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Diversity of *Trichoderma* species in chili rhizosphere that promote vigor and antagonism against virulent *Phytophthora capsici*

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ABSTRACT

The oomycete *Phytophthora capsici* causes chili pepper (*Capsicum annuum* L.) blight that is extremely hard to control. In this work, the diversity of *Trichoderma* species from chili farms and their antagonistic activity against virulent strains of *P. capsici* were studied. The maximum likelihood phylogenetic analysis based on translation elongation factor 1α (*TEF1α*) locus revealed divergent evolution in the population structure of *Trichoderma* species exhibiting antagonistic activities against *P. capsici*. *In vitro* confrontation analysis revealed that *Trichoderma harzianum*, *T. viride* and *T. reesei* displayed over 85.5% inhibition of mycelial growth of *P. capsici*. Furthermore, soil application of *Trichoderma* species under greenhouse conditions effectively suppressed root–rot severity by 11.24–26.50% ($P < 0.05$) hallmarked by a significant improvement in mean fresh weights (P = 3.57E-31, F = 486.98, $P < 0.05$) and length of the roots (P = 3.76E-28, F = 313.51, $P < 0.05$) compared to controls harboring *P. capsici*, not inoculated with *Trichoderma*. We provide evidence of genetic diversity of beneficial *Trichoderma* species in chili farms having both biocontrol potential against *P. capsici* coupled with growth promoting properties for chili roots.

1. Introduction

Chili pepper (*Capsicum annum* L.) is an important horticultural economic crop farmed over 19.89 million hectares worldwide (Majid et al., 2016) with an annual yield of 33.52 million tons (Patel, 2014). However, the soil-borne *Phytophthora capsici* Leonian severely limits chili production even though it is often controlled via fungicide application, crop rotation, fallow, and even an abandonment of the cropland (Morrison et al., 2011; Wu et al., 2013). *P. capsici* penetrates the root tissues and cause obstruction in the vascular system that prompt withering, damping-off, foliar blight and fruit rot (Latin and Rane, 1999; Behbudi et al., 2005), leading to an estimated annual loss of $1700–$3200 USD per hectare (Matthew et al., 2006).

At times, the occurrence of *P. capsici* mating types (A1 and A2) in the same farm and their ability to produce infectious oospores that are difficult to eradicate by chemical fungicides calls for alternative control strategies. Additionally, the extensive use of fungicides to combat *P. capsici* which are expensive and ecologically unfriendly, lead to the development of resistance (Fernández Pavía et al., 2004; Qi et al., 2012; Pang et al., 2013). An alternative to fungicides has been the use of biological control agents (BCA) for the management of *P. capsici* such as *Burkholderia cepacia* (Ezziyyani et al., 2004), *Pseudomona* species (Virgen-Calleros et al., 1997), *Glomus intraradices* (Zheng et al., 2005), *Bacillus* species (Guillén-Cruz et al., 2006), uncultured ascomycetes (Robles-Yerena et al., 2010) and *Trichoderma* species (Li-Destri et al., 2014; Saravanakumar et al., 2016; Jiang et al., 2016; Uniyal and Singh, 2017). Nevertheless, less studies have been performed on the interaction of *P. capsici* with *Trichoderma* species, although their antagonistic activities on *Phytophthora ramorum* (Widmer, 2014), *P. infestans* (Bouziane et al., 2016), *P. melonis* (Sabbagh et al., 2017) and *P. nicotianae* (Ros et al., 2017) are well known. Despite the on-going studies, a few *Trichoderma* species are known to confer both growth promoting and antagonistic activities for the management of *P. capsici in vivo*. Additionally, no *Trichoderma* biodiversity data is available on a chili farming region in Pakistan. In this study, we examine the diversity of *Trichoderma* species in chili rhizosphere that promote vigor and antagonism against virulent invasive *P. capsici strains*. On the basis of *in vitro* and greenhouse investigations, it is concluded that *T. viride, T.*

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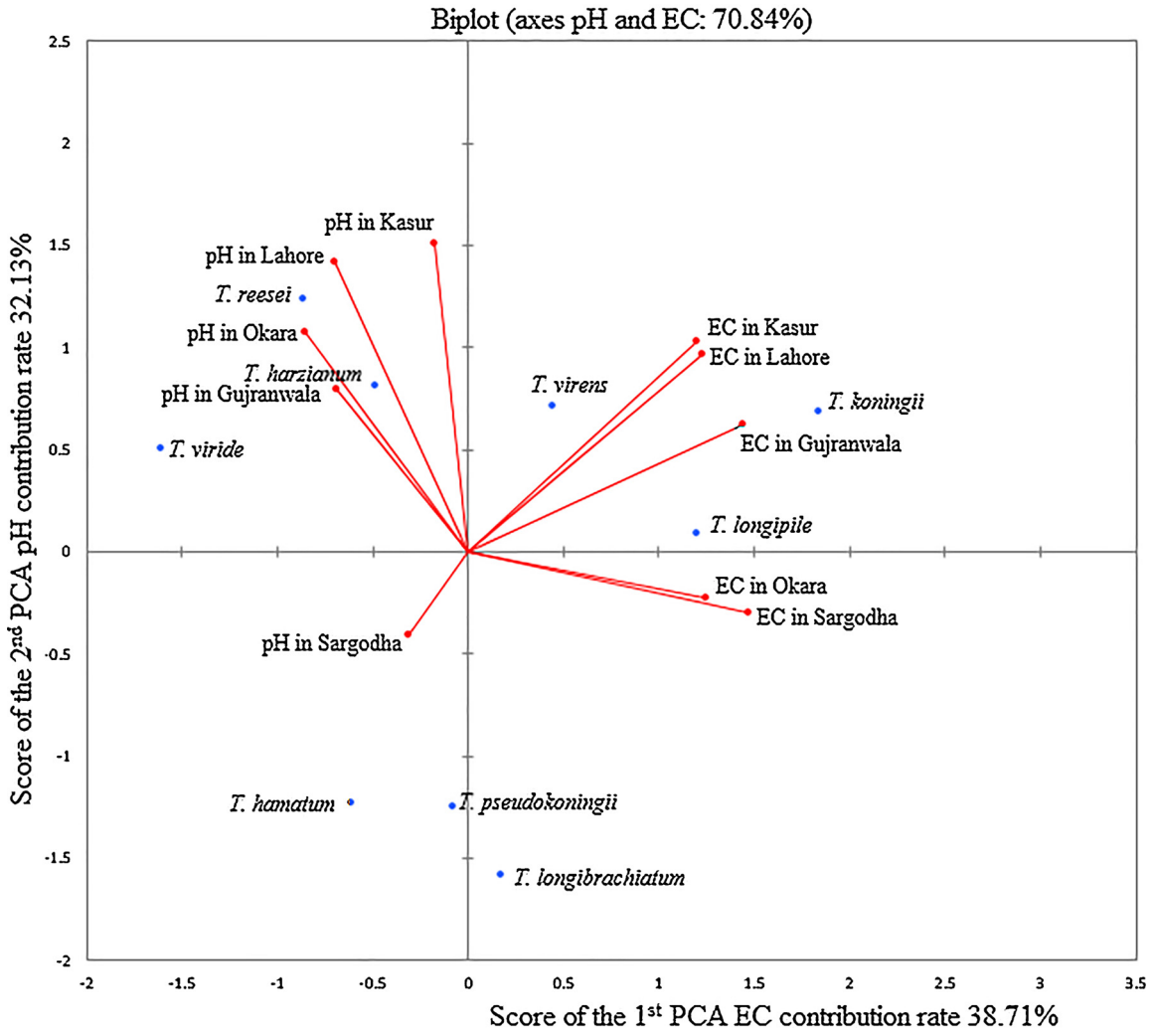

Fig. 1. Principal component analysis (PCA) biplot of the distribution of studied *Trichoderma* species with respect to location in Punjab Pakistan, soil electro-conductivity (EC), and pH.

reesei, T. harzianum and *T. longibrachiatum* are competitive soil fungi, and during interaction with *P. capsici* and chili pepper in the same biota promote plant vigor.

2. Materials and methods

2.1. Isolation of antagonists

The success of biocontrol agent chiefly depends on the ability of the antagonist to thrive in the biota harboring the prevailing invasive pest, thus, we sampled soil from chili farms solely. Surveys were conducted in 5 chili pepper regions (~25 ha each), in Punjab province (24–37 °N and 62–75 °E), Pakistan in the month of June 2014 and June 2016 during which mean temperature is 37 °C. During surveyed, thirty soil samples in Lahore, Kasur, Sargodha, Okara, and Gujranwala were collected in polythene bags in a randomized block manner. Preceding soil collection, the sampling site containing mix population (of healthy and diseased) chili plants was tagged with 5 m x 5 m dimension using a nylon robe and GPS reading was recorded. A 10 cm long stainless-steel cork-borer with 50 mm diameter was used to drill holes closer to roots of healthy plants. The soil in direct contact with the chili root herein refer to as rhizosphere was used for isolation of *Trichoderma* species. Sampling was performed prior to application of fungicides during each farming season. This region of Pakistan produces 400 tons of chili pepper from 300 ha annually (Anon, 2002). The soil samples were serially diluted and different *Trichoderma* species were isolated on *Trichoderma* specific medium (0.2 g $MgSO_4.7H_2O$, 0.9 g K_2HPO_4, 0.15 g KCl, 3.0 g NH_4NO_3, 3.0 g Glucose, 15 g Agar, 0.15 g Rose Bengal, 0.25 g Chloramphenicol, 1000 ml distilled water, pH 6.5) as previously described (Elad et al., 1980). *Trichoderma* species were subsequently grown on PDA medium and purification was done using a single spore isolation method (Bissett, 1991). The pure isolates were stored by cryopreservation in 20% glycerol at −80 °C in Institute of Agricultural Science, University of the Punjab, Lahore, Pakistan.

2.2. Morphological characterization and taxonomic placement

For macroscopic studies, 0.5 cm discs were removed from actively growing cultures of each isolate and kept at the center of Petri plate containing PDA medium at 28 °C for 4 days. The cultures were examined for the rate of colony growth, color, texture and identified as previously described (Bissett, 1991). Microscopic features such as branching and apex of the conidiophores, shape of the phialides, size, shape and color of conidia were scored for delimiting species. Furthermore, these morphological characters were compared with the reported literature (Bissett, 1991) and online interactive key (Samuels et al., 2002) based on the colony appearance, growth rate at 28 °C, the sizes of conidia, the branching patterns of conidiophores, and the presence (or absence) of chlamydospores.