Biological Control 90 (2015) 193–199

Contents lists available at ScienceDirect

Biological Control

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

The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode

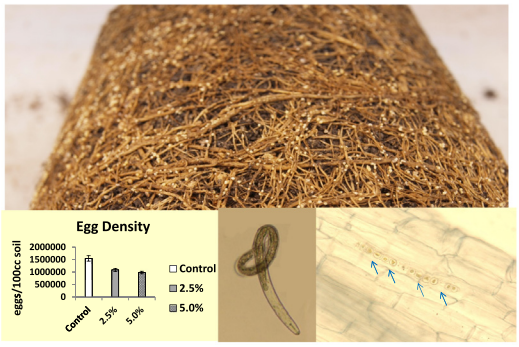
Ruchika Bajaj [a,b], Weiming Hu [c], YinYin Huang [b], Senyu Chen [c,d], Ram Prasad [a], Ajit Varma [a], Kathryn E. Bushley [b,*]

[a] Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Sector 125, Noida 201303, India
[b] Department of Plant Biology, University of Minnesota, Saint Paul, MN, 55108, United States
[c] Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[d] Southern Research and Outreach Center, University of Minnesota, Waseca, MN 56093, United States

HIGHLIGHTS

- Soil was inoculated with root endophyte *Piriformospora indica*.
- A significant decrease in egg population density observed in *P. indica* amended soil.
- *P. indica* is a promising biocontrol agent of the SCN.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 February 2015
Revised 29 May 2015
Accepted 31 May 2015
Available online 2 July 2015

Keywords:
Piriformospora indica
Soybean
Soybean cyst nematode
Biotic stress
Biocontrol

ABSTRACT

The soybean cyst nematode (*Heterodera glycines*) is a plant parasitic nematode that is a major plant pest worldwide and causes severe economic and yield losses. *Piriformospora indica*, a plant growth promoting fungus isolated from the Thar Deserts of western India, has been shown to protect a wide range of plants from various biotic and abiotic stresses. To evaluate the potential of *P. indica* to protect soybean (*Glycine max*) seedlings from damage by the soybean cyst nematode (SCN), we amended soil with two different concentrations of *P. indica* (2.5% and 5% w/w) and inoculated with second-stage juveniles (J2s) of SCN in each treatment. After 60 days, abundance of nematode eggs was measured by calculating SCN egg population densities. We found that egg density/100 cc soil was significantly decreased by 29.7% and 36.7% respectively in the soil amended with 2.5% and 5% *P. indica* compared to a control. Amendment with *P. indica* also had a strong growth and yield promoting effect in Soybean. Although root biomass was significantly decreased by 27.9% and 33.5% in the two treatments compared to the control, shoot biomass (dry weight) increased by 30.8% and 8.2% in the 2.5% and 5% *P. indica* treatments compared to the control. Additionally, plant development was accelerated and a 75% increase in flowering was observed between the 2.5% *P. indica* treatment and the control. We conclude that *P. indica* used as a soil amendment decreases abundance of the SCN in soil and has plant-growth promoting properties that may help offset yield losses due to plant parasitic nematodes.

* Corresponding author.
E-mail address: kbushley@umn.edu (K.E. Bushley).

http://dx.doi.org/10.1016/j.biocontrol.2015.05.021
1049-9644/

1. Introduction

The soybean cyst nematode (SCN), *Heterodera glycines*, is a plant-parasitic nematode and a destructive pest of soybean worldwide (Monson and Schmitt, 2004). The life cycle of the SCN has three stages: egg, juvenile, and adult. Soybeans are infected by the second-stage juveniles (J2s), the microscopic colorless worm stage that penetrates the roots with a stylet. After invading the roots, the nematodes migrate towards the vascular tissue where they feed and develop. Feeding causes changes in internal root structure and interferes with normal root function, causing plant disease (Lambert and Bekal, 2002). Approximately three weeks after infection, under optimum conditions (soil temperatures at 27–29 °C), female juveniles that are fertilized by males grow into mature females. The enlarged females burst through the root surface and lay eggs in a jelly like mass attached to their posterior end, retaining about two-thirds of the eggs within their swollen bodies. After the female dies, the cuticle becomes melanized to form a brown cyst that encloses approximately 200–400 eggs. The cysts protect the eggs from desiccation, chemicals, predators, and some parasites (Duan et al., 2009). The SCN is able to tolerate various environmental stresses, especially low temperature, primarily due to protection of eggs within the cysts.

The SCN causes the greatest yield loss worldwide of any disease or pest of soybean, with losses in the United States alone estimated at 1 billion US dollars annually (Riggs, 2004; Arelli and Wang, 2008). Various strategies have been employed to suppress the damage caused by the SCN (Porter et al., 2001). These include crop rotation and other cultural practices, resistant cultivars, and nematicides (Koenning et al., 1993; Riggs and Schuster, 1998; Schmitt, 1991; Young and Hartwig, 1992; Young, 1998a,b). Ross (1962) reported that crop rotation with non-hosts of the SCN such as corn (*Zea mays*), wheat (*Triticum aestivum*), or grain sorghum (*Sorghum bicolor*) was an effective management strategy. However, resistant cultivars are costly to produce and the SCN may evolve resistance within a short period of time (Zheng and Chen, 2011). While a number of highly effective chemical nematicides, including fumigants and non-fumigants, have been deployed over the past 30 years, the most effective compounds (e.g., methyl bromide) have been banned or restricted due to environmental and health concerns, as many are toxic to mammals, including humans. Various fungal and bacterial pathogens of nematodes have also been employed as potential biocontrol agents, with variable or limited success (Chen and Dickson, 2012). Consequently, plant-parasitic nematodes are currently among the most difficult crop pathogens to manage and there is a great need for development of nontoxic, inexpensive, and effective control methods.

Piriformospora indica is a root endophyte that was isolated from the Thar Desert of western India (Verma et al., 1998; Varma et al., 1999). This fungus has growth promoting effects mimicking those of arbuscular mycorrhizal fungi (Mishra et al., 2014; Chadha et al., 2014) and increases biomass and yield of many plant species (Malla et al., 2004; Varma et al., 2014). It colonizes a wide range of plants including gymnosperms, angiosperms and orchids (Ye et al., 2014) and improves growth through increased nutrient uptake (N and P) of the host plants (Sherameti et al., 2005; Yadav et al., 2010). It has also been shown to enhance the production of protective secondary metabolites like podophyllotoxins in *Linum album* (Baldi et al., 2008), bacosides in *Bacopa monniera* (Prasad et al., 2008a, 2013), and curcumin and volatile oils in *Curcuma longa* (Bajaj et al., 2014). All these compounds may induce local and systemic resistance (Deshmukh et al., 2006), providing increased resistance to biotic stresses such as cyst nematodes and other plant pathogens as well as abiotic stresses such as acidity, heavy metals and drought (Vadassery et al., 2009a,b). The fungus has been shown to confer resistance against root and leaf fungal pathogens including *Fusarium culmorum* and *Blumeria graminis* in barley by increasing antioxidant activity (Waller et al., 2005). It also decreased disease symptoms of *F. culmorum*, *Pseudocercosporella herpotrichoides*, and *B. graminis* on wheat (Deshmukh and Kogel, 2007; Serfling et al., 2007). Recently, Daneshkhah et al. (2013) reported that inoculation of *P. indica* onto *Arabidopsis* roots *in vitro* antagonized the infection and development of cyst nematodes. In this study, we tested the ability of *P. indica* amended to soil of soybean plants to decrease reproduction, as measured by egg density, of the SCN.

2. Materials and methods

2.1. Fungus cultivation

P. indica ATCC (204458) was cultured in potato dextrose broth with constant shaking at 100 rpm at 30 °C. The mycelium was harvested 8 days after inoculation by filtration to remove liquid media.

2.2. Preparation of soil

Field soil was collected from an agricultural field with no soybean cyst nematode infestation at the Southern Outreach and Research Station in Waseca, Minnesota, USA. Soil was mixed with 30% sand and autoclaved at 121 °C for 60 min. Mycelium of *P. indica* at concentrations of 2.5% (w/w) and 5% (w/w) was thoroughly mixed with soil and placed into four 16-cm-diameter clay pots. A control treatment consisting of the autoclaved soil mixture with no *P. indica* amendment was similarly prepared. Eight soybean seeds were surface sterilized with 0.5% NaOCl for 3 min, rinsed three times in sterile water, and sown in each pot. One week after planting, seedlings were thinned to keep only five seedlings of approximately the same size and developmental stage per pot. The pots were arranged randomly and maintained in a greenhouse with a temperature ranging from 26 °C ± 4 °C with 16 h light/8 h dark. Pots and seedlings were watered daily.

2.3. Preparation and inoculation of J2s

SCN race 3 nematodes, which were originally collected from a field at the Southern Outreach and Research Station in Waseca, Minnesota, USA, were cultured on soybean plants in sterilized soil in a greenhouse. A soil suspension containing newly formed cysts was poured into a 2-liter jug and sprayed with a strong jet of water. This suspension was allowed to settle for 5–10 s and then poured onto a 850-μm-pore (#20) sieve nested on top of a 250-μm-aperture (#60) sieve. This procedure was repeated 5 times to ensure that all cysts were collected. The cysts were then sprayed with water on the 850-μm-aperture screen to remove root debris and collected onto a 250-μm-aperture sieve. The cysts were washed from the 250-μm-aperture sieve into a 50 mL centrifuge tube with 63% (w/v) sucrose and centrifuged at 1100×*g* for 5 min. The cysts floating on the top of these tubes were collected into a tube mounted with a 150-μm-pore screen, and eggs were released by crushing the cysts on a 150-μm-aperture sieve with a rubber stopper mounted on a motor (Faghihi and Ferris, 2000). The eggs were cleaned and separated from debris by centrifugation in a 38% (w/v) sucrose solution for 5 min at 1500×*g* to remove most of the remaining debris. The collected eggs were transferred onto a 38-μm-aperture sieve, rinsed with sterile water, treated with SCQ antibiotic solution (100 ppm streptomycin sulfate, 50 ppm chlorotetracycline, and 20 ppm 8-quinolinol) for 24 h at 4 °C. They were then placed on a 35-μm-aperture nylon cloth on a screen immersed in 4 mM $ZnCl_2$ solution to hatch J2s. The