Crop Protection 35 (2012) 102–109

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Co-inoculation of *Dactylaria brochopaga* and *Monacrosporium eudermatum* affects disease dynamics and biochemical responses in tomato (*Lycopersicon esculentum* Mill.) to enhance bio-protection against *Meloidogyne incognita*

Udai B. Singh [a,*], Asha Sahu [b], Nisha Sahu [c], R.K. Singh [a], Renu [d], Ratna Prabha [d], Dhananjaya P. Singh [d], B.K. Sarma [a], M.C. Manna [b]

[a] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India
[b] Division of Soil Biology, Indian Institute of Soil Science, Nabibagh, Berasia Road, Bhopal 462 038, India
[c] Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India
[d] National Bureau of Agriculturally Important Microorganisms (NBAIM), Kushmaur, Maunath Bhanjan 275 101, India

ARTICLE INFO

Article history:
Received 9 September 2011
Received in revised form
4 January 2012
Accepted 8 January 2012

Keywords:
Nematophagous fungi
Dactylaria brochopaga
Monacrosporium eudermatum
Root gall
Total phenolic compounds
Phenylalanine ammonia lyase

ABSTRACT

The nematode trapping ability of nematophagous fungi *Dactylaria brochopaga* and *Monacrosporium eudermatum* to colonize tomato root and parasitize and kill root-knot nematode *Meloidogyne incognita* was evaluated, along with the capability of the fungi to induce the accumulation of defence-related biomolecules in tomato plants under the greenhouse and field conditions. Co-inoculation of *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 significantly reduced root-knot disease in tomato (89.63%) and increased the accumulation of total chlorophyll (125.34, 140.53 and 152.67 mg g^{-1} fresh wt.), total phenolic compounds (TPC) (37.40, 48.32 and 59.63 μg of gallic acid equivalent), and phenylalanine ammonia lyase (PAL) activity (58.45, 69.05 and 74.57 mM cinnamic acid h^{-1} g^{-1} fresh wt.) after 10, 20 and 30 days of inoculation, respectively, in the greenhouse. However, in the field experiments, the total chlorophyll content in leaves of *M. incognita* infected tomato plants co-inoculated with *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 (152.70 mg g^{-1} fresh wt.) was higher than the plants treated with *D. brochopaga* Dp-5 or *M. eudermatum* Mv-1 individually (130.87 and 144.73 mg g^{-1} fresh wt., respectively) and *M. incognita* treated plants (19.47 mg g^{-1} fresh wt.). TPC and PAL activity were found to be higher in tomato leaves co-inoculated with *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 (62.90 μg of gallic acid equivalent and 79.33 mM cinnamic acid h^{-1} g^{-1} fresh wt., respectively) than the plants treated with *D. brochopaga* Dp-5 or *M. eudermatum* Mv-1 individually, whereas in *M. incognita* inoculated and control plants, the induction of these two compounds was found to be very low after 30 days of inoculation. The results of this study showed that these fungi reduced root-knot disease and enhanced accumulation of defence related biomolecules in tomato plants and has potential to serve as a biocontrol agents against root-knot nematodes.

1. Introduction

Plants usually face several environmental stresses that affect crop quality and productivity (Jones, 2009), including root diseases which are often major constraints to profitable crop production. Plant parasitic nematodes attacking roots of plant are small microscopic soil living roundworms that cause root dysfunction, reducing rooting volume and foraging and utilization efficiency of water and nutrients (Walia and Bajaj, 2003). In addition, they have been shown to predispose plants to infection by fungal or bacterial pathogens or transmit viruses that contribute to additional yield reduction (Upadhyay and Dwivedi, 2008). Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops in the world, including India. Root-knot disease, caused by nematode *Meloidogyne incognita* (Kofoid and White) Chitwood, is among the most devastating and destructive diseases of tomato in Asia. Root-knot nematode caused 39.7% loss in yield at a preplant density of 20 juveniles (J_2) g^{-1} of soil (Reddy, 1981). Upadhyay and Dwivedi (2008) reported 28–47% yield loss due to root-knot nematodes in tomato.

Diseases caused by soilborne pathogens are more difficult to control than those caused by foliar pathogens and usually cause

* Corresponding author. Present address: National Bureau of Agriculturally Important Microorganisms (NBAIM), Kushmaur, Maunath Bhanjan 275 101, India. Tel.: +91 547 2530080; fax: +91 547 2530381.
E-mail addresses: udaiars.nbaim@gmail.com, udaibhu1@gmail.com (U.B. Singh).

0261-2194/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2012.01.002

more devastating losses to producers because they are difficult to detect before significant damage occurs (Rush, 1990). Genetic resistance is a possibility for managing soilborne pathogens. However, breeding for resistance to *M. incognita* is a difficult task because resistance is often multigenic and its heritability is low (Walia and Bajaj, 2003). The management of root-knot disease has long been carried out by integration of conventional practices with chemical pesticides (Khan et al., 2011). Chemicals may create imbalance in natural micro flora in the soil, which in turns adversely affect the whole ecosystem (Groth et al., 1990).

Nematophagous fungi are prominent inhabitants of agricultural soil and are the natural colonizers of tomato roots (Bordallo et al., 2002), where they potentially contribute towards various attributes like biological control of plant parasitic nematodes (Jansson and Lopez-Llorca, 2001; Lopez-Llorca et al., 2002) and improve plant health and crop productivity (Kumar and Singh, 2006; 2006b). They can benefit plant growth by reducing phytopathogenic nematodes population (Singh, 2007; Khan et al., 2011), evoking changes in metabolic status (Ahmad et al., 2008) and inducing systemic resistance against pathogenic attack to plants (Barriuso et al., 2008). The microbe-mediated induced systemic tolerance (IST) can enhance tolerance in plants to biotic stresses by physical and biochemical changes (Weir et al., 2004; Wu et al., 2009). Looking into the prospective of crop production losses due to the plant pathogenic nematodes, bio-protection provided by nematophagous fungi offers attractive and environmentally safe option (Oka, 2010; Niu and Zhang, 2011).

The nematode trapping fungi *Dactylaria brochopaga* Drechs. and *Monacrosporium eudermatum* (Drechsler) Subram. have been studied less intensively as biocontrol agents. These fungi have different nematode-trapping structures (Singh, 2007; Khan et al., 2011). In recent years, emphasis has been made on the application of binary and multiple mixtures of microbes (Bashan and de-Bashan, 2005; Meena et al., 2010) or application of microbial species with multi-pronged functions. Hence, looking into the previous gaps in research related to biocontrol of *M. incognita*, the present study is to determine the potentials of *D. brochopaga* and *M. eudermatum* alone or in combination to parasitize and kill *M. incognita* in *in vitro*, stimulate defense related biomolecules accumulation in plants and their efficacy as effective biocontrol agents against root-knot diseases of tomato under both greenhouse and field conditions.

2. Materials and methods

2.1. Sources of media, nematicide and plant material and growth conditions

Corn meal agar (CMA) medium (pH 7.0) was prepared (per liter) with 20 g of corn infusion containing 20 g agar powder (HiMedia, India) and used in 1:10 ratio for the *in vitro* evaluation of trap formation and parasitism of *M. incognita* by the selected nematophagous fungi. The nematicide, carbofuran (Syngenta, India), was used as chemical control. Carbon and nitrogen sources *viz*., glucose, fructose, dextrose, sucrose, mannitol, starch, sodium nitrate, potassium nitrate, calcium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate and other media ingredients were obtained from HiMedia Pvt. Ltd., India. All experiments were performed at 25 ± 2 °C.

Twenty-day old tomato (cv. HS 101) seedlings were obtained from Horticultural farm, Department of Horticulture, Institute of Agricultural Science, Banaras Hindu University (BHU), Varanasi, India. Tomato seedlings were transplanted to pots in a greenhouse and field plots (3 m × 2 m). Each pot containing 3 kg of experimental soil with and without inoculation was transplanted with one seedling. These experiments were carried out from October to December with relative humidity 80–85% and an 11/13 h light/dark photoperiod.

2.2. Laboratory study

2.2.1. Fungal strains and culture conditions

Root samples along with the rhizospheric soil samples were collected from tomato plants grown in nematode infected agricultural farms and farmer's field from different geographical locations in India in 2007–08 (Table 1) to isolate nematophagous fungi *D. brochopaga* and *M. eudermatum*. Isolation of *D. brochopaga* and *M. eudermatum* was performed using the methods described by Bandyopadhyay and Singh (2000) with slight modifications (Singh, 2007) on corn meal agar (CMA) and rabbit dung agar media. Monoconidial cultures of all isolates were made (Singh et al., 2004). Identification of the *D. brochopaga* and *M. eudermatum* isolates was carried out using the microscopic identification key described by Drechsler (1937), Cooke and Godfrey (1964), Haard (1968) and Barron (1977). Cultures were maintained on CMA medium at 25 ± 2 °C.

2.2.2. Mycelial growth

Radial growth of different isolates of *D. brochopaga* and *M. eudermatum* was observed for seven days on CMA medium at 25 ± 2 °C (Singh et al., 2007). Effects of different media and sources of carbon and nitrogen on the growth of the selected fungal isolates were recorded after five days of inoculation as per methods of Dayal (2000) with slight modifications (Singh, 2007). The media used in the tests included Martin's medium, nutrient agar medium, yeast peptone soluble starch medium, water agar medium, carrot agar medium, Czapek's agar medium, beef extract agar medium, CMA medium, glucose agar medium and potato dextrose agar (PDA) medium. The carbon sources included glucose, fructose, dextrose, sucrose, mannitol and starch and the nitrogen sources included

Table 1
Geographical location and enumeration of *Dactylaria brochopaga* and *Monacrosporium eudermatum* isolates from different parts of U. P., India.

S.N.	Isolates	Location	Coordinate	Isolated from
A. *Dactylaria brochopaga*				
1.	Dl-15	Horticultural farm, BHU, Varanasi, India	25°15′56.60″N 82°59′28.11″E	Root gall
2.	Dp-5	Agricultural farm, IIVR, Varanasi, India	25°10′58.24″N 82°52′30.69″E	Root gall
3.	Dp-47	Farmers field, Azamgarh, India	25°48′02.95″N 83°00′18.09″E	Rhizospheric soil
B. *Monacrosporium eudermatum*				
1.	Mv-1	Horticultural farm, BHU, Varanasi, India	25°15′56.60″N 82°59′28.11″E	Root gall
2.	Ml-4	Farmers field, Mirzapur, India	25°05′52.98″N 82°52′12.45″E	Root gall
3.	Mu-17	Farmers field, Ghazipur, India	25°35′00.45″N 83°34′29.83″E	Rhizospheric soil
4.	Mp-99	Agricultural farm, IIVR, Varanasi, India	25°11′00.72″N 82°52′29.44″E	Root gall

BHU represents Banaras Hindu University, IIVR- Indian Institute of Vegetable Research.