

EVALUATION OF PLANT GROWTH-PROMOTING AND BIOPROTECTING RHIZOBACTERIA ON WHEAT CROP

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ABSTRACT

Experiments were carried out under laboratory, growth chamber, and field conditions to evaluate the effect of Plant growth-promoting and bioprotecting rhizobacteria (PGPBR) seed treatment on seed pathogens, seed germination, plant growth, and grain yield of wheat (*Triticum aestivum*). Most of the PGPBR strongly reduced the recovery of the pathogens from infected wheat seeds. All treatments, except the chemical iprodione + thiram, significantly promoted plant growth over the nontreated control. *Pseudomonas putida* biotype A (11) and *P. agglomerans* (14) showed the greatest effects. Field experiments, carried out at two locations, indicated that all treatments, except *P. chlororaphis* (42), significantly increased seedling emergence

of wheat. In Pato Branco, PR, *P. putida* biotype A (11) and *P. putida* biotype B (44) presented the best results, both being superior to fungal biological and chemical treatments. In Passo Fundo *P. putida* biotype A (11) and *P. putida* biotype B (17 and 44) significantly improved yield over the nontreated control. Yield increases of these three PGPBR were similar to the chemical treatment iprodione + thiram. In Pato Branco, *P. putida* biotype A (11) and *P. putida* biotype B (17), as well as the chemical treatment, provided significant increase over the nontreated control. Yield increases by the PGPBR varied from 18% to 22% in Passo Fundo and from 27% to 28% in Pato Branco.

Key words: wheat, *Triticum aestivum*.

RESUMO

Avaliação de rizobactérias promotoras do crescimento e bioprotetoras em trigo

Experimentos foram realizados em laboratório, em câmara de crescimento e em campo com o objetivo de avaliar o efeito do tratamento de sementes com Rhizobactérias Promotoras de Crescimento de Plantas e Bioproteção (RPCPBs) nos patógenos de sementes, na germinação de sementes, no crescimento de plantas e no rendimento de grãos de trigo (*Triticum aestivum*). A maioria das RPCPBs reduziu drasticamente a contaminação com fungos patogênicos presentes nas sementes de trigo. Todos os tratamentos, exceto o controle químico, promoveram o crescimento de plantas quando comparados com a testemunha não tratada. Os tratamentos *Pseudomonas putida* biótipo A (11) e *Pantoea agglomerans* (14) apresentaram os melhores efeitos. Experimentos ao nível de campo, em dois locais, indicaram que todos os tratamentos, com exceção de *Pseudomonas chlororaphis* (42), aumentaram significati-

vamente a emergência de plântulas. Em Pato Branco, PR o melhor tratamento foi *P. putida* biótipo A (11). Em Passo Fundo, *P. putida* biótipo A (11) e *P. putida* biótipo B (44) foram os mais eficientes. Os tratamentos *P. putida* biótipo A (11) e *P. putida* biótipo B (17 e 44) significativamente melhoraram o rendimento de grãos quando comparados com a testemunha não tratada em Passo Fundo. Os aumentos de rendimento dessas três RPCPBs foram similares ao do tratamento químico. Em Pato Branco, *P. putida* biótipo A (11), *P. putida* biótipo B (17) e o tratamento químico proporcionaram aumentos significativos no rendimento de grãos quando comparados com o da testemunha não tratada. Os aumentos de rendimento devido às RPCPBs variaram entre 18% e 22% em Passo Fundo e entre 27% e 28% em Pato Branco.

INTRODUCTION

The most important seed-transmitted pathogenic fungi of wheat [*Triticum aestivum* (L.) Thell.] in Brazil are: *Bipolaris sorokiniana* Sacc. in Sorok. (Shoem.), *Stagonospora nodorum*

(Berk) Cast. & Germ., *Drechslera tritici-repentis* (Died.), and *Fusarium graminearum* Schw (Luz *et al.*, 1976; Luz, 1987). A generally recommended practice to protect against these fungi is the fungicide seed treatment. (Recomendações da Comissão Sul-Brasileira de Pesquisa de Trigo - 2000). However, chemical treatment is known to disrupt the natural equilibrium within living microbial communities. In addition,

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chemical pesticides have adverse effect on animal and human health. (Luz, 1996)

Plant growth-promoting and bioprotecting rhizobacteria (PGPBR) are one of the possibilities in overcome these problems. The concept of PGPBR, recently created by Luz *et al.* (1998), is intended to encompass both PBPR (Plant Bioprotection Promoting Rhizobacteria) (Luz, 1996), which are rhizobacteria that promote the protection against major plant pathogens, and PGPR (Plant Growth Promoting Rhizobacteria) (Kloepper & Schroth, 1978), which are rhizobacteria that promote beneficial effects on plant growth through control of deleterious microorganisms (minor pathogens). Several PGPBR have been studied for protecting wheat seeds in Brazil, and the potential of such microbial agents has already been reported (Luz, 1991, 1993a,b, 1994, 1996; Luz *et al.*, 1998).

The objective of the present study was to evaluate the effect of PGPBR on seed-borne pathogen, growth stimulation, seedling emergence, and grain yield of wheat.

MATERIAL AND METHODS

Seeds of wheat cultivar Embrapa 24 associated with *B. sorokiniana*, *D. tritici-repentis*, and *F. graminearum* were obtained from the Basic Seed Production Service-Embrapa, Passo Fundo, RS, Brazil. The following PGPBR were used: *Pseudomonas putida* (Trevisan) Migula biotype B (17 e 44), *P. putida* biotype A (11), *P. fluorescens* Migula biotype G (42), *Pse. chlororaphis* Guignard & Sauvageau (42), *Pantoea agglomerans* Gavini (14) (all from Embrapa Trigo, Passo Fundo, RS, Brazil), and *Bacillus subtilis* Ehrenberg (Kodiak HB, provided by Gustafson Co., Texas, USA at a dosage of 300 g per 100 kg of seed). Three checks were used: a nontreated control, a fungal control agent (*Trichoderma harzianum* Rifai T-22 Planter box, provided by Bioworks, Inc., Geneva, New York, USA), (180 g/100 kg of seed), and a chemical treatment (Iprodione + Thiram 150 g/100 kg of seeds).

Colonies of each PGPBR were grown on nutrient agar, for 24 h at 24 ± 2 °C. Bacterial cells were removed from the surface of the culture media with a brush and placed in sterile distilled water. The concentration of each PGPBR was approximately 10^7 CFU/ml. A suspension was then applied by dipping the seeds for 3 min and allowing them to dry at room temperature for 24 h. Nontreated seeds were embedded in sterile distilled water, for 3 min, and allowed to dry. For Kodiak HB, T-22 and iprodione + thiram, the correct amount of product was mixed in a plastic bag by continuous shaking for 3 min until the plastic became clear and the kernels were uniformly coated. For the laboratory experiment, each treatment was replicated four times (at 100 grains, ten grains per plate) and placed under UV light for a photoperiod of 12 h at 24 ± 2 °C. The experimental design was completely randomized. The presence of each pathogenic fungi was determined five days after plating on nutrient-agar. Data were expressed as a percentage of pathogens recovered from the plated seeds. The growth promotion experiment was done in

growth chamber using the same treatments as its laboratory counterpart. Treatments were applied on noninfected seeds of the wheat cultivar PG1. The experimental design was completely randomized with five replicates of 20 seeds sown, spaced 20 cm apart in autoclaved soil in aluminum trays. Plant height was evaluated 35 days after planting. The data were subjected to variance analysis and the means were separated by Fisher's LSD test at $p < 0.05$.

Field experiments were carried out at two locations: Passo Fundo, RS, and Pato Branco, PR. Seeds of each treatment were manually sown in plots of 12 rows, 3 m long. The space between rows was 20 cm and the amount of seeds was equivalent to 120 kg per hectare. Fertilizers were used following soil analysis and at recommended dosages of NPK. Treatments in each experiment were arranged in a randomized block design. Emergence was measured 21 days after sowing. At maturity, eight central rows of each plot were harvested and yield was determined by kg/ha. The data were subjected to variance analysis and the means were separated by Fisher's LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Data from the laboratory experiment (Table 1) indicated that nontreated wheat seeds were severely contaminated, showing 10% of *D. tritici-repentis*, 9% of *F. graminearum* and 7% of *B. sorokiniana*. Most of the PGPBR greatly reduced the recovery of pathogens from infected wheat seeds whereas *T. harzianum* (T-22) was slightly effective. The treatments *P. putida* biotype B (44 and 17), *Pse. chlororaphis* (42), and *P. putida* biotype A (11) were equivalent to the chemical treatment, providing the best results against the wheat seed pathogens (Table 1). Little is known about the mechanisms of biocontrol using the isolates of the present work. The following effects have been shown by other isolates: antibiosis, competition, niche exclusion, pathogen adherence, inactivation of fungal propagule stimulants present in the seed exudates and parasitism (Luz, 1996). Antibiotics, however, may be playing the most significant role in this particular experiment.

The effects of PGPBR on plant growth (Table 2) showed that all treatments, except the chemical iprodione + thiram, promoted plant growth when compared with the nontreated control. *Pseudomonas putida* biotype A (11) and *P. agglomerans* (14) showed the greatest effects. These data illustrate another way by which PGPBR act on plants, that is, stimulating direct growth without the presence of pathogens (Kloepper, 1991, 1993; Luz 1996). This may involve mechanisms such as soil mineralization, nitrogen fixation and phytohormones described for several PGPBR (Luz, 1996; Kloepper, 1991; 1993).

Data from field experiments (Tables 3 and 4) showed that both locations, all treatments, except *P. chlororaphis* (42), significantly increased seedling emergence of wheat (Table 3). In Pato Branco, the best treatment was *P. putida* biotype A (11). In Passo Fundo, *P. putida* biotype A (11) and *P. putida*

TABLE 1 - Effect of seed-applied PGPBR on pathogen recovery from infected wheat (*Triticum aestivum*) seeds. Passo Fundo, RS, Brazil

Treatment	Recovery ¹ from seeds (%)		
	D.t-r ²	F. gram. ²	B.s. ²
Nontreated	10 d	9 d	7 c
<i>Pseudomonas putida</i> biot B (44)	0 a	0 a	1 a
<i>P. putida</i> biot A (11)	0 a	0 a	1 a
<i>Bacillus subtilis</i> (Kodiak HB) ³	3 b	4 b	5 b
<i>Pantoea agglomerans</i> (14)	3 b	3 b	1 a
<i>P. fluorescens</i> biot G (42)	2 b	3 b	4 b
<i>P. putida</i> biot B (17)	0 a	0 a	0 a
<i>Trichoderma harzianum</i> (T 22 Planter box) ⁴	8 c	7 c	5 b
<i>P. chlororaphis</i> (42)	1 a	2 b	1 a
Iprodione + thiram ⁵	0 a	0 a	0 a
C.V.%	2.4	3.2	2.5

¹ Means of 5 replicates of 100 seeds. Means followed by different letters are different according to Fisher's LSD test at p < 0.05.

² D.t-r = *Drechslera tritici-repentis*, F. gram. = *Fusarium graminearum*. B.s. = *Bipolaris sorokiniana*.

³ 300 g/100 kg of seeds

⁴ 180 g/100 kg of seeds

⁵ 150 g/100 kg of seeds

TABLE 2 - Effect of seed-applied PGPBR on wheat (*Triticum aestivum*) growth under controlled conditions. Passo Fundo, RS, Brazil

Treatment	Plant height ¹ (cm)
Nontreated	26.5 c
<i>Pseudomonas putida</i> biot A (11)	29.6 a
<i>P. putida</i> biot B (17)	28.6 ab
<i>P. putida</i> biot B (44)	28.8 ab
<i>P. chlororaphis</i> (42)	28.2 ab
<i>P. fluorescens</i> biot G (42)	28.9 ab
<i>Pantoea agglomerans</i> (14)	29.2 a
<i>Bacillus subtilis</i> (Kodiak HB) ²	27.8 b
<i>Trichoderma harzianum</i> (T 22 Planter box) ³	27.7 b
Iprodione + thiram ⁴	26.2 c
CV %	9.6

¹ Plant height in centimeter of cultivar PG1, 35 days after sowing. Average of 5 replicates. Means followed by different letters are different according to Fisher's LSD test at p < 0.05.

² 300 g/100 kg of seeds

³ 180 g/100 kg of seeds

⁴ 150 g/100 kg of seeds

biotype B (44) were the best treatments, both being superior to a fungal biocontrol agent and chemical treatment.

The effects of the treatments on wheat grain yield at both locations (Table 4) showed that the treatments *P. putida* biotype A (11), and *P. putida* biotype B (17 and 44) significantly improved yield over the nontreated control in Passo Fundo. Yield increases of these three biological agents were similar to the chemical treatment iprodione + thiram. In Pato Branco, *P. putida* biotype A (11) and *P. putida* biotype B (17), as well as the chemical treatment, provided significant increase over the nontreated control (Table 4). Yield increases by the PGPBRs varied from 18% to 22% in Passo Fundo and from 27% to 28% in Pato Branco.

In the present experiments PGPBR reduced the population of pathogens associated with seeds, stimulated

TABLE 3 - Effect of seed-applied PGPBR on wheat (*Triticum aestivum*) seedling emergence under field conditions. Passo Fundo, RS, and Pato Branco, PR, Brazil

Treatment	Seedling emergence ¹	
	Passo Fundo	Pato Branco
Nontreated	251 d	241 d
<i>Pseudomonas putida</i> biot B (44)	285 a	263 bc
<i>P. putida</i> biot A (11)	284 a	276 a
<i>Bacillus subtilis</i> (Kodiak HB) ²	272 b	261 bc
<i>Pantoea agglomerans</i> (14)	268 bc	260 bc
<i>P. fluorescens</i> biot G (42)	268 bc	260 bc
<i>Pseudomonas putida</i> biot B (17)	267 bc	268 ab
<i>Trichoderma harzianum</i> (T 22 Planter box) ³	262 bc	257 bc
<i>P. chlororaphis</i> (42)	258 cd	253 cd
Iprodione + thiram ⁴	269 bc	263 abc
C.V.%	3.19	3.30

¹ Means of 2 central rows of 3 m. Means followed by different letters are different according to Fisher's LSD test at p < 0.05.

² 300 g/100 kg of seeds

³ 180 g/100 kg of seeds

⁴ 150 g/100 kg of seeds

TABLE 4 - Effect of seed-applied PGPBR on wheat grain yield under field conditions. Passo Fundo, RS, and Pato Branco, PR, Brazil

Treatment	Yield (kg/ha) ¹	
	Passo Fundo	Pato Branco
Nontreated	1817 b	1528 b
<i>Pseudomonas putida</i> biot A (11)	2180 a	1959 a
<i>P. putida</i> biot B (17)	2135 a	1938 a
<i>P. putida</i> biot B (44)	2213 a	1605 b
<i>P. chlororaphis</i> (42)	1869 b	1552 b
<i>P. fluorescens</i> biot G (42)	1821 b	1560 b
<i>Pantoea agglomerans</i> (14)	1872 b	1559 b
<i>Bacillus subtilis</i> (Kodiak HB) ²	1826 b	1581 b
<i>Trichoderma harzianum</i> (T-22 Planter box) ³	1873 b	1598 b
Iprodione + thiram ⁴	2277 a	1938 a
CV %	6.5	10.3

¹ Means followed by different letters are significantly different according to Fisher's

LSD test at p < 0.05.

² 300 g/100 kg of seeds

³ 180 g/100 kg of seeds

⁴ 150 g/100 kg of seeds

plant growth, improved seedling emergence, and increased wheat grain yield. Other studies using *Paenibacillus macerans* Ash (144) and another isolate of *P. putida* biotype B for wheat seed protection against *D. tritici-repentis* (Luz *et al.*, 1998) provided similar evidence. In that study, *T. harzianum* presented no significant efficacy against *D. tritici-repentis* under laboratory conditions, but showed wheat yield enhancement (Luz *et al.*, 1998). Similar benefits from *T. harzianum* have been shown in other crops (Harman *et al.*, 1989). Beneficial effects of PGPBR and fungal bioprotectants on plants have been previously reviewed (Bakker *et al.*, 1991; Harman, 1991; Kloepper, 1991, 1993; Luz, 1993b, 1996). Other mechanisms such as hydrocyanic acid, siderophores and induction of resistance may also play a role in the action of PGPBR. Rhizobacterial agents will probably be one of the

most significant strategies for disease management in the third millennium (Luz, 1996). Therefore, the PGPBR tested in the present experiments are promising as potential plant growth stimulators and bioprotectants against wheat diseases.

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