Biological Control 60 (2012) 297–304

Contents lists available at SciVerse ScienceDirect

Biological Control

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

Biocontrol of black scurf on potato by seed tuber treatment with *Pythium oligandrum*

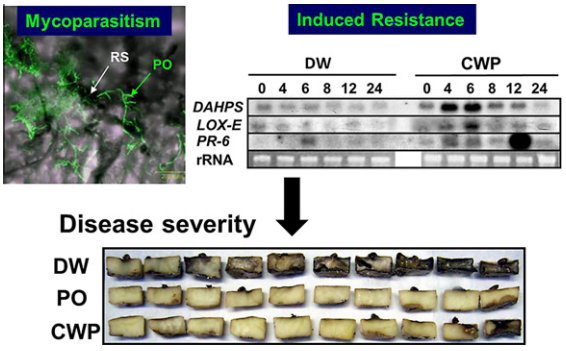
Sachiko Ikeda [a], Ayano Shimizu [b], Motoshige Shimizu [a,1], Hideki Takahashi [b], Shigehito Takenaka [c,*]

[a] Hokkaido, Research Organization, Tokachi Agricultural Experiment Station, Memuro, Hokkaido 082-0081, Japan
[b] Department of Life Science, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan
[c] Memuro Research Station, Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization (NARO), Shinsei, Memuro, Kasaigun, Hokkaido 082-0081, Japan

HIGHLIGHTS

- *Pythium oligandrum* (PO) is a practical biocontrol agent for black scurf of potato.
- Treatment of seed tubers with PO is an effective control alternative to fungicides.
- Mycoparasitism of *Rhizoctonia solani* by PO was observed on the seed tubers.
- Treatment of tubers with PO enhanced the expression of defense-related genes.
- The biocontrol mechanisms by PO include mycoparasitism and induced resistance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 September 2011
Accepted 30 October 2011
Available online 7 November 2011

Keywords:
Biological control
Black scurf
Pythium oligandrum
Colonization
Mycoparasitism
Induced resistance

ABSTRACT

The biological control activity of *Pythium oligandrum* against black scurf of potato caused by *Rhizoctonia solani* AG-3 was evaluated in field experiments after treatment of potato seed tubers with *P. oligandrum*. Seed tubers infected with black scurf sclerotia were dipped for a few seconds in a suspension of 10^3, 10^4 or 10^5 mL^{-1} *P. oligandrum* oospores and were then air-dried. Each level of *P. oligandrum*-treatment significantly reduced the disease rates of stolon at a level similar to that achieved by chemical control. When *P. oligandrum* populations adherent to the surface of seed tubers were determined, oospore counts on tubers treated with 10^4 or 10^5 oospores mL^{-1} were about 540/cm^2 or about 22,000/cm^2 just after dipping and decreased to about 170/cm^2 or 2900/cm^2 after a 3-week incubation, respectively. Confocal laser scanning microscopic observation with an immuno-enzymatic staining procedure showed that *P. oligandrum* hyphae had colonized the sclerotia and established close contact by coiling around the *R. solani* hyphae present on the surface of seed tubers, in a manner similar to that observed in the dual-culture test. Quantification of *R. solani* DNA by PCR indicated that the *R. solani* population was reduced on the seed tubers treated with *P. oligandrum* compared to untreated tubers. Furthermore, the ability of *P. oligandrum* to induce resistance against black scurf was determined using a potato tuber disk assay. Treatment of tuber disks with the cell wall protein fraction of *P. oligandrum* enhanced the expression of defense-related genes such as 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, lipoxygenase and basic *PR-6* genes, and reduced disease severity upon challenge with *R. solani* compared with untreated controls. These results suggest that biocontrol mechanisms employed by *P. oligandrum* against black scurf involve both mycoparasitism and induced resistance.

* Corresponding author. Present address: NARO Headquarters, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8517, Japan. Fax: +81 29 838 8525.
E-mail address: stake@affrc.go.jp (S. Takenaka).
[1] Present address: Hokkaido Research Organization, Central Agricultural Experiment Station, Naganuma, Hokkaido 069-1395, Japan.

1049-9644/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2011.10.016

1. Introduction

Pythium oligandrum Drechs, a soil-inhabiting and non-pathogenic oomycete (Martin and Hancock, 1987), has been demonstrated to be an effective biocontrol agent of damping-off and root diseases caused by soil-borne pathogens such as damping-off of sugar beet caused by *Pythium ultimum* Trow (Martin and Hancock, 1987) and *Aphanomyces cochlioides* Drechs (McQuilken et al., 1990), damping-off of cress caused by *Rhizoctonia solani* Kühn (McQuilken et al., 1990), Verticillium wilt of pepper (Al-Rawahi and Hancock, 1998), and bacterial wilt of tomato (Hase et al., 2006). This oomycete has been reported to be effective against even leaf diseases caused by aerial pathogens such as gray mold of tomato (Le Floch et al., 2003a,b) and grapevine (Mohamed et al., 2007). The process of *P. oligandrum* biocontrol of these diseases is complex and seems to be pathogen- and host plant-dependent through both direct effects such as mycoparasitism, antibiosis, and competition for colonization, as well as indirect effects such as stimulation of plant defense reactions and promotion of plant growth (Rey et al., 2008). *P. oligandrum* has been reported to have antagonistic activity against a wide range of plant pathogenic oomycetes and true fungi by mycoparasitism mediated by intimate hyphal interaction and antibiosis with alternation of the host hyphae prior to contact (Benhamou et al., 1999). However, different degrees of host hyphal interactions, ranging from markedly susceptible to highly resistant to *P. oligandrum* attack, have been demonstrated (Ribeiro and Butler, 1995). *P. oligandrum* can colonize the rhizosphere, although its population density varies in different crop species and cultivars (Al-Rawahi and Hancock, 1997). Our previous study on the relationship of *P. oligandrum* to the tomato rhizosphere indicated that this oomycete was rhizosphere-competent, but did not actively spread along roots (Takenaka et al., 2008).

Using microscopic examination of the interaction between the tomato and *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici*, Benhamou et al. (1997) first showed that *P. oligandrum* has the potential to induce plant defense reactions. Later studies also indicated that *P. oligandrum* can induce plant defense reactions and resistance to fungal and bacterial pathogens such as *Botrytis cinerea* Pers. in tomato (Le Floch et al., 2003b) and grapevine (Mohamed et al., 2007), and *Ralstonia solanacearum* (Smith) in tomato (Hase et al., 2006, 2008). Two types of elicitor protein have been identified in *P. oligandrum*, and their biological and molecular characteristics were determined. One type was an extracellular elicitor protein, termed oligandrin, which was secreted into the culture filtrates (Picard et al., 2000). Another type of elicitor protein from *P. oligandrum* was contained in the cell wall protein fraction (CWP) (Takenaka et al., 2003, 2006). Furthermore, this oomycete is also able to enhance plant root growth via production of auxin-like compounds, including tryptamine (Le Floch et al., 2003a). These advantageous traits of *P. oligandrum* suggested that it would be beneficial to test whether *P. oligandrum* is consistently able to control black scurf on potato under the field condition.

In Japan, potatoes are cultivated mainly in the Hokkaido region, where black scurf caused by *R. solani* Kühn is one of the most serious potato diseases reducing the marketable tuber yield. Isolates of *R. solani* from potato in the Hokkaido region most often belong to the strain AG-3 (Abe and Tsuboki, 1978), although other AG groups should be of the pathogen found in other countries (Tsror, 2010). Typical disease symptoms include death of pre-emergent sprouts, cankers on underground stem parts and stolons, diminished root systems, and production of progeny tubers with sclerotia. Seed tuber treatment of potatoes with fungicides can be effective in controlling black scurf and is the conventional method used in the Hokkaido region. This treatment consists of dipping seed tubers in a fungicide solution, air-drying for a few days, and chitting for 1–2 weeks. This approach, however, has raised concerns regarding the negative impact of the chemicals on the environment and the development of fungicide resistance. One alternative approach to controlling the disease without the use of fungicides may be the use of biological control agents suitable for practical application.

In this study, we first determined whether a *P. oligandrum* oospore suspension could be adapted in place of the conventional seed tuber treatment with fungicides for control of black scurf under field conditions. Next, the hypothesized mechanisms by which *P. oligandrum* acts as a biocontrol agent for black scurf on potato were evaluated by determining the population dynamics of *P. oligandrum* and *R. solani* on the surface of seed tubers, and by observing mycoparasitism of *P. oligandrum* against *R. solani* on the surface of tubers. Further, the question of whether *P. oligandrum* could trigger defense-related genes in potato and increase resistance against black scurf was addressed.

2. Materials and methods

2.1. Fungal isolates and preparation

For the production of large quantities of *P. oligandrum* oospores, *P. oligandrum* isolate NITE P-619 (MMR2: D-type, based on the number of major proteins in CWP; Takenaka et al., 2003), isolated from soybean soil in Hokkaido Prefecture, was grown in V8 broth (100 mL Campbell's V8 juice, 1.5 g $CaCO_3$ per liter) containing 0.1% wheat germ oil at 25 °C for 4 weeks. At harvest, mycelia were washed with sterile distilled water (DW) several times, then transferred aseptically to a Waring blender cup. After homogenization at 14,000 rpm for 5 min to disrupt hyphae without causing obvious physical damage to the oospores, the oospore concentration was adjusted to 1×10^5 oospores mL^{-1} with sterile DW and stored at 4 °C. For the extraction of CWP, the NITE P-619 isolate was grown in V8 broth at 25 °C for 3 weeks, and mycelial mats were collected by filtration, washed with DW, and blotted dry with a paper towel. CWP was extracted as described previously (Takenaka et al., 2006). *R. solani* AG-3 isolate 04k02, obtained from infected potato tubers from Hokkaido prefecture, was used for DNA extraction and for inoculum. The 04k02 isolate was grown in potato dextrose broth at 20 °C for 3 weeks for DNA extraction. For the production of inoculum for field trials and potato tuber disk bioassays for biological control of black scurf, the isolate was grown in an oat grain medium (Weller and Cook, 1983) and in a barley grain medium (Pierson and Gaskill, 1961) at 20 °C for approximately 4 weeks, respectively.

2.2. Field trials

Potato (*Solanum tuberosum* L.) cultivar "Irish Cobbler" was used in the following studies. Field experiments were conducted in 2007 and 2008 at the Hokkaido Research Organization, Tokachi Agricultural Experiment Station, located in the eastern region of Hokkaido, Japan. The soil type at the experiment station is Brown Andosol, which is a well-drained, fine volcanic ash soil. The plots were arranged in a randomized complete block design with three replicates. Each plot consisted of 10.1 m^2 blocks planted with 45 tubers each. Potato seed tubers infected with *R. solani* AG-3, with approximately 1% of the tuber surface covered with sclerotia, were dipped for a few seconds in either an oospore suspension of *P. oligandrum* at 10^3, 10^4, or 10^5 mL^{-1}, or in a flutalonil solution at 4 mg active ingredient mL^{-1}, and each tuber was air-dried for a few days. The treated seed tubers were then chitted by placing them in a light and cool place for approximately 2 weeks to sprout before planting in plots. Untreated potato seed tubers were planted in control plots. The treatment with 10^3 oospores mL^{-1} was only