Inoculation of *Herbaspirillum Seropedicae* Increases Biomass in Maize Roots in the Early Stages of Plant Development

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Research Article

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Abstract

*Herbaspirillum seropedicae* is a plant growth-promoting bacteria isolated from diverse plant species. In this work, the main objective was to investigate the efficiency of *H. seropedicae* strain SmR1 in colonizing and increasing maize growth in the early stages of development under greenhouse conditions. Inoculation with *H. seropedicae* resulted in 10.51 and 19.43% in mean of increase of root biomass concerning non-inoculated controls, mainly in the initial stages of plant development, at 21 days after emergence (DAE). Quantification of *H. seropedicae* in roots and leaves was performed by quantitative PCR. *H. seropedicae* was detected only in maize inoculated roots by qPCR, and a slight decrease in DNA copy number g\(^{-1}\) of fresh root weight was observed from 7 to 21 DAE, suggesting that there was initial effective colonization on maize plants. *H. seropedicae* strain SmR1 efficiently increased maize root biomass exhibiting its potential to be used as inoculant in agriculture systems.

Introduction

Plants are closely associated with an enormous variety of microorganisms. This association can be beneficial, neutral, or harmful (Backer et al., 2018; do Amaral et al., 2016). One beneficial interaction example is the association with plant growth-promoting bacteria (PGPB), a group of heterogeneous free-living microorganisms found in the rhizosphere, root surface, and endosphere of plants (de Souza et al., 2015; Rilling et al., 2019). PGPB can stimulate plant growth through several processes, including biological nitrogen fixation (BNF) performed by diazotrophic bacteria, phosphate solubilization, phytohormones, and siderophores production, protection against pathogens and abiotic stresses (Ferreira et al., 2019).

One of the significant challenges of agricultural production to meet the demand for food is maintaining high yields with the least possible environmental impact. However, the current agricultural system is dependent on chemical inputs, including fertilizers (Do Amaral et al., 2014). Exploring the PGPB is an economically viable and environmentally sustainable alternative to decrease the application of chemical fertilizers (Canellas et al., 2013) once they can associate with economically important crops, such as soybean, bean, rice, wheat, maize, canola, and sugarcane (Olivares et al., 2017; Ramakrishna et al., 2019).

*Herbaspirillum seropedicae* is an endophytic PGPB, aerobic, Gram-negative, diazotrophic, β-Proteobacteria (Baldani et al., 1986) that has been isolated from many grass species, such as maize, sorghum, rice, sugar cane, wheat, and other forage plants (Monteiro et al., 2012; Olivares et al., 1996). The most studied strain of *H. seropedicae* is strain SmR1, a spontaneous mutant of strain Z78 (ATCC 35893) isolated from sorghum by Baldani et al. (1986) resistant to the antibiotic streptomycin. *H. seropedicae* strain SmR1 has its complete genome sequenced and published in 2011 by Pedrosa et al. (2011).

Different studies have shown the potential of the *Herbaspirillum*-maize association. This PGPB can promote growth and increase the productivity of maize mainly to BNF and phytohormones production (Da Fonseca Breda et al., 2016; Monteiro et al., 2008; Roncato-Maccari et al., 2003). In an experiment
developed by Alves et al. (2015) under two growing conditions (greenhouse and field), 21 strains of *Herbaspirillum* in two maize varieties were evaluated. The strain that presented the best results for both conditions was *H. seropedicae* ZAE94, demonstrating that it can increase maize production up to 34% and provide 37% of the nitrogen (N) demand by plants by BNF. Assessing the effect of *Azospirillum brasilense* and *Herbaspirillum seropedicae* inoculation on maize nitrogen metabolism in the presence of two nitrogen levels, Breda et al. (2016) observed that *H. seropedicae* contributed to the change on the N metabolism and promoted maize plants development, as well as higher accumulation of phosphorus (P) and potassium (K) in the shoots when compared with *A. brasilense*.

An essential requirement for PGPB to stimulate plant growth is the inoculant survival used as biofertilizer in the plant surface in a sufficient cell number to establish satisfactory colonization (Berninger et al., 2018; Stets et al., 2015). Currently, molecular techniques based on DNA amplification are widely used to monitor bacterial populations. Quantitative PCR (qPCR) has been one of the most used techniques for quantifying microbial populations present in the rhizosphere because it provides fast results with high specificity and sensitivity (Couillerot et al., 2010; Dall’Asta et al., 2017, Dall’Asta et al., 2019). qPCR assay was developed to quantify *H. seropedicae* strain SmR1 in inoculated maize roots using species-specific primers (Pereira et al., 2014) and a hydrolysis probe (Dallasta et al., 2017; Dall’Asta et al., 2019).

The bacterial inoculant survival can be affected by biotic and abiotic factors before, during, and after plant application (Berninger et al., 2018). Furthermore, under field conditions, inoculants may behave differently from that observed in greenhouses or axenic conditions (Sammauria et al., 2020). Therefore, quantifying *H. seropedicae* DNA in maize plants when grown in non-sterile soil and with the inoculated seed would make it possible to evaluate bacteria in plant tissues and relate the benefits of plant growth and environment interaction. Therefore, the objective of the work was to evaluate the establishment and efficiency of diazotrophic bacteria *H. seropedicae* strain SmR1 in the early stages of the development of a maize cultivar.

### Material And Methods

#### Bacterial growth conditions

*Herbaspirillum seropedicae* strain SmR1 (strain Z78 ATCC 35893 Sm\(^R\)) was grown in NFbHPN medium supplemented with 5 g L\(^{-1}\) malic acid (Klassen et al., 1997) at 30 °C under aeration and 120 rpm shaking until OD\(_{600}\) 0.8, corresponding to \(\sim 10^8\) CFU mL\(^{-1}\). A correlation was obtained between the optical density (OD) measured at 600 nm using Hitachi U2910 Spectrophotometer (Tokyo, Japan) and the number of colony forming units obtained by plate counting in NFbHPN agar medium after ten-fold serial dilution (0.9% saline solution). Plate counting was performed after incubation for 48h at 30°C.

#### Maize inoculation and growth conditions

Seeds of maize variety DKB 390 were surface sterilized in biological safety cabinet by 3 washing in autoclaved distilled water, followed by submersion in 70% ethanol for 5 min, and 20 min shaken in 1%
sodium hypochlorite plus 0.01% Tween 20 solution. Then seeds were rinsed 5 times with sterile distilled water and treated with 10% sucrose solution (300 mL 50 kg\(^{-1}\) of seeds) for inoculant adherence. For inoculation, 1 mL of washed bacterial culture of \(H.\) \(seropedicae\) strain SmR1 (~10\(^8\) CFU.mL\(^{-1}\)) was applied in 1g of sterilized peat according to Ferreira et al. (2010) protocol instructions. The peat inoculant was applied in sterilized seeds considering recommended dosage (25 g kg\(^{-1}\)) (Hungria et al., 2010). Control seeds were Mock-inoculated under the same conditions. Inoculated and control seeds were transferred to 2-L pots containing soil, and plants were grown under a semi-controlled greenhouse and watered every two days. The soil used in the first experiment was collected from layer 0-20 cm in Curitibanos, Santa Catarina, Brazil (-27° 16' 58.01" S; -50° 35' 3.98" W), and used without sterilization. For the second experiment, the same soil was used after solarization method application.

The results reported represent two independent greenhouse experiments performed in different periods, first in late spring and second in early summer. On the third day before the collections, a potassium nitrate solution (KNO\(_3\)) was applied to provide two nitrogen (N) concentrations, high N (5 mM) and low N (0.5 mM) doses, for the control treatments, obeying the fertilization recommendations for maize seeding (CQFS-RS/SC, 2004). Both experiments were performed in a completely randomized design, factorial 3x2, with 3 biological replicates. The first factor consisted of bacterial inoculation (or control non-inoculated – High N and Low N), and the second factor was the growth period (collect time).

**Assessment of plant growth parameters**

Maize seedlings from each treatment were randomly collected on the 7th, 14th, and 21st day after emergence (DAE). Plant growth parameters of root and shoot length were determined with the aid of a graduated ruler. Dry weight was determined by drying of root and shoot at 65 °C for 72h until constant weights were achieved. Nitrogen content was determined by sulfuric digestion of shoot tissue according to Tedesco et al. (1995). The remained sampled tissues were frozen in liquid nitrogen and stored in -80 °C freezer until DNA extraction.

**DNA isolation**

Genomic DNA was isolated from bacterial cultures \(H.\) \(seropedicae\) strain SmR1 using Wizard ® Genomic DNA Purification Kit (PromegaTM, Madison, WI, USA) with modifications (Faleiro et al., 2013). Total DNA from maize leaves and roots was isolated using DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) as described by the manufacturer with modifications (Pereira et al., 2014). Soil DNA was isolated using PowerSoil DNA Isolation Kit (MoBio, Laboratories, Inc.). Each extract corresponded to a pool of four plants (~ 100 mg of tissue). DNA concentration and quality were measured using a Thermo Scientific Nanodrop™ 2000 spectrophotometer (Wilmington, DE, USA), at 260 and 280 nm.

**Quantitative PCR analysis**

\(H.\) \(seropedicae\) strain SmR1 DNA quantification in maize root and shoot samples was performed by qPCR on an ABI PRISM 7500 detection system (Applied Biosystems, Foster City, CA, USA). HERBAS1 species-specific primer pair was used to amplify a conserved hypothetical protein-coding region in \(H.\) \(seropedicae\)
strain SmR1 genome (Pereira et al., 2014). Amplification reactions contained 12.5 µL of 2X Taqman Universal PCR Master Mix (Applied Biosystems), 0.1 µM primer pair HERBAS1 R/F, 0.1 µM HERBAS1 probe, ultrapure water and 20 ng DNA template in a final volume of 25 µL. qPCR reactions were carried out in triplicate for inoculated and control samples under the cycling conditions of 2 min at 50°C, 10 min at 95°C followed by 40 cycles of 15 s at 94°C and 1 min at 60°C. All real-time PCR runs were analyzed using automatic software settings.

**Generation of standard curves from *H. seropedicae* genomic DNA**

Standard curves (Cq versus log DNA copy number) were obtained by ten-fold serial dilution of DNA isolated from *H. seropedicae* strain SmR1 culture in water. The DNA solutions ranged from 6.03 ng to 6.03 fg, corresponding to estimated DNA copy number from $10^6$ to $10^0$, based on the *H. seropedicae* strain SmR1 total sequence length (Genbank ASM14322v1) of 5,513,887 bp, 1 chromosome, assembly level: complete genome (Rose A Monteiro et al., 2012). Amplification efficiencies were determined as described previously by Pereira et al. (2014).

**Statistical analysis**

Statistical analysis was performed using Sisvar 5.3 software (DEX-UFLA). Data was first evaluated for normality and homogeneity using Lilliefors and Bartlet tests followed by the variance analysis and Tukey test ($P \leq 0.05$), used to determine statistically significant differences between plant growth parameters and quantification in maize by qPCR.

**Results**

**Plant-growth parameters**

Maize DKB 390 variety inoculated with *H. seropedicae* strain SmR1 under greenhouse conditions resulted in increased root biomass in the early stages of plant development, compared to the non-inoculated control for both experiments conducted in different periods.

For the first greenhouse experiment, shoot dry mass and root length were not influenced by treatments (inoculated or non-inoculated High N and Low N controls), just by the growth period (Figure 1A and 1B). In this assay, shoot length has increased by the inoculation with *H. seropedicae* strain SmR1 4.93% in mean compared to the non-inoculated High N control (Figure 1C), regardless of the growth period. Root dry mass was also significantly influenced by treatments and the inoculation increased root biomass by 19.43% in mean, at 21 DAE, compared with High N and Low N non-inoculated controls (Figure 1D). Inoculation with *H. seropedicae* strain SmR1 influenced the nitrogen content of the maize shoot only at 14 DAE (Figure 2), increasing in mean 20% of this content compared with High N and Low N non-inoculated controls. Besides it, a significant decrease in N was observed during the growth period (7 to 21 DAE).
For the second greenhouse experiment, shoot length and shoot dry mass were not influenced by treatments (Figure 3A and 3B). Regardless of the growth period, the inoculation with *H. seropedicae* strain SmR1 has increased root length (Figure 3C) and root dry mass (Figure 3D) in mean by 8% and 10.51%, respectively, in comparison to the non-inoculated Low N control. Inoculation treatment has shown no significant difference of non-inoculated High N control.

**qPCR reaction parameters for H. seropedicae strain SmR1 quantification**

Reaction parameters (efficiency, slope and correlation coefficient) of the qPCR assay using HERBAS1 were determined based on two independent standard curves obtained from DNA isolated from *H. seropedicae* strain SmR1 pure culture (Figure 4). The reaction parameters were calculated by plotting the Cq values against the log10 of the genome copy number. *H. seropedicae* strain SmR1 standard curves. Efficiency varied from 89 to 92% and sloped ranged from – 3.61 to – 3.53 to standard curve performed for quantification of control samples (Figure 4A) and inoculated samples or soil (Figure 4B), respectively. All standard curves presented a suitable linear correlation (R² > 0.99).

Limit of detection (LOD) of a qPCR is defined as the lowest amount of DNA that can be reliably detected at least 95% of repetitions (Gómez-Rojo et al., 2015). LOD using pure culture samples of *H. seropedicae* strain SmR1 was established as 10⁻¹ genome copies, corresponding to 60.3 fg of DNA, as previously described by Pereira et al. (2014).

**Quantification of H. seropedicae in maize roots and leaves samples**

Total DNA isolated from roots and leaves of maize grown in pots under greenhouse conditions (control and inoculated) was used as a template for qPCR to quantify *H. seropedicae* strain SmR1 DNA. This amount was estimated using standard curves Cq versus log DNA copy number (Figure 4). DNA isolated from control maize roots and leaves showed late Cq values (below 10⁻¹ genome copy number, LOD) or none amplification. No amplifications were observed for DNA isolated from soil samples. The late Cq values correspond to non-specific amplification (Table S1). Quantification results of inoculated roots showed that bacterial DNA copy number per gram of fresh root weight ranged from 3.48 ± 1.48 x 10⁵ (7 DAE) to 3.83 ± 0.9 x 10⁴ (21 DAE) (Table 1).
Table 1

Bacterial DNA copy number g\(^{-1}\) of root (fresh weight) of DKB 390 maize cultivated in soil, inoculated and control samples after inoculation with \(H.\) \textit{seropedicae} strain SmR1. Means represent the values detected over the limit of detection (>LOD) of 10 copies (n = 9).

| Sample | Sample | DAE | > LOD.n\(^{-1}\) | Bacterial DNA copy number g\(^{-1}\) | > LOD.n\(^{-1}\) | Bacterial DNA copy number g\(^{-1}\) |
|--------|--------|-----|----------------|-----------------------------------|----------------|-----------------------------------|
|        |        |     |                |                                   |                |                                   |
| Control|        |     |                |                                   |                |                                   |
| Root   | 7      | 0/9 | -              |                                   | 9/9            | 3.48 ± 1.48 x 10^5               |
|        | 14     | 0/9 | -              |                                   | 3/9            | 1.31 ± 0.14 x 10^4               |
|        | 21     | 0/9 | -              |                                   | 3/9            | 3.83 ± 0.9 x 10^4               |
| Leaf   | 7      | 0/9 | -              |                                   | 0/9            | -                                |
|        | 14     | 0/9 | -              |                                   | 0/9            | -                                |
|        | 21     | 0/9 | -              |                                   | 0/9            | -                                |

Discussion

Environmental health, maintenance of ecological balance, and conservation of soil biodiversity have gained more attention nowadays due to the growing food demands globally, seeking agricultural strategies that reduce negative impacts. The use of PGPB as inoculants contributes to the adoption of more sustainable agricultural practices without affecting the productivity of large crops, as they are ecologically correct (Alves et al., 2019; Mahdi et al., 2010; Prashar and Shah, 2016). Several studies have shown the potential of the \textit{Herbaspirillum}-maize association, however, a differential response between genotypes has been reported (Vacheron et al., 2013), so that further studies on the factors that influence this interaction are needed. Molecular methods have been widely used to detect and monitor the survival of microbial populations due to its high sensitivity and specificity. In the present study, we evaluated the effects of plant growth-promoting bacteria \(H.\) \textit{seropedicae} strain SmR1 in association with hybrid maize variety DKB 390 and monitored the bacterial survival during association using molecular tools such as quantitative PCR.

The plant growth results observed in this work show great variability in the responses on the initial plant development of DKB 390 maize concerning the inoculation treatment with \(H.\) \textit{seropedicae} strain SmR1 and the growing season. The association of PGPB with maize plants can promote an increase in the production of biomass and grains (Dobbelaere et al., 2002), however, the successful association appear to be dependent on the genotype used and the inoculated bacterial strain.

The inoculation of hybrid maize variety DKB 390 with \(H.\) \textit{seropedicae} strain SmR1 increased plant shoot length concerning the control treatments in the first greenhouse experiment performed (Figure 1C), however, this increase was not observed in the second experiment (Figure 3A). Similarly, significant
differences in root length were observed only in the second experiment in response to inoculation (Figures 1A and 3C), with additions compared to the non-inoculated Low N control. Inoculation with *H. seropedicae* strain SmR1 did not demonstrate significant differences in the shoot dry mass evaluation on the experiments performed (Figures 1B and 3B). Likewise, Do Amaral et al. (2014) also did not observe significant differences for the length and biomass of roots and leaves in variety DKB 240 maize plants inoculated with *H. seropedicae* strain SmR1 under *in vitro* conditions but observed a more significant number of lateral roots in the inoculated plants after 7 and 10 days. The effect of increasing the volume, biomass, number of lateral roots and other changes in the root architecture is a response commonly found in plants inoculated with several genera of PGPB and seems to be related to phytostimulation induced by hormones such as auxins (Bashan and de-Bashan, 2010; Radwan et al., 2004; Spaepen and Vanderleyden, 2011).

Root dry mass was the only growth parameter evaluated that showed a difference between the inoculated plants with *H. seropedicae* strain SmR1 and non-inoculated Low N plants for both experiments. Significant increases of 10.51 and 19.43% in mean, for root dry mass were observed, mainly in the initial stages of plant development, at 21 DAE (Figure 1D and 3D). For the second experiment, the values observed for root dry mass to inoculated plants were not statistically different from values of the non-inoculated High N control (Figure 3D), indicating that the increase in biomass provided by the inoculation with *H. seropedicae* strain SmR1 can be as efficient as that of the soil with high doses of nitrogen.

In this study, the nitrogen content of shoot was evaluated only for the first trial and significant increases of 20% in mean, were reported in response to inoculation with *H. seropedicae* strain SmR1 compared to High N and Low N non-inoculated controls at 14 DAE (Figure 2). Despite the increase in maize root biomass after inoculation with *H. seropedicae* strain SmR1, no significant contributions to FBN were observed in this work since there was no consistent increase in N during the growth period for the inoculated plants.

Similar results were observed for Da Fonseca Breda et al. (2016) when increments of N in hybrid maize varieties inoculated with *H. seropedicae* strain BR11417 were not significant. However, they did observe significant increases in plant growth regardless of the nitrogen fertilizer dosage, as well as an increase in root biomass. In another study, da Fonseca Breda et al. (2019) observed that the inoculation with *H. seropedicae* strain ZAE94 did not promote significant differences in the N accumulation in maize leaves concerning the control plants. However, the inoculation at high levels of N showed greater accumulation of this nutrient concerning inoculation at low levels of N. They also observed an increase in dry biomass for roots and leaves, indicating that the effect of promoting growth in these associations is more related to hormonal stimulation than to FBN itself (Pérez-Montaño et al., 2014). Studies carried out reported the presence of genes related to the synthesis of auxins in *H. seropedicae* genome, such as indole-3-acetic acid (IAA) (Fábio O Pedrosa et al., 2011). Like other auxins, IAA seems to trigger direct changes in plant root architectures, such as the development of lateral root and consequently an increase in root biomass,
contributing to the improvement of water and mineral absorption capacity in plants (Bashan and de-Bashan, 2010; Cohen et al., 2015; Duca et al., 2014).

In order to establish a reliable assay of qPCR for *H. seropedicae* DNA quantification in maize root and leaves, reaction parameters for SYBR Green assay were defined using standard curves obtained from diluted DNA isolated from pure bacterial culture. According to Zhang and Fang (2006), a reliable standard curve should present slope values between -3.9 and -3.0, corresponding to qPCR efficiency between 80 and 115%, and R² values higher than 0.95. In this work, standard curves presented suitable reaction parameters with efficiency values ranging from 89 to 92% (Figure 4). Similar results were already observed in qPCR assay using HERBAS1 for *H. seropedicae* quantification: 85 - 99% of efficiency and R² = 0.99 (Pereira et al., 2014).

Regarding the colonization of maize tissues (Table 1), DNA of *H. seropedicae* was detected only in the inoculated maize roots, and the results of quantification exhibit a tendency to decrease the bacterial DNA copy number per gram of root fresh weight with advancing plant growth period. A slight decrease in DNA copy number/g of root fresh weight was observed from 7 DAE (3.48 x 10⁵) to 21 DAE (3.83 x 10⁴), suggesting that there was initial effective colonization of *H. seropedicae* in the maize plants, however, some external factors may have affected the microbial population over the growth period. Pereira et al. (2014) using the same bacterial strain inoculated in maize variety DKB 240, have found values of DNA copy number/g of root that ranged from 5.16 x 10⁷ (1 DAI) to 1.42 x 10⁹ (10 DAI) for plants grown under *in vitro* conditions and from 3.25 x 10⁶ (4 DAI) to 3.5 x 10⁶ (10 DAI) for plants grown in pots under greenhouse conditions. Internal colonization by *H. seropedicae* strain SmR1 was previously demonstrated in roots of maize (DKB 240) grown *in vitro* and the bacterial population was around 10⁴ – 10⁵ MPN g soil⁻¹ to all collection times after inoculation (Do Amaral et al., 2014). The results observed suggest that under controlled conditions, *H. seropedicae* can quickly colonize and increase its population in plant tissues in the early growth period. In non-controlled or semi-controlled conditions, this bacterial population tends to decrease or not increase significantly after colonization. The microbial community in the soil is under influence of several environmental factors that influence the colonization and survival process, such as exposure to extreme temperatures, water availability, and fluctuating soil conditions, or as the interactions that occur with the autochthonous microbiota when using non-sterile soil, causing competition for the same colonization niche (Arora et al., 2011; Berninger et al., 2018; Shameer and Prasad, 2018).

On the other hand, we must also consider that the inoculation in this work was performed in maize seeds, and quantification results were based on the period after the plants emergence. This inoculation performed directly into the seeds may have hampered the colonization of maize roots concerning inoculation performed into pre-germinated seeds as observed in Pereira et al (2014) and do Amaral et al (2014).

In our study, even though bacterial population decreased, at 21 DAI the inoculated plants showed significantly higher root biomass increments compared to the non-inoculated plants. In conclusion, *H.
seropedicae strain SmR1 has shown that inoculation of maize plants cultivar DKB 390 occurred quickly and effectively, exhibiting its potential to be used as an inoculant. However, due to the variation of results obtained according to the planting season, additional studies are necessary to evaluate the effectiveness of the colonization of H. seropedicae strain SmR1 in field conditions throughout the crop cycle.

Declarations

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Competing Interests:

All authors declare that they have no conflict of interest.

Author contributions:

JCFB and TPP contributed in practical procedures. ETC contributed with analysis of data, confection of figures and tables, and writing the text. AMP also contributed writing the text. ACMA and CRFSS conceived and coordinated this study. All authors contributed by reading, revising and improving the text, especially the professors ACMA and CRFSS.

Data Availability:

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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Figures
Figure 1

Plant-growth parameters root length (A), shoot dry mass (B), shoot length (C) and root dry mass (D) in first greenhouse experiment performed with Dekalb 390 maize variety in response to inoculation with H. seropedicae strain SmR1. Parameters were determined at 7, 14 and 21 days after emergence (DAE) for controls (Low N and High N) and inoculated plants. Values are mean of factor which no significant difference was observed. Different letters indicate significant difference, by Tukey’s test $p \leq 0.05$. Lowercase letters compare treatments under same time and capital letters compare DAE for each treatment.
Figure 2

Nitrogen content of shoot tissue in the first greenhouse experiment performed with Dekalb 390 maize variety in response to inoculation with H. seropedicae strain SmR1. Parameters were determined at 7, 14 and 21 days after emergence (DAE) for controls (Low N and High N) and inoculated plants. Different letters indicate significant difference, by Tukey’s test $p \leq 0.05$. Lowercase letters compare treatments under same time and capital letters compare DAE for each treatment.
Figure 3

Plant-growth parameters shoot height (A), shoot dry mass (B), root length (C) and root dry mass (D) in the second greenhouse experiment performed with Dekalb 390 maize variety in response to inoculation with *H. seropedicae* strain SmR1. Parameters were determined at 7, 14 and 21 days after emergence (DAE) for controls (Low N and High N) and inoculated plants. Values are mean of factor which no significant difference was observed. Different letters indicate significant difference, by Tukey’s test $p \leq 0.05$. 
Figure 4

qPCR assay standard curves (Cq versus log DNA copy number) for H. seropedicae strain SmR1 using primers Herbas1. Quantification performed in two qPCR runs in different days using DNA extracted from H. seropedicae pure culture. A) standard curve performed for quantification of control samples, B) standard curve performed for quantification of inoculated samples and soil.
Supplementary Files

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