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BIOCONTROL OF COMMON ROOT ROT OF WHEAT WITH *LYSOBACTER ENZYMOGENES* AND BINUCLEATE *RHIZOCTONIA*

Cafer Eken[1] and Gary Yuen[2]

[1]Department of Agricultural Biotechnology, Faculty of Agriculture, Süleyman Demirel University, 32260 Isparta, Turkey. E-mail: cafereken@sdu.edu.tr

[2]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583

ABSTRACT

***Bipolaris sorokiniana* and *Rhizoctonia solani* AG-4 are associated with common root rot of wheat. Bacterial strain *Lysobacter enzymogenes* C3 and the binucleate *Rhizoctonia* strain BNR-8-2 were evaluated for their biocontrol activities against common root rot under greenhouse conditions. In greenhouse trials, the biocontrol agents were evaluated for reducing disease on wheat cultivars Russ and Alsen planted in sterilized soil artificially infested with the pathogens. Four weeks after sowing, disease severity was evaluated. *L. enzymogenes* C3 and the binucleate *Rhizoctonia* BNR-8-2 were applied as single strains and a 2-strain mixture. Statistical analysis of data indicated that, treating wheat cultivars with *L. enzymogenes* C3, the binucleate *Rhizoctonia* BNR-8-2 and mixture provided significant protection against common root rot. No differences ($P<0.01$) were found between Russ and Alsen for reaction to the diseases. The ability of these isolates to affect the infection of wheat seedling by *R. solani* AG-4 and *B. sorokiniana* may be of potential value in field trials.**

Key words: biological control, *Rhizoctonia solani*, *Bipolaris sorokiniana*, *Lysobacter enzymogenes*, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the world's most important crops. Common root rot is a well-known major disease of wheat in many areas, including the USA and Turkey (Wiese, 1987; Tunali et al., 2008). Disease symptoms include necrosis of basal stems, crowns, subcrown internodes and roots. Many different fungal species are associated with the disease, but the most prevalent pathogen is *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex. Dastur] (Wildermuth, 1986; Wiese, 1987; Eken and Demirci, 1998). *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is also associated with root rot (Wiese, 1987; Mazzola et al., 1996). *R. solani* isolates are divided into anastomosis groups (AG) based on hyphal fusion between compatible strains. Although strains of *R. solani* belonging to several anastomosis groups have been isolated from wheat roots (Mazzola et al., 1996), most isolates responsible for root rot belong to AG-4 in the USA and Turkey (Tuncer and Erdiller, 1990; Mathieson and Rush, 1991; Rush et al., 1994; Demirci, 1998).

Resistance, chemical treatments and agricultural practices such as crop rotation are the main strategies for disease management. The use of biocontrol agents, or biopesticides, for plant disease control may not be more effective than other control methods, but is commonly encouraged for environmental and other reasons (Whipps and Lumsden, 2001). Several bacterial and fungal strains have been identified as effective biocontrol agents (Fravel et al., 1998).

Previous research demonstrated that strain C3 of *Lysobacter enzymogenes* Christensen and Cook (formerly classified as *Stenotrophomonas maltophilia*) was described as an effective biological control agent for a number of fungal plant diseases, including brown patch of turfgrass caused by *Rhizoctonia solani* (Giesler and Yuen, 1998; Yuen and Zhang, 2001), leaf spot of tall fescue caused by *Bipolaris sorokiniana* (Zhang and Yuen, 1999), bean rust caused by *Uromyces appendiculatus* (Yuen et al., 2001)

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and Fusarium head blight of wheat caused by *Fusarium graminearum* (Jochum et al., 2006), but the strain was never tested for control of common root rot caused by *Bipolaris sorokiniana* and *Rhizoctonia solani* on wheat.

This bacteria controls fungal pathogens by various mechanisms, such as production of chitinases and b-1,3-glucanases (Zhang and Yuen, 2000a; Zhang et al., 2001; Palumbo et al., 2005), antibiotics (Zhang and Yuen, 2000b) or by induction of systemic resistance (Kilic-Ekici and Yuen, 2003; 2004).

Isolates of binucleate *Rhizoctonia* (BNR) have binucleate cells, and the perfect state is species of *Ceratobasidium*. BNRs, like *R. solani*, are grouped on the basis of hyphal anastomosis (García et al., 2006). BNRs have demonstrated control of Rhizoctonia diseases and other fungal pathogens on various crops under both controlled environment and field conditions (Sneh, 1996; García et al., 2006). Researchers have suggested that the mechanism of biocontrol may be due to competition for nutrients or induced host resistance (Burpee and Goulty, 1984; Cardoso and Echandi, 1987; Escande and Echandi, 1991; Herr, 1995; Poromarto et al., 1998; Jabaji-Hare and Neate, 2005).

The objectives of this research were to compare the efficacy of *L. enzymogenes* strain C3 and BNR isolate BN-8-2 for control of *B. sorokiniana* and *R. solani* AG-4 common root rot on wheat cultivars under the greenhouse conditions.

MATERIAL AND METHODS

General microbial methods

All strains of *Bipolaris sorokiniana*, *Rhizoctonia solani*, binucleate *Rhizoctonia* and *Lysobacter enzymogenes* C3 were obtained from the collection of Gary Yuen's biological control laboratory. Strain C3 was stored at -70^{o}C in nutrient broth containing 10% glycerol. For experiment, a fresh culture of a strain was grown on tryptic soy agar (TSA) for 2 days at 25^{o}C. For application to plants, cells were suspended in sterile 0.01 M potassium phosphate buffer, pH 7.0. The surfactant Soydex 937 (Setre Chemical Co., Memphis, TN) was added to suspensions at 0.25% v/v to aid cell dispersal. The surfactant did not affect the viability of *B. sorokiniana*, *R. solani*, binucleate *Rhizoctonia* and *L. enzymogenes* C3. Cell density was determined by turbidity measured on a spectrophotometer at 595 nm and then adjusted to 10^9 CFU/ml. *B. sorokiniana*, *R. solani* AG-4 and binucleate *Rhizoctonia* (BNR-8-2) were stored on colonized tall fescue seed at -20^{o}C and cultured on potato dextrose agar (PDA) at 25^{o}C. Virulence of *B. sorokiniana*, *R. solani* AG-4 and binucleate *Rhizoctonia* (BNR-8-2) on wheat was determined on water agar plate methods (Eken and Demirci, 2004). Binucleate *Rhizoctonia* was not pathogenic on wheat seeds (cvs. Alsen and Russ), whereas isolates of *B. sorokiniana* and *R. solani* were pathogenic to wheat seeds.

Preparation of *B. sorokiniana*, *R. solani* AG-4 and binucleate *Rhizoctonia* (BNR-8-2) inoculums were made as follows: tall fescue seeds were autoclaved (100 g in 500-ml Erlenmeyer flasks), and then were inoculated with 6-mm-diameter mycelia plug from the margins of 5-day-old PDA cultures and incubating the seed cultures at 22^{o}C for 1 month. Colonized seeds were air dried for 7 days at room temperature, milled in a blender to pass through a 4-mm sieve, and kept at 4^{o}C in darkness until used.

Greenhouse experiments

Wheat seeds (cvs. Alsen and Russ) were surface sterilized in 1% sodium hypochlorite for 5 minutes and then air dried in a laminar flow. Eight wheat seeds were sown per 15-cm-diameter pot, each containing steam-pasteurised growth medium (equal volumes loam soil, vermiculite, and sand). The plants were grown for 30 days in the greenhouse under natural light at an average temperature of 25^{o}C.

Experiments with isolates of *B. sorokiniana* and *R. solani* AG-4 were conducted separately using a randomised complete block design with four replications (one cup per replication). In the experiment with *B. sorokiniana*, treatments were *B. sorokiniana* alone, *B. sorokiniana* + *L. enzymogenes*, *B. sorokiniana* + binucleate

Rhizoctonia, *B. sorokiniana* + binucleate *Rhizoctonia* + *L. enzymogenes*, *L. enzymogenes* alone, binucleate *Rhizoctonia* alone and a control with only wheat. Similarly, in the experiment with *R. solani* AG-4, treatments were *R. solani* alone, *R. solani* + *L. enzymogenes*, *R. solani* + binucleate *Rhizoctonia*, *R. solani* + binucleate *Rhizoctonia* + *L. enzymogenes*, *L. enzymogenes* alone, binucleate *Rhizoctonia* alone and a control with only wheat.

The bacterial cells suspensions (50 ml per pot) were used for soil drenching. The fungal inoculums were added to all experiments at 1% (w/w). *L. enzymogenes* and binucleate *Rhizoctonia* were added to the soil in pot 24 h before sowing seeds. Control treatments were inoculated with sterile distilled water.

Thirty days after sowing, the plants were harvested and the roots were washed free of soil, disease was assessed on roots, crown and subcrown internode tissues. Severity of disease was evaluated on a scale of 0 to 4 in which 0 = no symptoms, 1 = traces of superficial discoloration, 2 = one or more small lesions (<0.5 cm), 3 = one or more large lesions (>0.5 cm) and 4 = girdling lesions (Demirci, 1998). After the termination of all greenhouse experiments, isolations were made from inoculated plants to determine the presence of *R. solani*, *B. sorokiniana*, *L. enzymogenes* and binucleate *Rhizoctonia*.

The experiment was conducted twice and data were analysed by ANOVA. Mean separation was accomplished using the LSD multiple range test (P<0.01). Since the results from repeated experiments were similar, the average of both trials was presented.

RESULTS AND DISCUSSION

Cultivars varied in response to inoculation with *R. solani* and *B. sorokiniana* (Table 1). The average disease severity of Alsen (3.50) was greater than the Russ (2.98) when inoculated with *R. solani*. However, the average disease severity of Russ (3.10) was greater than the Alsen (2.28) when inoculated with *B. sorokiniana*. Overall, *R. solani* AG-4 caused significantly greater disease severity than *B. sorokiniana*. On the other hand, *L. enzymogenes* strain C3 and BNR isolate BN-8-2 showed no differentiation in disease severity compared to control plants.

Table 1. Efficacy of *L. enzymogenes* strain C3 and BNR isolate BN-8-2 disease severity caused by *B. sorokiniana* and *R. solani* AG-4 on cvs. Russ and Alsen

Treatment	Wheat cultivars and disease severity[y]	
	Russ	Alsen
Bs[z]	3.10	2.28
Bs + C3	1.53	1.50
Bs + BNR	1.35	1.48
Bs + BNR + C3	1.58	1.30
Rs	2.98	3.50
Rs + C3	1.50	1.73
Rs + BNR	1.20	1.30
Rs + BNR + C3	1.25	1.08
C3	1.10	1.20
BNR	1.08	1.13
Control	1.10	1.13
LSD (P<0.01)	0.54	0.55

[y]Disease severity was on a scale of 0-4; 0= no symptoms, 1= traces of superficial discoloration, 2= one or more small lesions (<0.5 cm), 3= one or more large lesions (>0.5 cm) and 4= girdling lesions (Demirci, 1998).

[z]Bs: *Bipolaris sorokiniana*, C3: *Lysobacter enzymogenes* strain C3, BNR: Binucleate *Rhizoctonia* strain BNR-8-2 and Rs: *Rhizoctonia solani* AG-4

L. enzymogenes strain C3 treatments reduced disease severity (P<0.01) of wheat cultivars (Russ and Alsen) compared with treatments with *B. sorokiniana* and *R. solani* alone. Biocontrol potentials of fungal plant diseases by *L. enzymogenes* strain C3 have been reported by many researchers (Giesler and Yuen, 1998; Zhang and Yuen, 1999; Yuen and Zhang, 2001; Yuen et al., 2001; Jochum et al., 2006).

Similarly, BNR isolate BN-8 treatments reduced disease severity (P<0.01) of wheat cultivars compared with treatments with *B. sorokiniana* and *R. solani* AG-4 alone (Table 1). Some BNRs have been reported as

biocontrol agents of *Rhizoctonia* diseases and other fungal pathogens on various crops under both controlled environment and field conditions (Sneh, 1996; García et al., 2006). For example, BNR (AG-K) isolate BN-8-2 has been reported as potential candidate for biocontrol of *R. solani* (Khan et al., 2005).

L. enzymogenes strain C3 applied in combination with BNR isolate BN-8 reduced disease severity ($P<0.01$) of wheat cultivars (Russ and Alsen) compared with treatments with *B. sorokiniana* and *R. solani* AG-4 alone (Table 1). Our data suggest that combined use of *L. enzymogenes* strain C3 and BNR isolate BN-8-2 played a more important role than applied alone in biocontrol of wheat common root rot.

To improve biocontrol activity, use of mixtures of biocontrol agents may be important. Mixtures of biological control agents may enhance the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community, and effectiveness over a wider range of environmental conditions. For example, *L. enzymogenes* strain C3 has the potential to inhibit fungi through lytic activity from chitinases and β-1,3-glucanases (Zhang and Yuen, 2000a; Zhang et al., 2001; Palumbo et al., 2005), antibiotics (Zhang and Yuen, 2000b) or by induction of systemic resistance (Kilic-Ekici and Yuen, 2003; 2004). Additionally, researchers have suggested that the mechanism of biocontrol by BNR may be due to competition for nutrients or induced host resistance (Burpee and Goulty, 1984; Cardoso and Echandi, 1987; Escande and Echandi, 1991; Herr, 1995; Poromarto et al., 1998; Jabaji-Hare and Neate, 2005).

According to several studies, combined use of biocontrol bacteria and fungi can reveal improved biocontrol of different plant pathogens (Park et al., 1988; Lemanceau and Alabouvette, 1991; Duffy et al., 1996; Leeman et al., 1996; Dar et al., 1997).

CONCLUSIONS

In the present work, effects of *L. enzymogenes* strain C3 and BNR isolate BN-8-2 against wheat common root rot (*R. solani* and *B. sorokiniana*) were studied under greenhouse conditions. Especially, *L. enzymogenes* strain C3, alone or combined with BNR isolate BN-8, can be a potential tool in integrated control systems against wheat common root rot. The results suggested the potential of *L. enzymogenes* strain C3 and BNR isolate BN-8-2 to be developed as a promising commercial biological control agent in the future.

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