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Common bean growth and health promoted by rhizobacteria and the contribution of magnesium to the observed responses

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ABSTRACT

Abiotic effects, such as nutrient abundance in soil, may interfere with the performance of plant-associated rhizobacteria in terms of plant physiology as well as disease control. We aimed to evaluate the effectiveness of rhizobacteria in the promotion of bean growth and nutrient uptake and the contribution of magnesium (Mg) supplementation to photosynthetic rates, CO_2 assimilation, chlorophyll content, and bacterial wilt severity (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*). Bean plants from seeds treated with rhizobacteria were assessed for growth promotion-related variables, photosynthetic-related variables, as well as disease severity when plants were grown in soil with different magnesium contents (0–50 mg kg^{-1}). There was a 33–45% increase in root dry weight (*Bacillus subtilis* UFLA168* and *B. amyloliquefaciens* ALB629) and a 24–35% increase in relative growth index (*B. subtilis* UFLA285, UFLA168*, copper oxychloride, Pa*enibacillus lentimorbus* MEN2). At 25 mg kg^{-1} Mg, although the plant continued to take up Mg from the soil, increased accumulation of CO_2 was found in the leaf mesophyll of both the ALB629 and control treatments, indicating low CO_2 fixation and low Rubisco activity. Higher doses of Mg caused an increase in chlorophyll content as well as in photosynthetic rates in rhizobacterium-treated plants. Additionally, at 25 mg kg^{-1} Mg, there was an increase in chlorophyll content in ALB629 (30%) and a reduction in bacterial wilt severity (51%). Moreover, photosynthesis was negatively correlated with disease severity ($r = -0.53$, $P < 0.01$). Therefore, ALB629 is a promising bacterial strain to improve bean plant growth and nutrient uptake and reduce plant disease even under abiotic stress.

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1. Introduction

With increasing problems associated with the use of synthetic chemicals in agriculture (negative impacts on health and the environment), there has been ever-increasing interest in the use of beneficial microorganisms to improve plant health while ensuring that products are safe for human consumption and enabling protection of the environment (Zafar et al., 2011).

For instance, some of these beneficial microorganisms such as the endophytic bacterium *Pantoea agglomerans* (Beijerinck) Gavini et al. isolate LRC 8311 enhanced seedling growth when applied to common bean seeds (Hsieh et al., 2005). Additionally, *Rhizobium leguminosarum* bv. *viceae* R21 may increase seedling emergence and plant height (Huang et al., 2007).

While Gram-negative bacteria have the potential to promote bean plant growth and control disease, bacteria belonging to the *Bacillus* genus produce endospores, which confer a higher tolerance to sudden environmental changes and are easier to formulate in a product with a long shelf life (Hayat et al., 2010; Saharan and Nehra, 2001). They are free-living bacteria in the soil and are known as plant growth-promoting rhizobacteria (PGPR). When applied to seeds or roots, certain strains may benefit crops by stimulation of plant growth (Kloepper et al., 1989; Orhan et al., 2006), suppression of plant diseases (Martins et al., 2013), enhancement of plant nutrient uptake (Remans et al., 2008; Saharan and Nehra, 2001), and/or by phytoremediation (Khan, 2005).

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Magnesium (Mg) is an essential element for plant growth and reproduction. It has noteworthy functions in plants including its role as enzyme co-factor for peroxidase (POX), an enzyme involved in plant defense, and Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), a key enzyme for photosynthesis (Hawkesford et al., 2012). This element also has a central position in the chlorophyll molecule (Waraich et al., 2011). Therefore, magnesium plays an important role in photosynthesis, and its lack in many weathered soils is a matter of concern. Increasing nutrient uptake is a plausible strategy to remediate contaminated soils (Khan, 2005) or sustain plant yield in nutrient-deficient soils (Zafar et al., 2011).

Many PGPR have also potential for disease control. Currently, bacterial wilt of the common bean caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) (Hedges) Collins and Jones (Hedges, 1922, 1926) is a serious threat because it is a seed-transmitted disease of the common bean, which is cultivated in Brazil and in another countries around the world (Corrêa et al., 2014; Huang et al., 2007). Although no commercially resistant cultivars or chemical treatments are available to growers for management of bean bacterial wilt, we have shown that PGPR can be successfully used to manage this disease with up to 70% disease reduction (Martins et al., 2013) even when plants are incubated at different temperatures (Martins et al., 2014). Once *Cff* infects bean plants, it causes wilting and a reduction in Mg uptake (Maringoni, 2003), which results in reduced yield. Therefore, either supplementing the nutrient in the soil or increasing nutrient uptake by PGPR treatment enables plants to better tolerate the disease and sustains plant development. Furthermore, it is common sense that disease is an exception and not a rule (Staskawicz, 2001). Therefore, for commercial purposes, it is important to show growers that even in the absence of the disease, PGPR treatment may result in other benefits, such as growth promotion and enhanced nutrient uptake.

The aim of this work was to evaluate the contribution of PGPR strains to growth promotion, nutrient uptake and bacterial wilt control in the common bean at different Mg concentrations in the soil.

2. Materials and methods

2.1. PGPR strains

Experiments were conducted under greenhouse conditions (temperature ca. 30 °C, relative humidity ca. 63% and light intensity ca. 1000 $\mu mol\,m^{-2}\,s^{-1}$) at the Universidade Federal de Lavras (UFLA), in Lavras, Minas Gerais, Brazil (915 m altitude, 21°13′34″s and 44°58′31″W). The PGPR strains used in this study were as follows: *Paenibacillus lentimorbus* MEN2, *Bacillus amyloliquefaciens* ALB629, *B. subtilis* UFLA285, and *B. subtilis* UFLA168*, which were obtained from rhizosphere soil and endophytically from roots of field-cultivated cotton plants or donated by research centers (Medeiros et al., 2008; Medeiros et al., 2008) and selected based on their ability to provide biological control of bean bacterial wilt (Martins et al., 2013).

2.2. Seed treatment with PGPR

Selected PGPR preserved in peptone glycerol at −80 °C were cultivated on nutrient agar medium in Petri dishes and incubated at 28 °C for 48 h prior to every experiment. Cells were transferred to nutrient-broth medium and cultivated for 48 h on a shaker at 150 rpm at 28 °C. The endospore concentration was adjusted to 1×10^8 CFU mL^{-1} in a Neubauer chamber and used for seed treatment.

Seeds of the common bean cv. 'Pérola' were initially disinfested in alcohol (70% ethanol) for 30 s and sodium hypochlorite (5% active chloride) for 10 min and subsequently washed thoroughly with sterile distilled water (SDW) and air-dried in a flow cabinet for 8 h. Disinfested seeds were soaked for 30 min in the antagonist's suspension (2 mL g^{-1} seed) at 10^8 CFU/mL, in the fungicide copper oxychloride or in water (2 g seed L^{-1}). They were dried overnight and sown (10 seeds per pot) in 5 L pots containing a mixture of soil and sand (2:1). The soil mixture had the following characteristics: $pH_{(H2O)}$: 5.6, Mg: 0.2 $cmol_c\,dm^{-3}$, sum of bases (S value): 2.67 $cmol_c\,dm^{-3}$, organic matter: 11.8 g kg^{-1} and clay content: 400 g kg^{-1}.

Plants were kept in a greenhouse. Four replicates of each treatment were performed and arranged in a randomized block design.

2.3. Assessment of 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 the percent of seedling emergence (PSE) from the last evaluated period. At 12, 15, 18, 21, and 24 DAS, seedling height was recorded by measuring from the cotyledon insertion to the apical bud, and the obtained data were used to calculate the relative growth index (RGI) as RGI = (LnP2 − LnP1)/(T2 − T1), where *Ln* = natural logarithm, and *P*2 and *P*1 = seedling height at times *T*2 (end time) and *T*1 (initial time).

All plants were harvested, and shoots were separated from roots at 24 DAS, a time set based on a previous work showing colonization of the common bean by ALB629 (Martins et al., 2014). Roots were thoroughly washed in tap water, and both shoots and roots were wrapped and oven-dried at 70 °C for 72 h to a constant weight to obtain shoot (SDW) and root dry weight (RDW). This experiment was repeated three times.

Table 1
Effect of seed treatment with PGPR on mineral nutrient concentrations in common bean shoots using regular fertilizer as recommended for the crop. Values of each column followed by the same letter(s) are not significantly different according to Tukey's test. (Means of 2 experiments of 4 replicates of 10 seedlings each).

Treatments	$^{**}N^{3-}$ g kg^{-1}	$^{***}P^{3-}$ g kg^{-1}	$^{**}K^{+}$ g kg^{-1}	$^{ns}Ca^{2+}$ g kg^{-1}	$^{**}Mg^{2+}$ g kg^{-1}	$^{***}S^{2-}$; g kg^{-1}	$^{**}B^{3+}$ g kg^{-1}	$^{***}Cu^{2+}$ mg kg^{-1}	$^{ns}Mn^{2+}$ mg kg^{-1}	$^{ns}Zn^{2+}$ mg kg^{-1}	$^{***}Fe^{2+}$ mg kg^{-1}
ALB629	48.8a	1.5a	24.2a	9.5	2.8a	3.8a	59.4a	6.5b	119.8	23.5	512.4a
MEN2	48.3a	1.3ab	24.2a	10.0	2.5b	3.7a	44.5ab	5.9b	118.1	20.9	394.6a
UFLA168*	45.6ab	1.3ab	23.8a	9.7	2.6ab	3.3ab	59.4a	5.7b	111.8	20.5	411.5a
UFLA285	47.5ab	1.3ab	24.3a	8.7	2.7ab	3.4ab	54.9a	6.0b	109.9	21.2	473.6a
Copper oxychloride	44.6b	1.1b	21.6b	6.3	2.5b	3.0b	30.3b	9.8a	104.1	20.1	216.1b
Water^{nc}	49.1a	1.3ab	24.2a	8.9	2.5b	3.6a	54.7a	5.9b	104.6	20.5	489.3a
CV (%)	5.1	10.3	5.9	31.7	7.6	9.9	31.1	11.7	13.4	10.8	19.1

ns: not significant; nc: negative control (seeds treated with sterile water).
* Significant at the 0.1 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

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