITS region was amplified by the use ITS1 and ITS4 primers (White *et al.*, 1990). PCR products were purified and sequenced by Bioneer Co. DNA sequences were aligned with ClastalW and Mega6 software (Tamura *et al.*, 2007) and analyzed by the second version of TrichOKEY program available online at www.isth.info (Druzhinina *et al.*, 2005). Data were analyzed within PHYIP and Mega6 programs using distance and maximum likelihood methods (Tamura *et al.*, 2007). The phylogenetic tree was obtained by the use of Mega6 program and Stability of clades was assessed with 1000 bootstrap replications (Sandoval-Denis *et al.*, 2014).

Results

Isolation and identification of *Trichoderma* spp.

In this study, totally 99 isolates of *Trichoderma* were isolated from soils samples and plant tissues in 11

different areas of South Khorasan province. On the basis of morphological characteristics, these 99 isolates of were divided into Four speciese (Fig. 1, 2 , 3).

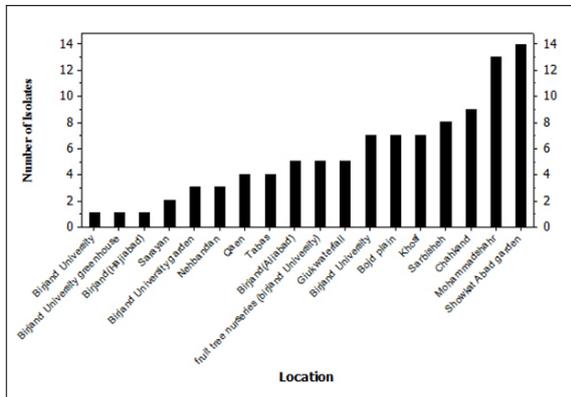


Fig. 1. Comparison of *Trichoderma* isolates in different Locations.

Seventy four isolates of *T. harzianum* from the section of Trichoderma were recovered from barberry, jujube, pistachio, pines, wild olives, lentils, grapes, camel's thorn, and soil (using the mushroom baiting method) in different areas of Birjand, Sarayan, Khosf townships, Giuk waterfall, and Bojd plain (Fig. 1, 2 , 3). This species were previously isolated from Iran especially in the wet areas and we isolated *T. harzianum* from eastern desert region of Iran, especially from barberry and jujube trees for the first time.

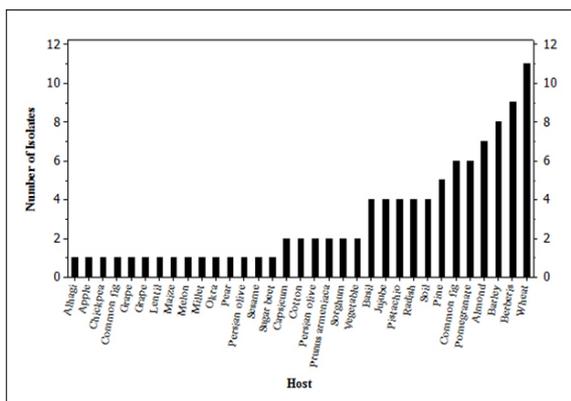


Fig. 2. Comparison of *Trichoderma* isolates with different hosts.

Subsequently, *T. virens* and *T. brevicompactum* were isolated from the Pachybasium section. Thirteen isolates of the *T. virens* species were recovered from barberry, jujube, wheat, radish, pomegranate, cotton, and pears in Sarbisheh and Qaen regions,

Mohammadshahr, Showkat Abad garden, and Chahkand village in Birjand (Fig. 1, 2 , 3). Nine isolates of the *T. brevicompactum* species were identified and isolated from barberry, wild olives, peas, radish, sesame seeds, melon, and almond in Sarbisheh, Qaen, and Khosf areas, Giuk waterfall in Birjand, and Birjand University greenhouse (Fig. 1, 2 , 3). *T. virens* and *T. brevicompactum* species were isolated from barberry in Iran for the first time during this research.

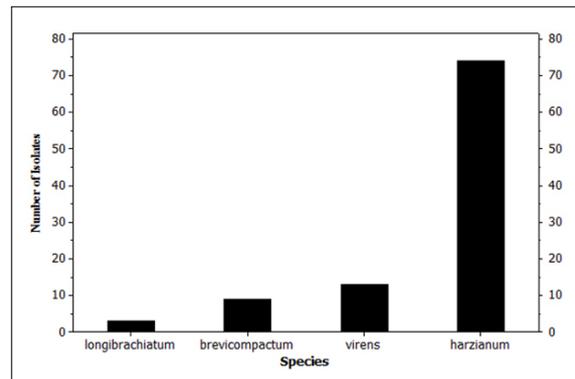


Fig. 3. Frequency of isolation of *Trichoderma* species.

Furthermore, three isolates of *T. longibrachiatum* were recovered from sugar beet and barberry in Qaen and Tabas. Having 3% of the isolates, this species showed the least abundance among the other species (Fig. 1, 2 , 3). Based on our data, *T. longibrachiatum* strains were isolated from barberry in Iran for the first time during this survey.

Phylogenetic Inference

Based on the ITS sequences of the isolates of this research and isolates from the gene bank, phylogenetic trees were prepared by the neighbor-joining method and thus they were grouped into 4 clade (Fig. 4).

T10 (for Sesame) and T24 (from Berberis) isolates were quite similar with *T. brevicompactum* species based on ITS sequences and were thus identified as *T. brevicompactum* for their consistency of morphological and growth characteristics. Moreover, the sequence of this isolates were absolutely identical to the sequence of *Hypocrea lutea* species. Therefore, these isolates, probably belong to the same sexual

stage as *H. lutea* species. The sequence similarity rate of this group and the other strains isolated in this study was less than 96%.

T12 and T13 isolate (from Berberis) of the 2nd group had completely identical sequences resembling to *T. longibrachiatum* species and thus were identified as *T. longibrachiatum* strains due to their similarities of morphological characteristics and ITS sequences.

T1 (Pomegranate), T23 (Jujube), T5 (Maize), T6 (Radish) and T8 (Pear) isolate were placed in the 3rd

group together with *T. crassum*, *T. virens*, and *T. koningiopsis*. These isolates had absolutely identical sequences as *T. virens* and *T. koningiopsis* species. However, the similarity rate of these isolates with *T. crassum* species was about 99.8%. One of the fundamental differences existing in this group relates to the sterile mycelium of *T. crassum* species. However, since it was not found in the above-mentioned isolates, these isolates were accepted relevant to *T. virens* species.

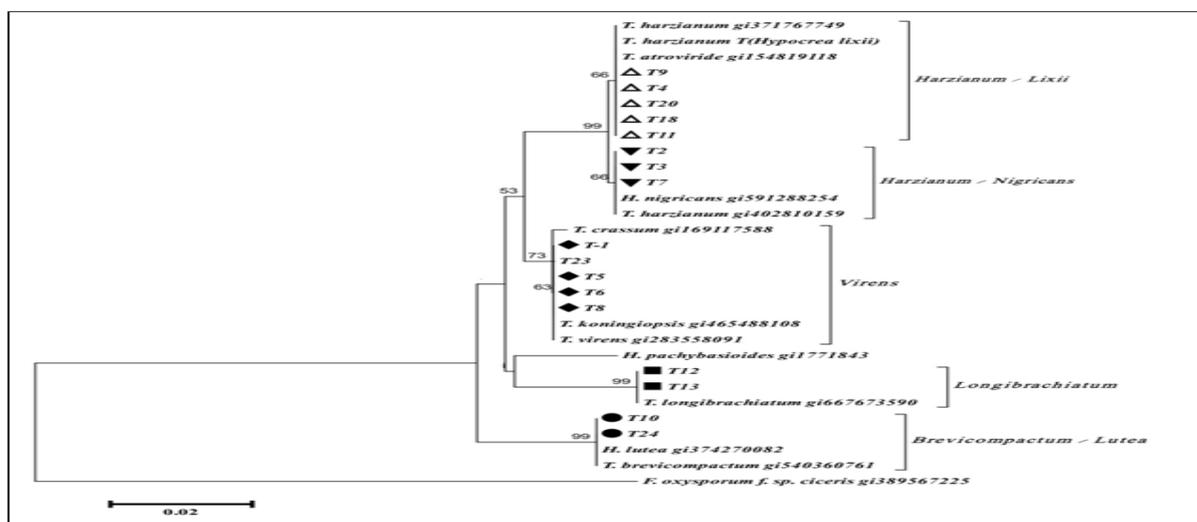


Fig. 4. Phylogenetic analysis of *Trichoderma* isolates using MEGA6 with 1000 number of replications and Neighbour-joining statistical method.

The 4th group of isolates was *T. harzianum* that was divided into 2 clades based on the sequences of ITS regions. The first class included T2 (Barley), T3 (Okra), and T7 (Pine) isolate since they were fully similar in sequences. The sequences of these isolates, *T. harzianum* gi402810159 of the gene bank, and *H. nigricans* were entirely alike. Therefore, this group is different from the other group based on sexual forms, though lying in the same group as *Harzianum* species. Another group of the *Harzianum* isolates, including T9 (Basil), T4 (Radish), T20 (Common fig), T18 (Almond), and T11 (Berberis) were placed in the same groups of *T. harzianum* gi3717767749 taken from the gene bank and *T. atroviride* based on sequences. The sequences of this group and *H. lixii* with the same sexual form of *T. harzianum* were completely similar. Therefore, the difference between

Harzianum group and the former group probably relates to their sexual forms. *T. atroviride* species also falls in this group. Nevertheless, coconut odor as one of the important characteristics of the *T. atroviride* (Joshi and Misra, 2013) was not smelled in the isolates of this study.

Discussion

The present study is a preliminary domestic assessment of *Trichoderma* diversity in the South Khorasan province. In this research, maximum of 74 isolates were belonged to *T. harzianum* from different areas of South Khorasan province. These species previously have been isolated from other parts of our country and this the first report of from this region of Iran. Our data showed that this the first isolation of *T. harzianum* from Barberry and Jujube. In our study

and in previous researchs, *T. harzianum* was the most commonly reported species in the genus, and was the most abundant species occurring in diverse ecosystems and ecological niches.

T. virens with 13 isolates was the 2nd abundant species after *T. harzianum* that was isolated from such hosts as jujube, barberry, corn, cotton, and pears in different regions of Sarbisheh, Mohammadshahr, Qaen, Nehbandan, and Bojd plain. Nine isolates of *T. brevicompactum* species were segregated from barberry, wild olives, peas, sesame, and almond in Sarbisheh, Qaen, and Khuf areas. In this study, this species was isolated from barberry and wild olives for the first time in Iran.

The largest number of *Trichoderma* isolates was collected from wheat (11 isolates), which belonged to *T. harzianum* and *T. virens* species and the second largest number of isolates was recovered from barberry (9 isolates), in which all the 4 species obtained in this investigation were seen.

In this research, 4 isolates of *T. harzianum* related to the hosts of grapes, figs, wild olives, and inner climate soil were isolated using *A. bisporus* baiting method. These isolates, which were not separated from specific media, seem to use edible mushroom as a food source due to the presence of the polysaccharides such as chitin, chitosan, glucan, and mannan in the cell wall of the mushroom.

Despite the great number of samples, the number of isolates collected was not too large and thus it can be concluded that *Trichoderma* species exist to a lesser degree with much less diversity in this region of the country due to dryness and low humidity.

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