Available online at www.sciencedirect.com
ScienceDirect
Crop Protection 26 (2007) 1682–1688
Crop Protection
www.elsevier.com/locate/cropro

Growth enhancement and Phytophthora blight (*Phytophthora capsici* Leonian) control by arbuscular mycorrhizal fungal inoculation in pepper

H. Ozgonen[a,*], A. Erkilic[b]

[a]*Department of Plant Protection, Agricultural Faculty, Suleyman Demirel University, Cunur 32260, Isparta, Turkey*
[b]*Department of Plant Protection, Agricultural Faculty, Cukurova University, Balcali 01330, Adana, Turkey*

Received 11 July 2006; received in revised form 8 February 2007; accepted 23 February 2007

Abstract

The effects of selected arbuscular mycorrhizal (AM) fungi, *Glomus mosseae*, *Glomus etunicatum*, *Glomus fasciculatum* and *Gigaspora margarita*, on growth of pepper seedlings and Phytophthora blight caused by *Phytophthora capsici* and the role of the phytoalexin, capsidiol were investigated. Root colonization by AM fungi reached between 61.3% and 68.1% in roots of pepper 4 weeks after transplanting. All tested AM fungi increased the shoot height between 23.4% and 31.7% and fresh and dry weights of shoots and roots of plants were enhanced by *G. etunicatum*, *G. fasciculatum* and *Gigaspora margarita* compared to uninoculated plants in pot experiments. *G. fasciculatum* increased yield significantly by 22% under greenhouse conditions. *G. mosseae* reduced the disease severity of *P. capsici* by 91.7%, 43.0% and 57.2% under pot, greenhouse and field conditions, respectively. Compared to the control, the capsidiol level was increased by preinoculation with *G. mosseae* and in the necrotic stems of *P. capsici*-inoculated pepper plants. In conclusion, AM fungi, especially *G. mosseae* enhanced the development of plants and reduced Phytophthora blight of pepper.

Keywords: Pepper; *Phytophthora capsici*; Growth enhancement; Arbuscular mycorrhizal fungi; Capsidiol

1. Introduction

Phytophthora blight of pepper caused by *Phytophthora capsici* Leonian is the most important fungal disease of pepper growing areas worldwide (Ristaino and Johnston, 1999). The pathogen is soilborne and causes blight on single or groups of plants in the field, especially in soil saturated with water after irrigation or rainfall. In the early stages, the first signs of disease are brown necrotic areas on the root and crown of plants, after which the disease develops rapidly, causing plants to wilt and die (Black et al., 1991).

The roots of most plants are generally infected by arbuscular mycorrhizal (AM) fungi which are beneficial to their host plants (Agrios, 1997). AM fungi have the effect of promoting host plant growth mainly by enhancing mineral uptake through symbiosis in plant roots (Marschner and Dell, 1994). In addition, there are many reports on their role in controlling plant disease, especially soilborne fungal pathogens. The disease severity reducing effects by AM fungi are known in some plant–pathogen systems as reported in peanut—*Sclerotium rolfsii* (Krishna and Bagyaraj, 1983), eggplant—Verticillium wilt (Matsubara et al., 1995), tomato—*Phytophthora nicotianae* var. *parasitica* (Trotta et al., 1996), pea—*Rhizoctonia solani* (Karagiannidis et al., 2002) and tomato–Fusarium wilt (Caron et al., 1986; Akkopru and Demir, 2006).

Phytoalexins are low molecular mass antimicrobial compounds that are synthesized and accumulated in response to some abiotic factors or after pathogen infection in plants (Paxton and Groth, 1994). In some Solanaceous plants, the phytoalexin, capsidiol was isolated from pepper (Ward, 1976) and tobacco (Chappell et al., 1997). Phytoalexin accumulation has an important role as part

*Corresponding author. Tel.: +90 246 211 46 15; fax: +90 246 237 16 93.
E-mail addresses: hozgonen@ziraat.sdu.edu.tr (H. Ozgonen), erkilic@cu.edu.tr (A. Erkilic).

0261-2194/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2007.02.010

of the multicomponent disease resistance mechanisms in plants (Mansfield, 1984; Kuc and Rush, 1985; Hammerschmidt, 1999).

The aim of the present work is to study the effects of the AM fungi, *Glomus mosseae*, *Glomus etuniatum*, *Glomus fasciculatum* and *Gigaspora margarita* on development of pepper plants and on *P. capsici* under pot, greenhouse and field conditions and to analyse the effect of capsidiol in disease resistance.

2. Materials and methods

2.1. Plant, pathogen and AM fungi

Pepper (*Capsicum annuum* L.) cv. Charliston Bagci was used in pot, greenhouse and field experiments. *P. capsici* Leonian was isolated from diseased tissue of naturally infected pepper plants on corn meal agar with pimaricin (10 mg l^{-1}), vancomycin (200 mg l^{-1}) and PCNB (100 mg l^{-1}). AM inoculum was obtained from the Soil Department, Agricultural Faculty, University of Cukurova and inoculum of four AM fungi *G. mosseae* (Nicol.&Gerd.) Gerdemann&Trappe, *G. etunicatum* Becker&Gerdemann, *G. fasciculatum* (Thaxter) Gerd.&Trappe and *Gigaspora margarita* Becker&Hall was bulked up on maize (*Zea mays* L.) and used in the experiments.

2.2. AM fungus inoculation, seedling production and plant growth conditions

Pepper seeds were surface disinfested in 2% NaOCl solutions for 5 min and thoroughly washed twice with sterile distilled water. Pepper seedlings were produced in plastic containers (30 × 40 cm). The mixture of soil, sand, and pumice (1/1/1, v/v/v) was autoclaved at 121 °C and 100 kPa twice for 1 h each time and used as growth medium. For the AM seedling production, AM inocula, soil infected with spores mixed with root fragments, was incorporated 2–3 cm below the seeds for each AM fungus (Menge and Timmer, 1982). The inoculum amount used was determined after quantifying the spore numbers for each AM fungus (Gerdemann and Nicholson, 1963) and the inoculum was added as a band in plastic containers with spore inocula of *G. mosseae* (188 spores 10 g^{-1} soil, 2.1 kg), *G. etunicatum* (268 spores 10 g^{-1} soil, 1.3 kg), *G. fasciculatum* (229 spores 10 g^{-1} soil, 1.7 kg) and *Gigaspora margarita* (235 spores 10 g^{-1} soil, 1.6 kg). Pepper seeds were sown in containers without AM fungus inoculation using same growth medium. The plastic containers were placed in a growth room at 25 ± 2 °C temperatures. During seedling production until 3–4 leaf stage, seedlings were watered with deionized water.

When seedlings were transplanted into pots, greenhouse and field, AM fungal spore inoculum for each fungus was added to seedling beds (*G. mosseae* (50 g), *G. etunicatum* (35 g), *G. fasciculatum* (45 g) and *Gigaspora margarita* (40 g)).

2.3. Plant growth and yield

For the determination of the effect of mycorrhizal fungi on development of the plant, important plant growth parameters such as plant height, shoot and root fresh and dry weights in pot conditions and yield under greenhouse conditions were examined.

Plants with 2–3 leaves infected with or without AM fungi were transplanted to 15 cm-diameter pots containing an autoclaved mixture of soil, sand and pumice (1/1/1, v/v/v). The following treatments with four replications and five plants in each treatment were included: *G. mosseae* (GM), *G. etunicatum* (GE), *G. fasciculatum* (GF), *Gigaspora margarita* (GiM) and uninoculated control. All pots were maintained in a growth room at 25 ± 2 °C temperatures with 8.000 lux illumination for 12 h a day in a completely randomized block experimental design.

Four weeks after transplanting, the colonization percentage of pepper roots by the four AM fungi was determined. The roots were cleared and stained as described by Koske and Gemma (1989) and the percentage of root colonization was estimated by a gridline intersect method (Giovannetti and Mosse, 1980). Plant height was measured weekly for 8 weeks. For determining the fresh and dry weights of shoots and roots, plants were harvested at the end of 8 weeks and shoots were separated from roots. The roots were washed with tap water and then with distilled water, and washed roots were left on two layers of filter paper to remove excess water. The shoots and roots in each treatment were weighed to determine fresh weight and then dried at 70 °C for 2 days to determine dry weight.

For determining the effect of AM fungi on pepper yield, another experiment was conducted in greenhouse conditions. In the experimental area (250 m^2), plots were prepared and uniform seedlings with 2–3 leaves with and without mycorrhizal fungus were transplanted into plots. Treatments were: GM, GE, GF, GiM and an uninoculated control. In the experimental area, a drip irrigation system was used. The experiment was designed as a completely randomized design with four replications and each plot contained 70 plants. At the time of harvesting from 10 weeks, ripened fruits were harvested in each plot and total yield was determined at the end of experiment.

2.4. Determination of the effects of AM fungi on P. capsici under pot conditions

Mycorrhizal and non-mycorrhizal plants with 3–4 leaves were transplanted into 15 cm-diameter pots containing autoclaved soil. The following treatments, with four replications and five plants in each treatment were included: GM+PC, GE+PC, GF+PC, GiM+PC and PC.

Four weeks later, plants were inoculated with the pathogen, *P. capsici*. The fungus was grown on oatmeal agar plates at 28 °C for 7 days and placed under fluorescent light for sporulation. Culture plates were incubated in sterile distilled water for 40 min at 4 °C and then for 30 min