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# Inoculation with the endophytic bacterium *Herbaspirillum seropedicae* promotes growth, nutrient uptake and photosynthetic efficiency in rice

Alessandro C. Ramos<sup>1</sup> · Juliana Melo<sup>2</sup> · Sávio B. de Souza<sup>1</sup> · Amanda A. Bertolazi<sup>1</sup> · Renderson A. Silva<sup>1</sup> · Weverton P. Rodrigues<sup>3</sup> · Eliemar Campostrini<sup>3</sup> · Fábio L. Olivares<sup>4</sup> · Frederico J. Eutrópio<sup>1</sup> · Cristina Cruz<sup>2</sup> · Teresa Dias<sup>2</sup>

1 - Environmental Microbiology and Biotechnology Lab; Universidade Vila Velha (UVV), Vila Velha, ES, Brazil

2 - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

3 - Plant physiology lab, Universidade estadual do Norte Fluminense (UENF), Campos dos Goytacazes, RJ, Brazil

4 - Cell Tissue and Biology Lab, Universidade Estadual do Norte Fluminense (UENF), Campos dos Goytacazes, RJ, Brazil

## Abstract

**Main conclusion** Higher vacuolar proton pump activity may increase plant energy and nutrient use efficiency and provide the *nexus* between plant inoculation with *Herbaspirillum seropedicae* and growth promotion.

**Abstract** Global change and growing human population are exhausting arable land and resources, including water and fertilizers. We present inoculation with the endophytic plant-growth promoting bacterium (PGPB) *Herbaspirillum seropedicae* as a strategy for promoting growth, nutrient uptake and photosynthetic efficiency in rice (*Oryza sativa* L.). Because plant nutrient acquisition is coordinated with photosynthesis and the plant carbon status, we hypothesize that inoculation with *H. seropedicae* will stimulate proton (H<sup>+</sup>) pumps, increasing plant growth nutrient uptake and photosynthetic efficiency at low nutrient levels. Plants were inoculated and grown in pots with sterile soil for 90 days. *Herbaspirillum seropedicae* endophytic colonization was successful and, as hypothesized, inoculation (1) stimulated root vacuolar H<sup>+</sup> pumps (vacuolar H<sup>+</sup>-ATPase and vacuolar H<sup>+</sup>-PPase), and (2) increased plant growth, nutrient contents and photosynthetic efficiency. The results showed that inoculation with the endophytic bacterium *H. seropedicae* can promote plant growth, nutrient uptake and photosynthetic efficiency, which will likely result in a more efficient use of resources (nutrients and water) and higher production of nutrient-rich food at reduced economic and environmental costs.

**Keywords:** Endophytic plant-growth promoting bacteria; Nutrients; Photosynthesis; Rice plants; Vacuolar proton pumps

## Introduction

Rice (*Oryza sativa* L.) is the most important crop worldwide and a primary food source for more than half of humanity. However, to meet the demands of the growing human population, the world rice production has to increase 25% over the next 2 decades (Chen et al. 2017). So far, production has been increased by: (1) increasing the farming area, which is no longer sustainable and, therefore, not recommended under the United Nations 'zero net land degradation' goal (<https://sustainabledevelopment.un.org>); (2) using more productive varieties, including genetically modified rice varieties; and (3) using larger amounts of chemical

fertilizers, which creates serious environmental and health issues (Sutton et al. 2011). Arable land and resources are being exhausted (Maurino and Weber 2013), and the urgency for more sustainable agricultural practices generating higher crop yields is consensual (Dias et al. 2015). One important approach is to implement or revitalize eco-friendly technologies, such as inoculating crops with plant-growth promoting bacteria (PGPB), which improve crop production through synthesis of growth promoting substances, increased nutrient availability and stimulation of hormone production and plant defence (Cocking 2003; Bhattacharyya and Jha 2012).

Because nitrogen (N) is the main limiting nutrient to cereal production (Ladha and Reddy 2003), rice and other non-legume crops (e.g., maize, wheat) have been inoculated with non-rhizobial diazotrophic PGPB (e.g., *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*). These bacteria colonize roots endophytically (James et al. 2002) and fix and provide N to the host plant, increasing crop productivity and quality (Cocking 2003), namely higher contents of protein and of 'digestible' nutrients (Skonieski et al. 2017). Endophytic PGPB are attracted to carbon-based substances excreted by non-legume roots (e.g., rice), and after they attached to the root surface, they initiate the endophytic colonization from newly emerged lateral roots. As in the legume-rhizobial symbiosis, chemical communication between the host plant and the bacteria also occurs (Monteiro et al. 2012). Besides N enhancement, phosphate solubilization and/or phytohormone production can also be improved through PGPB inoculation (Araujo et al. 2013; Baldani et al. 2000) as observed for rice (greenhouse trial—Gyaneshwar et al. 2002), maize (greenhouse and field trials—Canellas et al. 2013) and sugarcane (greenhouse trial—Aguiar et al. 2016) following inoculation with the PGPB *H. seropedicae*.

Several mechanisms may be involved in plant growth promotion by PGPB. The interactions between *A. brasilense* and soybean and cowpea plants involve increased proton ( $H^+$ ) efflux at the root surface (Bashan et al. 1992), showing the importance of cell membranes as reliable sensors of bacterial presence and mediators of bacterial effects on plants. Three types of  $H^+$  pumps (plasma membrane  $H^+$ -ATPase, vacuolar  $H^+$ -ATPase and vacuolar  $H^+$ -PPase) are responsible for  $H^+$  efflux into the vacuole or apoplastic space, which generates the  $H^+$  electrochemical gradient involved in plant cellular nutrition and growth (Felle 2001; Netting 2002; Gao et al. 2004; Shavrukov and Hirai 2016). The plant vacuole builds up cellular turgor and is actively involved in cellular responses to different stimuli. Furthermore, solute uptake into or release out of the vacuole allows cytosolic metabolite levels to adapt to changing physiological requirements and specific cellular demands. Solute transport through the vacuolar membrane is, therefore, tightly regulated (Neuhäus and Trentmann 2014), also by vacuolar  $H^+$  pumps. Indeed, genetic engineering approaches targeting increased  $H^+$  influx into the vacuole (transgenic overexpression of vacuolar  $H^+$ -PPase in *Arabidopsis thaliana*, tomato and rice plants) have been shown to generate higher crop yields (Paez-Valencia et al. 2013).

*Herbaspirillum seropedicae* was originally thought to be a new *Azospirillum* species (James and Olivares 1998). The similarity between *Herbaspirillum* and *Azospirillum* made further isolation and work on the former somewhat difficult, until Baldani et al. (1992) developed a new semi-solid malate medium (JNFb medium) that allowed to more easily differentiate the two. As already mentioned, *A. brasilense* colonization increases root  $H^+$  efflux (Bashan et al. 1992). Our aim was, therefore, to test if inoculation with *H. seropedicae* would also stimulate  $H^+$  pumps, especially at the vacuole, and thus promote growth, nutrient uptake and photosynthetic efficiency in rice plants growing at low nutrient levels.

## Materials and methods

### Experimental design

Our experimental design consisted of one factor, *H. seropedicae* inoculation, resulting in two treatments (non-inoculated—control; and inoculated) with five replicates (pots) per treatment. *Herbaspirillum seropedicae* strain HRC54, originally isolated from sugarcane roots, has been extensively tested on several crops (Olivares et al. 2017). The inoculum was grown at 28 °C for 36 h at 150 rpm in JNFb liquid medium (Baldani et al. 1992).

*Oryza sativa* seeds (kindly provided by Prof. Roberto Gaxiola, Arizona University, USA) were surface sterilized with 70% ethanol for 3 min, followed by 2% sodium hypochlorite (v/v) for 5 min, rinsed five times

with sterile water, sown on 1 L pots filled with autoclaved sand, and placed in a growth chamber (16/8 h light/dark; 25/20 °C day/ night; 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). After 15 days, seedlings were transferred to 1 L pots (four seedlings per pot) filled with autoclaved sand and a red-yellow oxisol (3:1), and inoculated. Half of the plants were inoculated with a living *H. seropedicae* suspension ( $10^9$  cells  $\text{mL}^{-1}$ ), added directly onto the root system (Baldotto et al. 2011), and the other half with the same but autoclaved suspension (control plants). After 30 days, *H. seropedicae* was re-inoculated (Rakiami et al. 2019). All plants were watered twice a week with 50 mL of 1/4 strength modified Clark solution (Clark 1975), establishing nutrient limitation, and twice a week with 50 mL of tap water, in alternating days. Because plant growth promotion can only be observed at later stages of plant-bacterium interaction (de Souza et al. 2016), plants were grown for 90 days (following the first inoculation), in a growth chamber under the same conditions as described for germination. Pots were randomized once a week.

## Harvest and analysis

The effect of *H. seropedicae* inoculation on rice photosynthetic efficiency was analysed immediately before harvesting. Net photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ) and intracellular  $\text{CO}_2$  concentration ( $C_i$ ) were measured in young fully developed leaves (second or third leaf) in four plants per treatment (from different pots), between 8:00 and 10:00 h using a LI-6200 portable photosynthesis system (LI-COR, Lincoln, NE, USA) with a 6  $\text{cm}^2$  leaf chamber. Air temperature inside the chamber was set at 25 °C, and  $\text{CO}_2$  concentration was kept at 390 ppm (ambient  $\text{CO}_2$  levels) using a  $\text{CO}_2$  injector system (LI-COR, Lincoln, NE, USA). Photosynthetic photon flux intensity was 850  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , provided by a red/blue LED light source (6400-02B).  $C_i$  and  $g_s$  were measured at the same time as  $A$ , and  $A/C_i$  (instantaneous carboxylation efficiency) was calculated.

Plants were harvested 105 days after sowing, and separated into roots and shoots. The effect of *H. seropedicae* inoculation on rice growth efficiency was analysed by measuring shoot length, biomass and stem diameter. The effect of *H. seropedicae* inoculation on rice nutrient uptake was analysed by quantifying shoot concentrations of P, K, Ca, Mg, S, Fe, Zn, Mn, Cu and B. Shoots were dried at 60 °C until constant weight was achieved, digested with  $\text{HNO}_3$  (Merck) and  $\text{H}_2\text{O}_2$  (Merck) in an open digestion system (Peters 2005), and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES), with plasma flow rate of 8.0  $\text{L min}^{-1}$ , auxiliary gas flow rate of 0.70  $\text{L min}^{-1}$  and carrier gas flow rate of 0.55  $\text{L min}^{-1}$ . N was quantified using the Nessler method (Jackson 1965) as described in the Handbook of Soil and Plant Analysis (Kalra 1997)

## Natural

ATP- and  $\text{PP}_i$ -dependent  $\text{H}^+$  transport across membranes were measured as the initial fluorescence quenching rate of 9-amino-6-chloro-2-methoxyacridine (ACMA) at 25 °C, using a fluorimeter (model F-3010, Hitachi, Tokyo), according to Façanha and de Meis (1998).

## Calculations and statistics

Nutrient contents were calculated using shoot nutrient concentrations and biomass values. The effect of *H. seropedicae* inoculation was calculated as follows:

$$\text{Inoculation effect (\%)} = (\text{Parameter}_{\text{inoculated}} - \text{Average parameter}_{\text{non-inoculated}}) / \text{Average parameter}_{\text{non-inoculated}} \times 100.$$

where  $\text{parameter}_{\text{inoculated}}$  is the value of a given parameter determined for inoculated plants;  $\text{Average parameter}_{\text{non-inoculated}}$  is the average value for the same parameter determined for non-inoculated plants. Means were compared using the Student's  $t$  test, at  $p \leq 0.05$ . In all cases, preliminary analyses were performed to ensure no violation of statistical assumptions (including the Levene test for equality of variances). All statistical analyses were performed using the GraphPad Prism 7.0 software.

## Results

Endophytic colonization of rice roots by the PGPB *H. seropedicae* and lateral root proliferation were observed in all inoculated plants (data not shown). Inoculating rice plants with *H. seropedicae* increased plant growth (Fig. 1), nutrient contents (Table 1), and photosynthetic (Fig. 2) and H<sup>+</sup>-pumping (Fig. 3) activity. Inoculated plants grew more than non-inoculated plants: they were 30% taller, presented a 70% wider diameter and accumulated 190% more biomass than non-inoculated plants (Fig. 1). Shoot macro and micronutrient contents were also much higher for inoculated than for non-inoculated plants, except for Mg, which was higher for non-inoculated plants (Table 1). Only non-inoculated plants showed pale-yellow leaves, especially older ones, indicating nitrogen deficiency (data not shown). *Herbaspirillum seropedicae* inoculation resulted in <sup>15</sup>N enrichment ( $\delta^{15}\text{N} = 5.7 \pm 0.5$ ; Table 1) of inoculated plants, contrasting with a <sup>15</sup>N impoverishment ( $\delta^{15}\text{N} = -2.7 \pm 0.3$ ) in non-inoculated plants. The  $\delta^{15}\text{N}$  values for inoculated plants were closer to those for the soil ( $\delta^{15}\text{N}_{\text{soil}} = 7.9 \pm 0.1$ ), whereas the  $\delta^{15}\text{N}$  values for non-inoculated plants were closer to those for the nutrient solution ( $\delta^{15}\text{N}_{\text{nutrient solution}} = -1.7 \pm 0.3$ ).

Net CO<sub>2</sub> assimilation rate ( $A_{390}$ ) and instantaneous carboxylation efficiency ( $A_{390}/C_i$ ) were 80% higher for inoculated than for non-inoculated plants (Fig. 2). No significant differences were observed in internal CO<sub>2</sub> concentration (data not shown), whereas stomatal conductance was lower for inoculated plants ( $g_s$ —i.e., 40% higher resistance to CO<sub>2</sub> diffusion across stomata).

*Herbaspirillum seropedicae* inoculation had no effect on root plasma membrane H<sup>+</sup>-ATPase (ATP hydrolytic activities or H<sup>+</sup> transport), but greatly increased the hydrolytic activities and H<sup>+</sup> transport of vacuolar H<sup>+</sup> pumps: a 740% increase in ATP hydrolytic activity and 430% increase in H<sup>+</sup> transport was observed for the vacuolar H<sup>+</sup>-ATPase and a 3970% increase in PP hydrolytic activity and 1860% increase in H<sup>+</sup> transport for the H<sup>+</sup>-PPase (Fig. 3).

Natural <sup>15</sup>N abundance in rice shoots, soil and nutrient solution were determined by mass spectrometry (IRMS, Micromass-GV Instruments, UK), using the following equation:  $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ , where  $R$  is the <sup>15</sup>N/<sup>14</sup>N ratio. The N standard (<sup>15</sup>N/<sup>14</sup>N) was atmospheric N (air) (Sandberg et al. 2012).

Root endophytic colonization by *H. seropedicae* was immediately (after harvest using fresh roots) confirmed using fluorescence microscopy (Canellas and Olivares 2017).

The effect of *H. seropedicae* inoculation on rice plant H<sup>+</sup> pumping efficiency (plasma membrane H<sup>+</sup>-ATPase—EC 3.6.3.6; vacuolar H<sup>+</sup>-ATPase—EC 3.6.1.3; and vacuolar H<sup>+</sup>-PPase—EC 3.6.1.1) was determined in fresh roots. Membrane vesicles were isolated from 10 g FW of rice roots as described by Giannini and Briskin (1987), frozen in liquid N<sub>2</sub> and stored at -80 °C until analysis. Protein concentrations were determined using the Bradford method (1976). ATPase and PPase activities were determined colorimetrically by measuring P<sub>i</sub> release (Fiske and Subbarow 1925).

## Discussion

*Herbaspirillum seropedicae* endophytically colonized rice roots and promoted plant growth by increasing nutrient uptake and photosynthetic capacity. For the first time, we showed the importance of vacuolar proton pump activity as a possible mechanism to explain plant growth promotion by PGPB at an early stage of plant-microbial interaction.

Previous studies have shown that endophytic PGPB provide greater crop enhancement than non-endophytic ones (Cocking 2003; Baldani and Baldani 2005), because they are more efficient colonizers, outcompeting other microorganisms (Verma et al. 2004). The observed plant growth stimulation by *H. seropedicae* was 15–20% higher than previously reported for rice (Bhattacharjee et al. 2008). This may be due to the fact that in the present case plants were grown under limiting nutrient availability, and PGPB effects are usually higher under stressful or non-favourable conditions (Fonseca et al. 2017; Dias et al. 2018; Al-Garni et al. 2019). However, growth and nutrient uptake increases similar to those observed are

not expected under field conditions, since PGPB field trials can have inconsistent effects on plant productivity (Kong et al. 2018; Ji et al. 2019; Timmusk et al. 2017). This is because: (1) PGPB isolated from a given crop may not be equally efficient/beneficial to other crops; and (2) variable biotic and abiotic conditions can affect the success of plant colonization by PGPB, which is not always stably maintained (Ji et al. 2019). Invasiveness should be considered when assessing the ecological impacts of field PGPB inoculation, especially how the inoculated strains survive or colonize the rhizosphere of host crops, how they interact with the indigenous soil microbiome and function, and how the indigenous soil microbiome responds to them, structurally and functionally (Kong et al. 2018). Few studies have evaluated the effects of bacterial inoculation on plant growth promotion and soil microbial community composition under field conditions (Cipriano et al. 2016). Some studies observed no changes in the rhizosphere microbial

community in response to PGPB inoculation (Armada et al. 2018), whereas others observed changes, although without counterproductive effects (Cipriano et al. 2016). Further studies are, therefore, needed.

All plants were supplied with growth-limiting N (0.39 mmol per week), which is consistent with the pale-yellow leaves (especially older leaves) observed in non-inoculated plants. However, inoculated plants showed no visual symptoms of nutrient deficiency, and presented higher growth and nutrient contents. Inoculated plants overcame nutrient limitations through better exploitation of soil resources, at least for N, as indicated by the shoot  $\delta^{15}\text{N}$ : inoculated plants presented shoot  $\delta^{15}\text{N}$  values that were more similar to those for the soil than non-inoculated plants, showing that they used a higher proportion of the soil N than non-inoculated plants.

Several mechanisms may contribute to more efficient nutrient uptake, namely increased lateral root development. If the effect of *H. seropedicae* inoculation was only due to increased root development, then the increases in nutrient

contents and growth should be proportional and identical for all nutrients. However, this was not the case, as inoculated plants were significantly more enriched in phosphorus, potassium, sulphur and zinc than in other nutrients when compared to non-inoculated plants, showing a putative effect of the bacterial inoculation on plant nutritional balance. The effect on plant nutritional balance could, either alone or together with hormonal changes due to *H. seropedicae* activity, may be responsible for changes in plant physiological activity such as photosynthesis (Peng et al. 2002; Zhou et al. 2006; del Amor and Cuadra-Crespo 2012; Bhattacharjee et al. 2008; de Souza et al. 2016). During photosynthesis,  $\text{CO}_2$  moves from the atmosphere to the sub-stomatal chamber (*C<sub>i</sub>*) through stomata, and from there through the leaf mesophyll to the carboxylation site inside the chloroplast stroma (Flexas et al. 2008). In our study, *H. seropedicae* inoculation stimulated net carbon assimilation and carboxylation efficiency despite lower stomatal conductance and similar *C<sub>i</sub>* (data not shown). Even though we did not measure mesophyll conductance, *H. seropedicae* inoculation may have stimulated the biochemical pathway associated with photosynthetic carbon assimilation, as observed for *A. thaliana* inoculated with *Gluconacetobacter diazotrophicus*, another endophytic diazotrophic PGPB (de Souza et al. 2016). Furthermore, by decreasing stomatal conductance *H. seropedicae* inoculation may decrease whole canopy transpiration, which is another advantage, especially considering plant water use efficiency.

In contrast with our results, Canellas et al. (2013) reported higher stomatal conductance in maize plants inoculated with *H. seropedicae*. However, they associated this to the activation of plasma membrane  $\text{H}^+$ -ATPase, a  $\text{H}^+$  pump that was not affected by *H. seropedicae* in our study. Because ATPase activity is an energy demanding process, the lack of activation of plasma membrane  $\text{H}^+$ -ATPase by *H. seropedicae* inoculation may be a symptom of sugar deprivation of plant root cells (Ramos et al. 2009). On the other hand, *H. seropedicae* inoculation stimulated vacuolar  $\text{H}^+$ -pumps (V- $\text{H}^+$ -ATPase and especially  $\text{H}^+$ -PPase), which may be an important part of the plant host response to energy consumption by the endophyte, as previously shown for arbuscular mycorrhizal fungi (Ramos et al. 2005). Vacuolar  $\text{H}^+$ -pumps have been shown to replace the pivotal role of plasma membrane  $\text{H}^+$ -ATPase under stress conditions (Ramos et al. 2009; Maeshima 2000). Our data showed that the energy backup system represented by vacuolar  $\text{H}^+$  pumps is tightly regulated in roots due to the increased energy demand of 'hosting' the endophytic PGPB and increased lateral root proliferation. In particular, activation of the energy-saving PPi metabolism (vacuolar  $\text{H}^+$ -PPase) in roots is an advantage, because it saves ATP that is needed for cells with higher metabolic activity located in physiologically strategic root regions (e.g., elongation zone and root hairs) (Ramos

et al. 2009). Non-exclusively, higher H<sup>+</sup> pumping activity would enable greater vacuolar compartmentation of solutes and metabolites (including compounds that could inhibit cytoplasmic enzymes) (Neuhaus and Trentmann 2014; Zhang et al. 2017), increasing vacuolar volume and allowing root cells to grow without de novo production of cytosolic components (Dünser and Kleine-Vehn 2015). It is, therefore, likely that *H. seropedicae* changed the abundance of vacuolar H<sup>+</sup> pumps through differential gene expression and/ or changed their activities due to post-translational modification or by interacting proteins (Neuhaus and Trentmann 2014).

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The observed stimulation of H<sup>+</sup>-PPases, and associated increased growth, nutrient contents and photosynthetic efficiency, was within the range reported for transgenic overexpression of vacuolar H<sup>+</sup>-PPases (from either *Arabidopsis* or *Thellungiella halophila*) in a variety of agriculturally important crops, including rice, grown under normal or stressful conditions, such as drought, salinity, and nutrient limitation (Gaxiola et al. 2001; Li et al. 2005, 2008, 2010; Yang et al. 2007; Pasapula et al. 2011; Paez-Valencia et al. 2013). Our results showed a similarity between the processes involved in plant growth stimulation by the endophytic bacterium *H. seropedicae* or by genetic modifications. This simple inoculation of the endophytic PGPB *H. seropedicae* can promote growth

and photosynthetic efficiency in rice, which will likely result in a more efficient utilization of resources (nutrients and water) and increase production of nutrient-rich food at reduced economic and environmental costs (Paez-Valencia et al. 2013).

**Author contribution statement** ACR, JM, SBS, CC and TD designed the experiment. JM, SBS and RAS conducted the experiment. FLO confirmed microscopically the endophytic colonization of rice roots by *H. seropedicae*. WPR and EC conducted the gas exchange measurements. ACR, JM, SBS and AAB extracted the membranes and quantified the proton pumps activity, and quantified the nutrients. SBS and FJE analysed the data. ACR, JM, SBS, CC and TD wrote the manuscript and all co-authors reviewed it.

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Table

**Table1** Effect of *H. seropedicae* inoculation on rice shoot mineral contents and isotopic N signature ( $\delta^{15}\text{N}$ )

|   | Non-inoculated | Inoculated | Inoculation effect |
|---|----------------|------------|--------------------|
| <b>Macronutrients (mg shoot<sup>-1</sup>)</b> |                |            |                    |
| N*  | 15 ± 1         | 33 ± 1     | + 120%             |
| P*  | 1 ± 0          | 6 ± 1      | + 350%             |
| K*  | 14 ± 1         | 77 ± 1     | + 430%             |
| Ca*   | 4 ± 1          | 11 ± 1     | + 150%             |
| Mg*   | 3 ± 0          | 6 ± 1      | + 110%             |
| S*  | 2 ± 0          | 6 ± 1      | + 300%             |
| <b>Micronutrients (µg shoot<sup>-1</sup>)</b> |                |            |                    |
| Fe*   | 323 ± 12       | 576 ± 19   | + 80%              |
| B*  | 15 ± 1         | 50 ± 2     | + 230%             |
| Cu*   | 10 ± 1         | 39 ± 0     | + 280%             |
| Mn*   | 682 ± 28       | 465 ± 17   | - 30%              |
| Mo*   | 0 ± 0          | 2 ± 0      | + 1020%            |
| Ni*   | 11 ± 0         | 50 ± 1     | + 340%             |
| Zn*   | 95 ± 13        | 3385 ± 193 | + 3460%            |
| $\delta^{15}\text{N}^*$                       | - 2.7 ± 0.3    | 5.7 ± 0.5  | Na                 |

Values are means ± SE ( $n=5$ )

na means non applicable

\*Mark significant effects at  $p \leq 0.05$

## Figures

Fig.1 Effect of *H. seropedicae* inoculation on rice growth. Growth was evaluated by determining shoot length (a), stem diameter (b) and biomass (c). Bars are means  $\pm$  SE (n = 5)

Fig.2 Effect of *H. seropedicae* inoculation on rice photosynthetic activity. Photosynthetic activity was evaluated by determining a net carbon assimilation at ambiente CO<sub>2</sub> concentrations (A390=, b – Stomatal conductance (gs), and c – carboxylation efficiency (A390/Cix 103) Bars are means  $\pm$  SE (n=5).

Fig. 3 Effect of *H. seropedi- cae* inoculation on rice root H<sup>+</sup>-pumping activity. Root H<sup>+</sup>-pumping activity was evaluated by determining the hydrolytic activity ( $\mu\text{mol P mg}^{-1}$  root  $\text{min}^{-1}$ ) and pro- ton pumping initial velocity (relative fluorescence units) of a, b plasma membrane H<sup>+</sup>-ATPase (P-H<sup>+</sup>-ATPase), c, d vacuolar H<sup>+</sup>-ATPase (V-H<sup>+</sup>-ATPase), and e, f vacuolar H<sup>+</sup>-pyrophosphatase (H<sup>+</sup>-PPase). Bars are means $\pm$ SE (n=5)

Fig 1

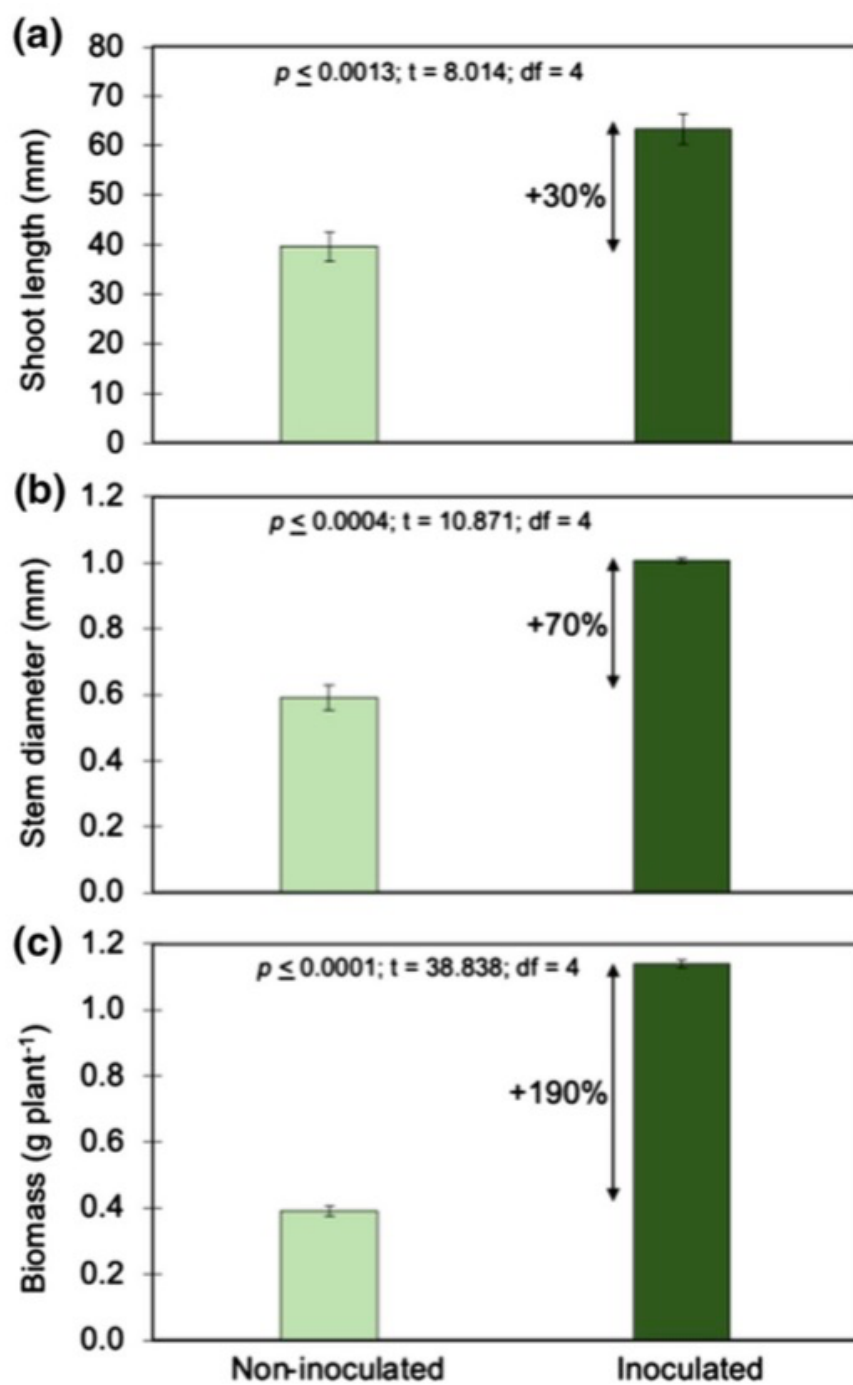


Fig2

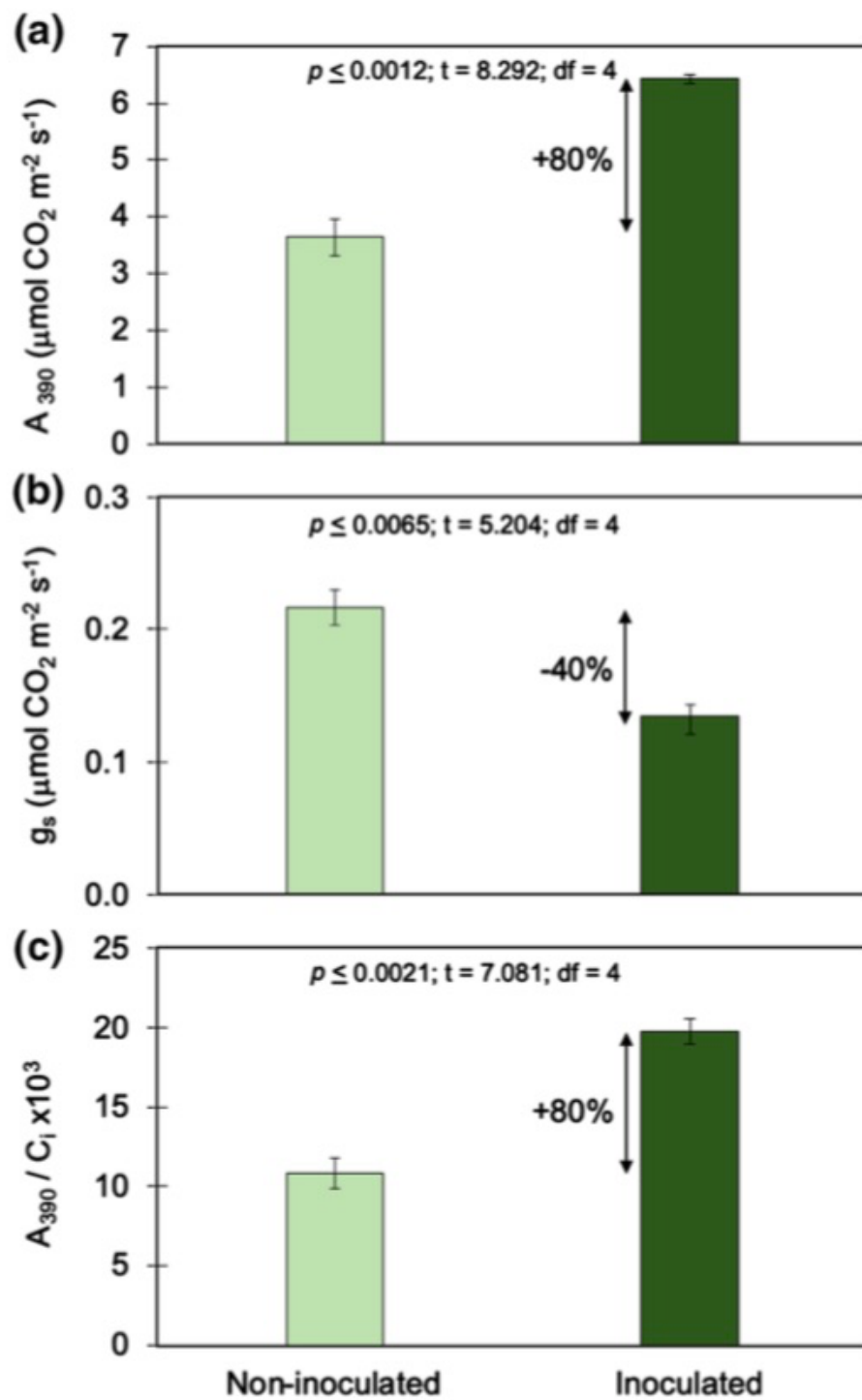


Fig 3

