Crop Protection 28 (2009) 533–538

Contents lists available at ScienceDirect

Crop Protection

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

Evaluation of systemic acquired resistance inducers for control of *Phytophthora capsici* on squash

D. Koné [1], A.S. Csinos, K.L. Jackson, P. Ji*

Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, 115 Coastal Way, Tifton, GA 31794, USA

ARTICLE INFO

Article history:
Received 22 August 2008
Received in revised form
13 February 2009
Accepted 16 February 2009

Keywords:
Cucurbita pepo
DL-3-aminobutyric acid
2,6-Dichloroisonicotinic acid
Induced resistance
Salicylic acid

ABSTRACT

Phytophthora blight induced by *Phytophthora capsici* is a major constraint in vegetable production worldwide. Limited information is available regarding potential systemic acquired resistance (SAR) inducers that may provide protection of squash (*Cucurbita pepo*) plants against the disease and the direct effect of the products on the pathogen. In this study, the effect of DL-3-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), Saver (a.i. salicylic acid), Syrup (nutrient supplement), and acibenzolar-*S*-methyl (ASM) on mycelial growth, zoospore germination and sporangium production of *P. capsici* was evaluated. The products were tested in *in vitro* studies at concentrations ranging from 25 to 2000 $\mu g\,ml^{-1}$. Mycelial growth and zoospore germination were generally not significantly affected by BABA and ASM and sporangium production was not significantly affected by BABA. INA and Saver reduced mycelial growth and sporangium production significantly at 100 $\mu g\,ml^{-1}$ or higher concentrations and zoospore germination at 500 and 1000 $\mu g\,ml^{-1}$. In greenhouse studies, all the products applied as a soil drench or foliar spray at 25 or 50 $\mu g\,ml^{-1}$ significantly reduced disease severity on squash, compared with the pathogen-only control, and zoospores at a concentration of 10^3 spores ml^{-1} were used to inoculate the leaves. INA, BABA, and ASM also reduced disease significantly when zoospores at 10^3 spores ml^{-1} were used to inoculate the root. The results indicated that most of the SAR inducers did not inhibit the growth of the pathogen at concentrations generally recommended for use but had the potential to suppress the disease on squash significantly.

1. Introduction

Phytophthora blight caused by *Phytophthora capsici* is a devastating disease and a major constraint in the production of cucurbits, peppers, tomatoes, and several other vegetable crops throughout the world. The pathogen causes plant wilt, root rot, crown rot, seedling damping-off, leaf and stem blight, and fruit rot and results in significant yield and quality loss (Erwin and Ribeiro, 1996). The efficacy of current strategies for management of this disease is limited. No single fungicide has consistently and effectively suppressed losses related to *P. capsici* epidemics. While fungicides containing the active ingredient mefenoxam provide some level of control of *P. capsici*, mefenoxam-resistant strains of *P. capsici* have developed that challenge the usefulness of this compound (Gevens et al., 2007; Lamour and Hausbeck, 2000; Mathis, 1999). On squash (*Cucurbita pepo*), cultivars with resistance to this disease are not available. Due to its destructive nature and lack of efficient control measures, development of alternative or complementary approaches for management of this disease is highly desirable.

A control practice that has shown promise for plant disease management is the use of systemically induced plant resistance. The plant possesses a range of defences that can be activated to protect it from diseases. This defence response, termed systemic acquired resistance, can be localized at the site of application of an inducer and can also be transmitted systemically to other plant tissues (Kessmann et al., 1994). Systemic acquired resistance (SAR) inducers can be chemical compounds, metabolic substances of the host plant, or microorganisms, which induce plant resistance through activation of a plant's signalling pathways such as the salicylic acid pathway (Achuo et al., 2004; Métraux et al., 1990).

A number of SAR inducers have been evaluated for control of different plant diseases. For example, salicylic acid was shown to induce resistance to Cucumber mosaic virus (CMV) in squash and tobacco (Mayers et al., 2005). 2,6-Dichloroisonicotinic acid (INA) was effective against a wide range of pathogens and was mediated

* Corresponding author. Tel.: +1 229 386 3160; fax: +1 229 386 7285.
E-mail address: pji@uga.edu (P. Ji).
[1] D. Koné is a Fulbright visiting scientist from the University of Cocody, Ivory Coast.

0261-2194/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2009.02.005

by a salicylic acid-dependent process (Walters and Boyle, 2005). Bokshi et al. (2006) indicated that INA increased chitinase and peroxidase activities and reduced powdery mildew and downy mildew on leaves of melons. DL-3-aminobutyric acid (BABA) is a non-protein amino acid that appeared to enhance resistance in pepper against *P. capsici* and in tomato against late blight disease caused by *Phytophthora infestans* (Jeun et al., 2000; Lee et al., 2000). A BABA and mancozeb mixture exhibited a synergetic effect compared to the application of BABA or mancozeb alone in disease control (Baider and Cohen, 2003). Enhanced resistance by BABA in tomato against *P. infestans* was correlated with the accumulation of pathogenesis related proteins such as PR-1 chitinase, β-1,3-glucanase, and AP24 (Cohen et al., 1994; Jeun and Buchnauer, 2001). In recent years, a SAR inducer acibenzolar-*S*-methyl (ASM) had been studied for control of plant diseases. Application of ASM significantly reduced Phytophthora blight on pepper (Matheron and Porchas, 2000). ASM acts as a functional analog of salicylic acid in the SAR signalling pathway and enhanced expression of resistance related genes and accumulation of lignin and phenolic compounds. It also increased the activity of peroxidase, phenylalanine ammonia lyase, chalcone isomerise and reactive oxygen species (Benhamou and Nicole, 1999; Bokshi et al., 2003; Buzi et al., 2004; Malolepsza, 2006; Soylu et al., 2003). Integrated use of ASM and soil fumigation enhanced suppression of root-knot and bacterial wilt on tomato (Ji et al., 2007). Induced host resistance has the potential as an effective means for suppression of a number of diseases. However, the effect of SAR inducers on *P. capsici* and Phytophthora blight on squash has not been well documented.

This study was conducted to evaluate several SAR inducers to determine if they suppress the growth of *P. capsici in vitro* and disease development on squash. Identifying effective SAR inducers will not only provide an alternative means for control of Phytophthora blight on squash, but also provide new information to develop strategies integrating this technology with standard fungicides and other disease management approaches.

2. Materials and methods

2.1. SAR inducers

"Saver" is a product provided by Plant Food Systems, Inc. (Zellwood, FL, USA) that contains salicylic acid as the active ingredient. Maui liquid compost factor (LCF) "Syrup", provided by ABR, LLC (Puunene, HI, USA), contains a number of compounds including nitrogen (0.3%) and soluble potash (2.25%). Acibenzolar-*S*-methyl (ASM, Actigard 50WG) was provided by Syngenta Crop Protection (Greensboro, NC, USA). BABA (DL-3-aminobutyric acid) was purchased from Fluka (Buchs, Switzerland) and INA (2,6-dichloroisonicolinic acid) was purchased from Aldrich (Natick, MA, USA). Concentrations of Saver, ASM, BABA, and INA mentioned in this study are concentrations of active ingredients of the products.

2.2. Effect of SAR inducers on mycelial growth of P. capsici

A *P. capsici* strain isolated from squash in Tifton, GA, USA, was grown on V8 juice agar (V8 juice, 50 ml; $CaCO_3$, 2 g; agar, 17 g; distilled water, 950 ml) at 25 °C for 5 d. An agar plug (7 mm in diameter) was taken from the edge of the colony and placed at the centre of a potato dextrose agar (PDA) plate amended with each product at a final concentration of 0, 25, 50, 100, 500, 1000, and 2000 μg ml^{-1}. Triplicate plates were used for each concentration and the plates were incubated at 25 °C in the dark. Two perpendicular colony diameters were measured per plate 8 d after incubation and diameter of colonies was calculated for each concentration using the mean of the two perpendicular colony diameters (Keinath, 2007). The diameter of the agar plug was subtracted from the colony diameter for calculating diameter of colony. The experiments were conducted twice under the similar conditions.

2.3. Effect of SAR inducers on zoospore germination

Zoospores were produced using the methods described previously with minor modifications (Keinath, 2007). A 7-mm-diameter plug removed from the edge of an actively growing culture was placed on a V8 agar plate. The plates were incubated at 25 °C for 4 d with a 16-h photoperiod. The plates were flooded with 10 ml sterile distilled water, chilled at 4 °C for 45 min, and then held at 23–25 °C for 20 min to allow zoospore release. Five drops of zoospore suspensions were streaked on PDA amended with SAR inducers at concentrations of 0, 25, 100, 500, and 1000 μg ml^{-1}. The experiment was repeated twice with triplicate plates used for each

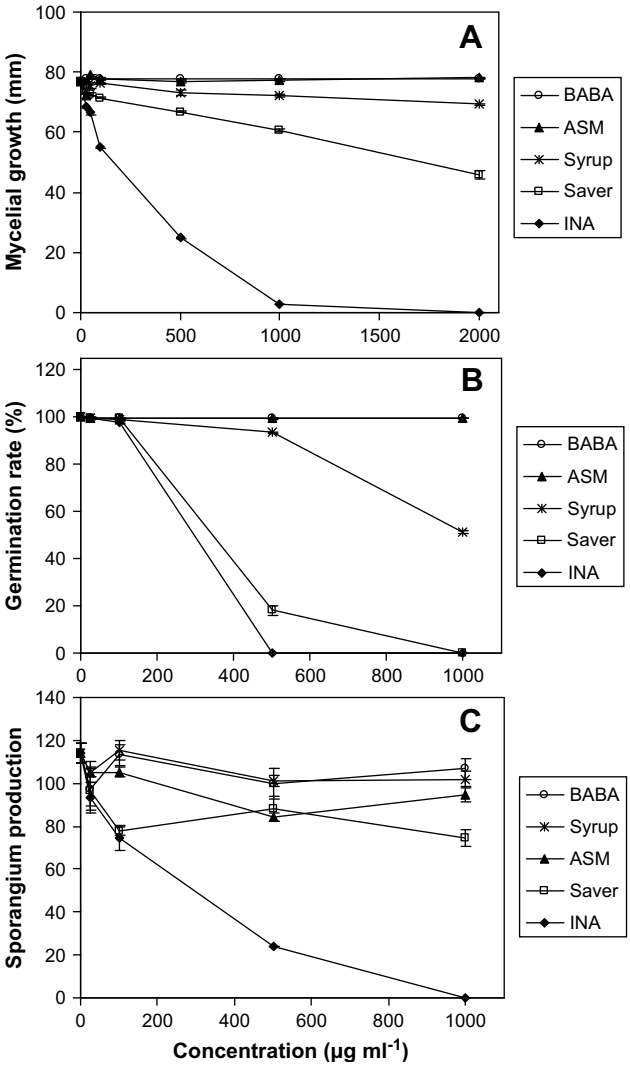

Fig. 1. Effect of different concentrations of SAR inducers on mycelial growth, zoospore germination and sporangium production of *P. capsici*. (A) Colony diameter (mm) of *P. capsici* on PDA amended with SAR inducers 8 d after incubation. (B) Zoospore germination rate (%) of *P. capsici* on PDA amended with SAR inducers 2 h after incubation. (C) Sporangium production (mean number per field under microscope) of *P. capsici* at different concentrations of SAR inducers 2 d after incubation. Data are means of two repeated experiments. The bar on each point represents the standard error of the mean and bars with length less than the diameters of the data points are not visible.