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Biological control of bacterial wilt of common bean by plant growth-promoting rhizobacteria

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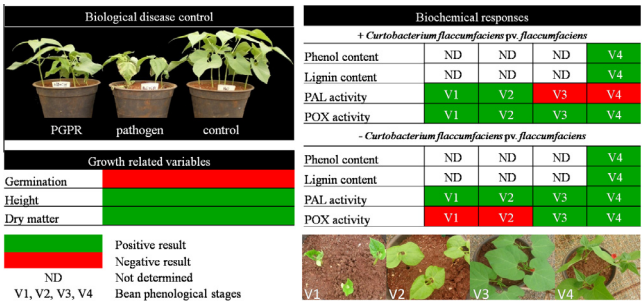
HIGHLIGHTS

- Four PGPR strains reduced bacterial wilt severity on common bean by seed treatment.
- The four PGPR also increased plant growth promotion.
- PGPR led to a significant increase in the phenolics' content and lignin accumulation.
- *Cff* seams to block plant response defense by decreasing PAL activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacterial wilt (BW) caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) is an emerging, seed-transmitted disease of common bean (*Phaseolus vulgaris*) in Brazil, and plant growth-promoting rhizobacteria (PGPR) have the potential to be used in disease management. The present work aimed at determining the potential of selected PGPR on the biological control of BW through seed treatment, growth promotion and induced resistance. Bean seeds cv. 'Pérola' were artificially inoculated with *Cff*, immersed in a PGPR suspension, and sown in 4 L pots containing a soil: sand mixture (2:1). Plants were assessed for seedling emergence (SE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), shoot dry weight (SDW), as well as biochemical plant responses in the presence or absence of *Cff*. The disease control ranged from 42% to 76%, respectively, for *Bacillus subtilis* UFLA285 and ALB629 compared to the untreated control. PGPR treatments also increased RGI, SDW, and RDW. Upon *Cff* inoculation, UFLA285 increased phenolics' content and ALB629 in the lignin accumulation compared to the untreated control. Without the pathogen inoculation, both PGPR promoted an increase in phenylalanine ammonia lyase activity and total phenolics content and UFLA285 in the lignin accumulation. Our findings demonstrated the potential of selected PGPR for disease control, enhancement of the RGI and biomass accumulation. Surprisingly, instead of a priming effect of PGPR, *Cff* apparently blocks the defense response development although the overall phenotype is disease control, suggesting there is a complementary and/or compensatory mode of action involved.

1. Introduction

Among the common bean diseases, bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) (Hedges) Collins and Jones (Hedges, 1922, 1926) is considered an emerging menace. *Cff* was first reported in South Dakota (Hedges, 1922) and although the pathogen still is considered a quarantine microbe

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in many countries it has been found in diverse geographical areas around the world (Harveson and Schwartz, 2007; Hedges, 1926; Hsieh et al., 2004; Krause et al., 2009a). In Brazil, the disease was first reported in 1995 (Maringoni and Rosa, 1997) and, thereafter it became of emerging importance to common beans in different regions (Herbes et al., 2008).

The bacterium is seed- or soil-borne and causes reduction in seed germination and seedling height emergence, as well as wilt of infected plants (Hall, 1994). Once it enters the plant, the bacterium colonizes the vascular tissue and causes wilting (Hedges, 1926; Souza and Maringoni, 2008).

Although, some works have been published currently regarding differences in levels of resistance to *Cff* (Conner et al., 2008; Huang et al., 2007; Krause et al., 2009a,b; Schwartz et al., 2010), there are no commercial genetic cultivars or chemical treatments available in Brazil against this disease. Currently, recommended managements for bacterial wilt rely on pathogen-free seeds and crop rotation with non-host crops (Mohan and Hagedorn, 1989). Therefore, other methods such as biological control might have potential for management of pathogenic bacteria. A promising disease control strategy is the use of plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1989; Shankar et al., 2009).

Besides disease control, PGPR may promote plant growth indirectly by suppressing plant pathogens and their harm (Hahm et al., 2012). Plant pathogen suppression by PGPR may occur through a combination of mechanisms including induced resistance (Sundaramoorthy et al., 2012) and a priming effect, i.e., the antagonist sets the plant in an "alert" state to pathogen detection with the response occurring faster and/or stronger compared to plants not previously exposed to the priming stimulus (Jung et al., 2012).

Crop losses due to bacterial wilt can be severe, especially when infection occurs early in the crop season (Mohan and Hagedorn, 1989). Thus, control strategies undertaken at early crop development may be more efficient especially for a seed-transmitted pathogen. Considering the importance of seeds in the transmission of pathogens and the need to reduce fungicide loads in the environment, PGPR seed treatment may result in a practical and cost-effective strategy to reduce seed-borne pathogens (Machado, 2000) such as *Cff* (Hsieh et al., 2003).

The present study was aimed at investigating the potential of selected PGPR for biological treatment of *Cff* contaminated seeds, to evaluate its potential to increase percentage seedling emergence (PSE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), shoot dry weight (SDW) as well as to evaluate the biochemical plant defense responses in the presence or absence of *Cff*.

2. Materials and methods

2.1. Artificial seed inoculation

The *Cff* isolate used for this study was the yellow variant of *Cff* from Santa Catarina State, Brazil (*Cff* SC – Feij-2928, isolated in March 23rd 2003 at Campos Novos, Santa Catarina State, Brazil from common bean *Phaseolus vulgaris* cv. Pérola), which was obtained from the culture collection of the plant bacteriology laboratory at the Universidade Estadual Paulista (Botucatu, Brazil), preserved for long-term in peptone-glycerol and for short-term in dried leaves from where it was recovered before each experiment.

The pathogen isolated from the dried leaves was grown on 523 medium (Kado and Heskett, 1970) in Petri dishes and incubated at room temperature (28 °C) for 48 h. Seeds of cv. 'Pérola' were initially disinfested in a series of 70% ethanol for 30 s, sodium hypochlorite (0.5% active chloride) for 10 min, and in sterile distilled water (SDW). Seeds were then air-dried in a flow cabinet for 8 h. Disinfested bean seeds were artificially inoculated with *Cff* by the physiological conditioning technique (Deuner et al., 2011).

2.2. Screening test

Eight PGPR strains were used for the initial screening test: *Paenibacillus lentimorbus* MEN2, *Bacillus subtilis* ALB629, *B. subtilis* UFLA285, *B. subtilis* sp. UFLA168*, *B. subtilis* UFLA246, *B. subtilis* UFLA373, *B. subtilis* UFLA116, and *B. subtilis* UFLA29 which were obtained from rhizosphere soil and endophytics of roots of field-cultivated cotton plant or donated by research centers (Medeiros et al., 2008, 2009). The screening tests were carried out under greenhouse conditions (Temperature ca.30 °C, relative humidity ca.63% and light intensity ca.1000 μmol m^{-2} s^{-1}). The experiment was repeated with the most efficient strains.

2.3. Biological seed treatment with PGPR

Selected PGPR were preserved in peptone glycerol at −80 °C and before every experiment, they were cultivated on agar nutrient medium in Petri dishes and incubated at room temperature (28 °C) for 48 h. Cells were transferred to the nutrient-broth medium and cultivated for 48 h on a shaker at 150 rpm at room temperature (28 °C). The endospore concentration was adjusted in a Neubauer chamber to 1×10^8 CFU ml^{-1} and used to treat seeds by soaking them for 30 min in the antagonist's suspension (2 ml g^{-1} seed) 10^8 CFU ml^{-1}, fungicide copper oxychloride or water (2 g seeds L^{-1}). They were dried overnight and sown in pots of 5 L containing a mixture of soil and sand (2:1), and with 10 seeds per pot. Plants were kept under greenhouse conditions as described previously and watered to field capacity.

2.4. Biocontrol of bacterial wilt

Selected PGPR from the screening test were tested under greenhouse conditions as described previously for their effectiveness to control common bean artificially inoculated with *Cff* to measure the percentage seedling emergence (PSE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), as well as shoot dry weight (SDW).

A total of four replicates for each treatment were used and arranged in a randomized block design. The experiment was performed three times.

2.5. Assessment of the analyzed variables

Seedling emergence from the 5th to the 12th day after sowing (DAS) was recorded daily and used to calculate the speed emergence index (SEI) according to Teixeira and Machado (2003), as well as percentage of seedling emergence (PSE) from the last evaluated period.

At 12, 15, 18, 21, 24 DAS, plants were assessed for disease severity of bacterial wilt with disease scores ranging from 0 to 5, where 0 = no wilt symptoms; 1 = wilt on one of the primary leaves; 2 = wilt on both primary leaves but not on the first trifoliolate; 3 = wilt on the first trifoliolate; 4 = death of seedling after development of primary leaves and 5 = unemerged seedling or death of seedling before development of primary leaves. With the values of this scale, AUDPC was calculated (Hsieh et al., 2003).

At the same day of disease evaluations, plant height was also recorded by measuring the distance from the cotyledon insertion to the apical bud and the obtained data was used to calculate the relative growth index (RGI) as $RGI = (\mathrm{Ln}P2 - \mathrm{Ln}P1)/(T2 - T1)$, where