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ORIGINAL PAPER

Improvement of growth, under field conditions, of wheat inoculated with *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1

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Abstract Effects of inoculation of wheat (*Triticum aestivum* L.) with the rhizobacterium *Pseudomonas chlororaphis* subsp. *aurantiaca* strain SR1 (termed SR1) were studied at an experimental field site in Río Cuarto, Argentina. Treatments involved SR1 inoculation with or without nitrogen/phosphorus fertilization. Inoculation produced a significant increase in plant height and root length in early growth stages. Inoculation plus fertilization with 40 kg ha^{-1} urea/30 kg ha^{-1} diamonic phosphate ("50% dose") gave a yield increase of 636 kg ha^{-1} relative to control, and an increase of 472 kg ha^{-1} relative to fertilization with 80 kg ha^{-1} urea/60 kg ha^{-1} phosphate without inoculation. SR1 inoculation without fertilization, compared to control, produced increases of 6% in weight of 1,000 grains, 13% in number of spikes per plant, and 30% in number of grains per spike. Inoculation plus 50% dose fertilization also improved these parameters. Results of the study indicate that inoculation of wheat with SR1 improves various growth and yield parameters, and allows reduced dosage of nitrogen/phosphorus fertilizers in the field.

Keywords *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1 · Wheat · Inoculation · Plant growth promotion

Introduction

Bacteria displaying high efficiency in improving plant development and increasing tolerance to pathogenic microorganisms have been designated as "plant growth-promoting rhizobacteria" (PGPR) (Kloepper and Schroth 1978). Recent controversy has arisen regarding the types of rhizobacteria that should be considered PGPR. Suggested characteristics to define the group include high population density in the rhizosphere after plant inoculation (since a rapidly declining population has low competitive ability against native soil microflora); effective colonization potential on the root surface; promotion of plant development; suppressing effect on other soil microorganisms that are plant pathogens; absence of harmful effects on humans. Experimental and field application of rhizobacteria has resulted in significant promotion of plant development, as observed in terms of emergence, vigor, biomass, development of root systems, and increased yield (up to 30%) of crop species such as corn (Fulchieri and Frioni 1994), soybean (Dashti et al. 1998), chickpea (Del Gallo and Fabbri 1990), wheat (Luz 2001), and others (Okon and Labandera-Gonzalez 1994; Bashan 1998; Lucy et al. 2004).

The positive effects of PGPR have been correlated with increased mobilization of insoluble nutrients and consequent improvement in plant nutrient uptake (Lifshitz et al. 1987); production of plant growth regulators (Dubeikovsky et al. 1993); suppression of deleterious bacteria and phytopathogenic fungi (Weller 1998; Weller and Thomashow 1993), mediated by production of antibiotics (Hebbar et al. 1992; Thomashow et al. 1990), iron-sequestering compounds (Loper and Buyer 1991), extracellular lytic enzymes (Fridlender et al. 1993), cyanhydric acid (Voisard et al. 1989), induced systemic resistance (ISR) (Andrade et al. 1998), or competition for infection sites and nutrients (Bull et al. 1991). These mechanisms all require direct contact between the bacteria and the surface or interior of root tissues, and active state of the inoculated bacteria (Weger et al. 1995; Höflich et al. 1995).

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Studies to date suggest that positive growth responses of wheat (*Triticum aestivum* L.) to inoculation with PGPR are due in part to increased root absorption capacity. Bacterial genera studied in this regard include *Azospirillum* (Bashan and Levanony 1990; Caballero-Mellado et al. 1992), *Azotobacter* (Rai and Gaur 1988), *Bacillus* (de Freitas 2000), *Pseudomonas* (Zaidi and Khan 2005), *Clostridium* (Gasoni et al. 2001), and *Herbaspirillum* (Baldani et al. 2000).

Pseudomonas spp. have been shown to contribute to increased plant yield and reduced levels of deleterious microorganisms, in field and greenhouse studies (Rovera et al. 2008), and have therefore been extensively studied (Duijff et al. 1997; Raunskov et al. 1999; Sastry et al. 2000; Vázquez et al. 2000; Kohler et al. 2006).

The species *Pseudomonas aurantiaca* was recently reclassified as *P. chlororaphis* subsp. *aurantiaca* (Peix et al. 2007). *P. chlororaphis* subsp. *aurantiaca* strain SR1 (hereafter termed "SR1") was isolated from soybean rhizosphere by our group, in the area of Río Cuarto, Córdoba, Argentina. SR1 has the abilities to inhibit various fungal species in vitro (Rosas et al. 2001); to produce siderophores, HCN, and phytohormone-like substances; to solubilize phosphates; and to colonize root systems of various crops (Rosas et al. 2005; Rovera et al. 2008). We showed previously that SR1 maintains stable populations in the rhizosphere and the internal structure of plants (Rosas et al. 2005).

In the present study, we evaluated the effects of inoculating wheat plants with SR1 under field conditions, in terms of the above properties.

Materials and methods

Study site and field inoculation technique

Studies were performed at the experimental field site of the Universidad Nacional de Río Cuarto, Río Cuarto, Argentina, (35°07′S, 64°14′W, 421 m.s.n.m.). The soil was Haplustol fine franc-sandy type, with the following physicochemical characteristics: pH (in water), 6.30; electrical conductivity (dS/m), 0.28; organic matter, 2.56%; nitrogen from nitrates, 11.60 ppm; nitrates, 51 ppm; available P, 19.7 ppm. The site has a temperate climate, with average annual rainfall 800 mm and mean annual temperature 16–17°C.

We used a randomized complete block design with seven blocks. Each block consisted of six plots (one per treatment; each 7.20 m^2); plots were separated by a distance of 1 m. Six rows (separated by 0.20 m) per block were sowed using a plot seed drill. Seed sowing density was 120 kg ha^{-1}. Seeds were inoculated with SR1, according to a formulation prepared by *Laboratorios Biagro S.A.* (10^9 CFU g^{-1} of peat), by the following procedure: 40 g inoculant, 20 g S2 adherent (*Laboratorios Biagro S.A*), and 5 g cell protector S1 (*Laboratorios Biagro S.A.*) were mixed in 80 ml water. Twelve grams of this mixture was added to 1 kg wheat seeds, to obtain a colony count of 10^5 CFU g^{-1} seeds.

Six treatments were established in order to evaluate the promoting effect of *P. chlororaphis* subsp. *aurantiaca* SR1: (1) seed inoculated with SR1 in soil without fertilization; (2) seed without inoculation in soil fertilized with 80 kg ha^{-1} urea and 60 kg ha^{-1} diamonic phosphate ("100% dose"); (3) seed inoculated with SR1 in soil fertilized with 100% dose; (4) seed without inoculation in soil fertilized with 40 kg ha^{-1} urea and 30 kg ha^{-1} diamonic phosphate ("50% dose"); (5) seed inoculated with SR1 in soil fertilized with 50% dose; (6) seed without inoculation in soil without fertilization (control).

Weeds were removed manually. Plants were watered by a sprinkler, as needed, in all growth stages.

Growth and yield parameters

Parameters were recorded at the growth stages termed emergence of seedlings, 1.5 (5 leaves), 3.0 (tillering), and 11.4 (ripe for harvest) (Feekes International Scale—Large 1954). At emergence of seedlings stage, the number of seedlings emerging per m^2 was evaluated using a ¼ m^2 iron ring. At Feekes 1.5 and 3.0 stages, the following growth parameters were measured: length from base to tip of leaf (cm), root length (cm) (Newman 1966), number of tillers, root volume (cm^3) (measured by volume displacement of water) (Díaz Vargas et al. 2001), shoot and root fresh and dry weight (72 h at 60°C). For each parameter, mean value was calculated from 5 plants per plot (seven plots per treatment).

Yield parameters evaluated were: kg ha^{-1}, weight of 1,000 grains, number of spikes per plant, and number of grains per spike. These parameters were determined after creating clearances of 1 m at the edges of each plot, and 2 sowing lines at each side.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). When ANOVA showed treatment effects ($P < 0.05$), the least significant difference test (LSD) was applied to make comparisons among the means ($P < 0.05$). The Statgraphics Plus 4.1 program (Statistical Graphics Corp.) was used for all analyses.

Results

Effects of inoculation of wheat with SR1 were evaluated over all stages of the crop cycle. Inoculation had no effect on germination or emergence of seedlings. The number of plants per m^2 was larger for the inoculation treatment than for fertilization without inoculation (Table 1).

For Feekes 1.5 stage, increase in shoot length (up to 14%) was observed for the inoculated/ unfertilized treatment and for 50% dose fertilization. For Feekes 3.0 stage, statistically significant increases relative to control were observed, particularly for inoculation plus 100% dose fertilization, in which shoot length increased 60% (Table 2).

SR1-inoculated plants showed root length increase up to 78% in Feekes 1.5 and 75% in Feekes 3.0 stages. Root volume increased significantly in Feekes 1.5 stage for inoculation plus 100% dose fertilization (Table 3).

Results for shoot biomass were variable, but all mean values for inoculation and/or fertilization treatments were higher than those of control. Root biomass at Feekes 1.5 stage was greatly increased by inoculation plus 50% dose fertilization, to the same degree as 100% dose fertilization without inoculation. At Feekes 3.0 stage, root biomass was significantly increased by inoculation even without fertilization (Table 4).

Number of tillers was increased relative to control at Feekes 3.0 stage by inoculation with or without fertilization (Table 5).

Regarding the yield parameters, kg ha^{-1} value was significantly higher than control (by 636) for SR1 inoculation plus 50% dose fertilization, and by 865 for inoculation plus 100% dose fertilization. Inoculation with or without fertilization increased yield up to 217 kg ha^{-1} relative to control. Relative to fertilization-only treatments, inoculation increased yield by 5.5 kg ha^{-1} (Table 6). Yield-promoting effects of inoculation were also reflected in weight of 1,000 grains and number of spikes per plant. Values of these parameters for inoculation plus 50% dose fertilization were consistently higher than for control, although the differences were not statistically significant. Regarding number of grains per spike, values for inoculation treatments were always higher than for control; the highest value was observed for inoculation plus 50% dose fertilization (Table 6).

Table 1 Wheat emergence (plants per m^2)

Treatments	Emergence
1. Inoculated seeds, unfertilized soil	534.86^a
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	357.7^b
3. Inoculated plus 100% dose fertilized	571.43^a
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	384.57^b
5. Inoculated plus 50% dose fertilized	540.00^a
6. Uninoculated seeds, unfertilized soil (control)	542.00^a

^{a,b} Significant differences by LSD test ($P < 0.05$)

Table 2 Shoot length at Feekes 1.5 and 3.0 stages

Treatments	Shoot length (cm)	
	Feekes 1.5	Feekes 3.0
1. Inoculated seeds, unfertilized soil	16.94^a	20.06^{ab}
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	13.78^c	20.89^{ab}
3. Inoculated plus 100% dose fertilized	14.33^c	29.72^a
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	16.02^a	20.44^{ab}
5. Inoculated plus 50% dose fertilized	14.89^{bc}	22.11^{ab}
6. Uninoculated seeds, unfertilized soil (control)	1,483^c	18.67^b

^{a,b,c} Significant differences by LSD test ($P < 0.05$)

Table 3 Root length and volume at Feekes 1.5 and 3.0 stages

Treatments	Root length (cm)		Root volume (cm^3)	
	Feekes 1.5	Feekes 3.0	Feekes 1.5	Feekes 3.0
1. Inoculated seeds, unfertilized soil	160.40^a	339.64^a	1.77^a	4.47^a
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	87.15^b	357.50^a	1.29^b	4.23^a
3. Inoculated plus 100% dose fertilized	181.49^a	346.28^a	2.09^a	3.97^a
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	159.59^a	235.52^b	1.20^b	2.46^b
5. Inoculated plus 50% dose fertilized	150.16^a	359.30^a	1.66^a	3.69^a
6. Uninoculated seeds, unfertilized soil (control)	101.89^b	204.92^b	0.91^c	2.71^b

^{a,b,c} Significant differences by LSD test ($P < 0.05$)

Discussion

This is the first field study in Argentina of *P. chlororaphis* subsp. *aurantiaca* strain SR1 inoculation effects. The results are promising. Effects of SR1 inoculation were variable, depending on the growth or yield parameter, and the plant growth stage recorded.

The decrease in seedling emergence we observed for 50% and 100% dose fertilization without inoculation (treatments 2 and 4, Table 1) may be related to application methodology, e.g., a phytotoxic effect by direct contact of seeds with phosphorus and nitrogen. Perhaps components of some formulations used (peat, S1, S2, SR1) reduced

Table 4 Fresh and dry weight of shoots and roots at Feekes 1.5 and 3.0 stages

Treatments	Feekes 1.5				Feekes 3.0			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	Shoot (g)	Root (g)	Shoot (g)	Root (g)	Shoot (g)	Root (g)	Shoot (g)	Root (mg)
1. Inoculated seeds, unfertilized soil	0.97 ns	0.33[a]	223.43[a]	190.00[a]	7.30[ab]	1.71[bc]	1.31[ab]	536.43[a]
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	1.01 ns	0.25[bcd]	217.71[a]	128.86[bcd]	9.49[a]	2.31[ab]	1.51[a]	498.29[ab]
3. Inoculated plus 100% dose fertilized	1.19 ns	0.31[ab]	252.29[a]	183.86[ab]	7.29[ab]	1.97[ab]	1.31[ab]	420.14[abc]
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	1.39 ns	0.16[cd]	228.14[a]	109.57[cd]	6.01[bc]	1.31[c]	1.08[bc]	377.00[bc]
5. Inoculated plus 50% dose fertilized	1.20 ns	0.26[abc]	262.14[a]	156.00[c]	7.84[ab]	2.83[a]	1.37[ab]	512.57[a]
6. Uninoculated seeds, unfertilized soil (control)	0.94 ns	0.12[d]	198.43[a]	80.29[d]	5.26[bc]	1.63[bc]	0.79[c]	326.71[c]

[a,b,c,d] Significant differences by LSD test ($P < 0.05$), ns: no significant differences

Table 5 Number of tillers

Treatments	Number of tillers
1. Inoculated seeds, unfertilized soil	3.00[a]
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	3.17[a]
3. Inoculated plus 100% dose fertilized	2.63[ab]
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	2.46[ab]
5. Inoculated plus 50% dose fertilized	2.69[ab]
6. Uninoculated seeds, unfertilized soil (control)	2.00[b]

[a,b] Significant differences by LSD test ($P < 0.05$)

phytotoxicity by avoiding direct contact between the chemical fertilizer and seeds. However, such difference in number of plants per m^2 did not alter final yield because of the compensation that took place during the Feekes 3.0 stage. Moreover, other authors reported emergence promotion effects in wheat inoculated with fluorescent *Pseudomonas* (Luz 2001); our results didn't show significant differences on this parameter in the inoculated treatments, relative to control.

SR1 inoculation increased shoot length up to 14% at Feekes 1.5 stage, and even more at Feekes 3.0 stage. Our results suggest that inoculation could allow the dosage of inorganic fertilizer to be reduced. García-González et al. (2005) found that treatment with *Azospirillum lipoferum*, *A. beijerinckii*, or a combination of the two, plus a 50% dose of urea, had an effect equivalent to treatment with 100% urea without inoculation, in regard to wheat leaf length.

The root growth parameters (length, volume, dry biomass) increased >60% during Feekes 3.0 stage in SR1-inoculated plants, compared to controls. The effect of SR1 plus 50% dose fertilizer was not significantly different from that of 100% dose fertilizer. Previous studies have shown that rhizobacteria increase root absorption capacity of gramineous plants when the dosage of nitrogen fertilizer applied to soil is reduced (Trân Van et al. 2000; Whitmore 2000). Increased root weight, mediated by rhizobacteria, is generally associated with inoculation, and enhances access of the plant to soil nutrients (Kohler et al. 2006).

Values of the yield component kg ha^{-1} were, on average, 5.5 kg ha^{-1} higher for SR1 inoculation than for

Table 6 Wheat yield components

Treatments	Yield (kg ha^{-1})	Weight of a thousand grains	Number of spikes per plant	Number of grains per spike
1. Inoculated seeds, unfertilized soil	2,249.72[ab]	34.48 ns	1.30 ns	42.45[b]
2. 100% dose fertilized (80 kg ha^{-1} urea – 60 kg ha^{-1} diamonic phosphate)	2,169.40[ab]	34.00 ns	1.25 ns	39.24[b]
3. Inoculated plus 100% dose fertilized	1,776.66[b]	36.40 ns	1.25 ns	41.70[b]
4. 50% dose fertilized (40 kg ha^{-1} urea – 30 kg ha^{-1} diamonic phosphate)	2,264.91[ab]	33.12 ns	1.10 ns	40.20[b]
5. Inoculated plus 50% dose fertilized	2,641.58[a]	37.84 ns	1.45 ns	45.75[a]
6. Uninoculated seeds, unfertilized soil (control)	2,005.82[b]	32.64 ns	1.15 ns	32.70[c]

[a,b,c] Significant differences by LSD test ($P < 0.05$) , ns: no significant differences

fertilization treatments. The value for inoculation plus 50% dose fertilization was significantly higher than either control or 100% dose fertilization (Table 6).

Important conclusions from this study are: (i) inoculation with SR1 promoted both growth and yield of wheat; (ii) the dosages of chemical fertilizers currently applied in commercial wheat fields in Argentina could be reduced through proper combination of SR1 inoculation plus fertilization.

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