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BASIC AREAS
Article

Root development and nitrogen acquisition of maize inoculated with two species of diazotrophs

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ABSTRACT: Plant-growth-promoting bacteria of the genus *Azospirillum* and *Herbaspirillum* can improve crop yields of maize in tropical environments. The main mechanism proposed for its promotion is related to the auxins produced by these species. The aim of this study was to compare the maize growth response of inoculation using two species, *Azospirillum baldaniorum* Sp245 (Ab-Sp245) and *Herbaspirillum seropedicae* ZAE94 (Hs-ZAE94) during the initial growth under controlled conditions. A pot experiment was conducted with five harvests measuring bacterial counts, root morphology, biomass, and nitrogen content, and soluble metabolites for 38 days. Depending on the harvest period, a differential growth response between the two strains test was compared to the control. Plants use the bacterium applied to improve nitrogen acquisition and transform it into higher biomass and nitrogen accumulation, especially for Ab-Sp245. On the other side, Hs-ZAE94 altered the soluble metabolites resulting in higher NO_3^-, NH_4^+, and N-amino and lower soluble fractions compared to Ab-Sp245 and the control. The observed plant-bacteria interaction is closely fine-tuned and regulated by the strain used and differences in growth promotion effects cannot solely attributed to the root morphology.

Key words: plant-bacteria interaction, nitrogen metabolism, root architecture.

INTRODUCTION

Maize is an important crop planted on 19,094 million hectares in Brazil, producing more than 88 million tons per year with two to three planting seasons (Faostat 2021). Nitrogen is the nutrient used in larger amounts, followed by potassium (Ye et al. 2022), which is applied in quantities related to the estimated productivity and represents 25% of the total fertilizer sold in Brazil (Heffer 2013). As a basic recommendation, for each ton of biomass produced, 25-27 kg of nitrogen is exported from the field (Sangoi et al. 2016). Nitrogen represents 15-20% of the maize production costs (Debruin and Butzen 2014) and is responsible for N_2O gas emissions (Martins et al. 2015) or is leached to the subsoil (Liu et al. 2020), as it is one of the most unstable elements present in the soil and is used as a fertilizer in higher amounts.

To overcome problems of nitrogenfertilization in the tropics, new technologies based on the biological process of nitrogen fixation or plant growth promotion have been adopted with selected bacteria used as an inoculant in Brazil since 2010 (Hungria et al. 2010), and even earlier in South America (Cassán and Dias-Zorita 2016). The Brazilian inoculant industry is based on a single bacterial species, *Azospirillum brasilense*, and the work done by several authors worldwide use the Sp245 strain (Spaepen and Vanderleyden 2015) now reclassified as *Azospirillum baldaniorum* by Ferreira et al. (2020). In 2010, a group containing two strains was tested and recommended for maize application, Abv5 and Abv6 (Hungria et al. 2010). Since then, both strains have been used in Brazil as a combined inoculant (Galindo et al. 2017; Martins et al. 2017).

In 1986, other diazotroph genera and species were discovered. *Herbaspirillum* differs from the previous genus *Azospirillum* in several attributes, being a β-proteobacteria that colonizes roots and shoots of several crops that lives within the inner spaces between plant cells (Monteiro et al. 2012). The first species was isolated from maize, wheat, and rice by Baldani et al. (1986) and is considered a true endophyte characterized by microscopy work done by James and Olivares (1998). The strain ZAE94 was selected for maize application among the other 25 strains of *Herbaspirillum* spp. (Alves et al. 2015) and tested in several locations for at least two (Alves et al. 2020) and three consecutive years (Alves et al. 2021).

While both microbial species show a similar auxin-based mechanism of action, it is unclear whether they have a differential effect on the growth of maize (Dias et al. 2021; Spaepen and Vanderleyden 2015). To understand the bacterium species interaction, an experiment was conducted in a sterile substrate to compare maize growth using a triple hybrid SHS5050 cultivar, previously tested by Alves et al. (2015) inoculated with *A. baldaniorum* strain Sp245 (Ab-Sp245) and *Herbaspirillum seropedicae* ZAE94 (Hs-ZAE94) to determine their effect on root architecture, nitrogen absorption, and biomass accumulation.

METHODS

A pot experiment was conducted at Empresa Brasileira de Pesquisa em Agropecuária (Embrapa), Centro Nacional de Pesquisa em Agrobiologia (CNPAB), Seropédica, RJ-Brazil (22°44'38"S and 43°42'28"W; 26 m altitude). The experiment was planted in October and harvested 38 days after sowing and during this period the average sunlight was around 12 hours per day. Pots were the experimental units and were arranged in five complete casualized blocks (repetitions) in an automatic greenhouse with temperature and humidity control.

Plastic pots of 10 kg capacity were filled with 7 kg of sand/vermiculite sterile substrate (2/1 v/v). The maize cultivar used was the triple hybrid SHS5050 produced by Santa Helena Seed Company. The inoculation treatments were: uninoculated control (NI), inoculated with *H. seropedicae* (Hs-ZAE94); and *A. baldaniorum* (Ab-Sp245) using five plants per harvest time, totaling 20 pots for each inoculation treatment and H2, H3, H4, and H5 were destructive (Fig. 1). Harvest 1 used 1g of the roots, which were collected from the existing pots. Nitrogen was supplied during the experiment with seven applications at different growth periods.

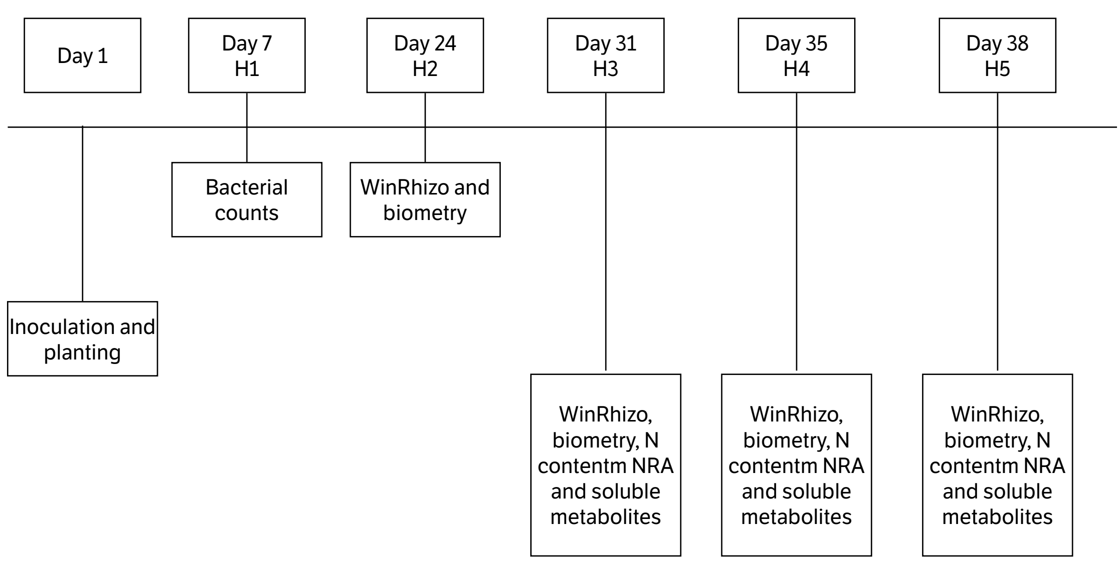

Figure 1. Measurements and harvest schedule of maize inoculation experiment using two strains of diazotrophs for 38 days. Legend: H – Harvest; WinRhizo = root analysis using software WinRhizo Pro™; NRa – Nitrate reductase activity

Source: Elaborated by the authors.

Inoculation and bacterial counting

The bacteria used were *H. seropedicae*, strain ZAE94 (= BR11417), and *A. baldaniorum* strain Sp245 (= BR11005), both acquired from the Centre of Biological Resources Johanna Döbereiner - CRB-JD (BR label). Inoculant was produced from a single colony grown on NFb 3x as described by Baldani et al. (2014). Inoculation used the DYGS medium with malic acid pH 6.5 (Baldani et al. 2014) grown at 30 oC in a rotary shaker at 175 rpm for 20 h. This inoculum was transferred to 150 mL of the NFB (Ab-Sp245) and JNFb (Hs-ZAE94) liquid media (Baldani et al. 2014) using 2 mL each. After cell growth, bacteria were counted using a Neubauer counting plate to equalize both populations to 10^6 cells·mL^{-1} using saline solution to dilute the cells if necessary. The inoculant was applied on the seeds at the time of planting at a dosage of 1 mL per seed, and the control received the same amount of phosphate buffer (pH 7.0).

At 7 and 24 days after planting, 1 g of roots was sampled for bacterial counting using the nitrogenfree semi-solid media JNFb and NFb for the strains Hs-ZAE94 and Ab-Sp245, respectively (Baldani et al. 2014) and bacterial counting (Most Probable Number technique - MPN) with the application of McCrady's Table using three replicates (Baldani et al. 2014).

Pot experiment procedures and analysis

The seeds were washed superficially with running water until the complete removal of the fungicide and after that, three washes were carried out with distilled water. Pots with 10 L capacity were used containing 7 kg of a sterile substrate, sand + vermiculite (2:1 v/v) autoclaved at 121 °C for 20 min and repeated this operation 2 days later. Three seeds were sown per pot, inoculated, and after five days of seedling emergence, thinning was performed for homogenization, keeping one plant per pot.

The substrate chemical analysis had the following characteristics: pH (H_2O) 6.70; macro elements (in cmol$_c$·dm^{-3}): Ca = 0.39; Mg = 2.81; Al = 0.0; (mg·dm^{-3}) K = 48.72 and P = 5.97; N = 0.01%. Fertilization was carried out in a fractional way in applications of a maximum of 100 mL each per pot of modified Hoagland solution with a pH of 5.8 (Hoagland and Arnold 1950) and supplemented with simple superphosphate (P_2O_5). The concentrations added per pot were: 0.043 g of N; P = 0.25g; K = 0.12g; Mg = 0.024 g; Ca = 0.080 g and S = 0.1g. Micronutrients were added as follows: B = 0.25 mg; Cu = 0.010 mg; Mn = 0.312 mg; Mo = 0.0041 mg; Zn = 0.025 mg, and Fe = 1.5 mg. All nutrients were applied as a complete Hoagland's solution at 7, 11, 14, 17, and 27 DAP and two nitrogen applications at 28, and 30 DAP as follows: 5 times 8.4 mg of nitrogen each time, and at 17 and 28 DAP 2 times 16.8 mg of nitrogen. The total amount of nutrients applied were (g per pot): 0.075 N, 0.26 P, 0.14 K, 0.031 Mg, 0.322 Ca, 0.16 S, and micronutrients (mg): 0.3 B, 0.012 Cu, 0.375 Mn, 0.005 Mo, 0.03 Zn, and 1.8 Fe. Plants were cultivated with 0.0756 g of nitrogen per pot using a solution divided as 5/6 using NO_3^- and 1/6 using NH_4^+ (0.014 g). As NO_3^- the source was 0.014 g of $Mg(NO_3)_2$ and 0.056 g of $Ca(NO_3)_2$ per pot. The experiment had a total duration of 38 days in which the plants were evaluated in four harvests using methods described in Fig. 1. A complete list of nutrients and chemical products used is described by Dias et al. (2021).

Root architecture

Sampled roots immersed in 50% ethanol was scanned and characterized by image analyses using *WinRHIZO Pro*® software (Regent Instruments, QC, Quebec, Canada) coupled to an Epson Expression 11000XL LA2400 image scanner, as described by Bouma et al. (2000). Roots were laid out in an acrylic container (30 × 40 cm), with water at an approximate depth of 1 cm, and placed onto the scanner. Total length (TL, m), surface area (SA, m^2), root volume (RV, m^3), and the number of tips, forks, and crossings were recorded.

Nitrate reductase activity (NRA – EC 1.7.1.1)

Nitrate reductase activity (NRa) was assessed using the in vivo method described by Jaworski (1971) and adapted for maize according to Breda et al. (2020). The amount of NO_2^- produced was measured, to estimate NRa, and expressed as nmol $NO_2^{-}\cdot h^{-1}\cdot g^{-1}$ fresh weight. NRa was blocked during the high incidence of daylight to avoid circadian effects, as measured using a luxmeter (Testo 540™).

Nitrogen and sugar soluble fractions

One gram of leaf or root tissue was sampled and added to 20 mL of ethanol /Milli-Q water™ (80% v/v), crushed for 3 min using a Turratec homogenizer (model TE-102 – Tecnal, Sao Paulo, Brazil) equipped with a 12 mm helix, and filtered through 4 layers of sterilized gauze and filter paper ø = 150 mm (Whatman™). The filtrate was then collected and transferred to a separating funnel with chloroform to form polar and nonpolar phases. The nonpolar phase was discarded, and the polar phase was used for the analyses. The levels of nitrate (plus nitrite) were determined according to Miranda et al. (2001), free amino-N content by the ninhydrin method according to Yemm and Cocking (1955), $N\text{-}NH_4^{+}$ levels by the colorimetric method (Mitchell 1972; Felker 1977), and soluble sugar contents according to Yemm and Willis (1954).

Biometric evaluation

The dry biomass of shoots and roots was quantified after 72 h in a forced air oven at 65 °C. Total nitrogen was measured after the root and shoot were dried and grounded in a Wiley mill to < 2 mm. The ground material was analyzed for nitrogen content (%) following the Kjeldhal procedure, as described by Bremner and Mulvaney (1983). The N accumulated in the plant was quantified by the product of n content ($g\cdot kg^{-1}$ of dry weight) and corresponding plant dry weight.

Statistical analysis

Statistical analysis including analysis of variance for the assumptions of normality (Lilliefors test) and homogeneity of error variances (Bartlett test) and the means of the variables were submitted to analysis of variance. Two software were used: SISVAR 5.3 (normality and homogeneity) and R Core Team (2020) for the ANOVA and if there was significance, the means were compared using the Fisher LSD test at a 5% probability level. Pearson's linear correlation coefficient (r) was calculated in the Excel software between the variables accumulated nitrogen (Total N - $mg\cdot plant^{-1}$) and root weight (root dry weight - g) over time.

RESULTS AND DISCUSSION

Bacterial counts of the two inoculated strains were performed using the inoculant applied and the fresh roots evaluated at 7 and 24 days after planting (Fig. 2). The initial population applied to the plant was calculated as 6.65 and 6.40 Log_{10} cell number$\cdot mL^{-1}$ for Ab-Sp245 and Hs-ZAE94, respectively. Seven days later, the population of the treatment with Hs-ZAE94 was superior to the others, 100 times higher than for Ab-Sp245. At 24 DAP these differences in cell number could not be detected. The cell number of bacteria using a semisolid medium tends to decrease, reaching equilibrium as the plant grows.

Biomass accumulation varied among the different treatments at 24 DAP (Fig. 3a). Both strains Ab-Sp245 and Hs-ZAE94, significantly increased the dry weight of roots compared to the control, with a 45% increase observed for Ab-Sp245 and a 47% increase for Hs-ZAE94. This increase in root dry weight was consistent across subsequent harvests at 31, 35, and 38 DAP (Fig. 3b). In terms of shoot dry mass accumulation, inoculation with Ab-Sp245 resulted in an 18% increase compared to the control, followed by Hs-ZAE94 with a 9% increase at 38 DAP. The final harvest also revealed differences in total dry weight, primarily due to the root dry mass of plants inoculated with Hs-ZAE94 (Fig. 3c).

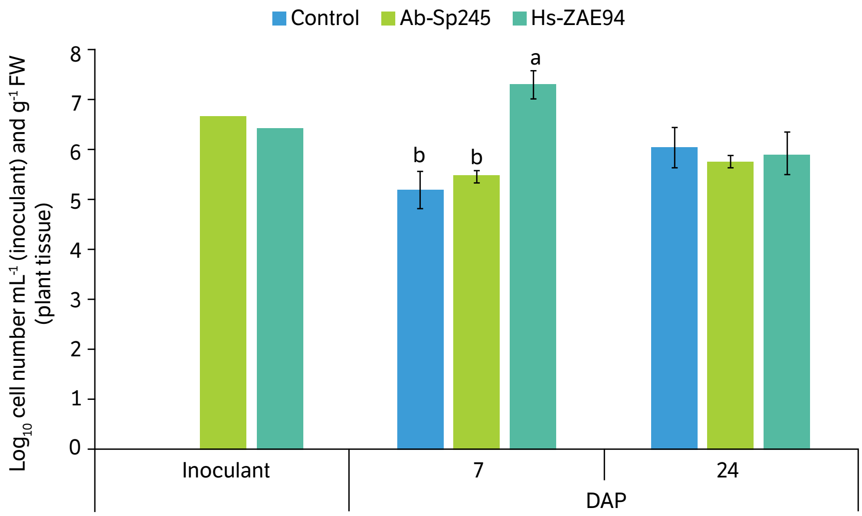

Figure 2. Bacterial counts of maize roots using two semisolid media, JNFb for Hs-ZAE94 and NFB for Ab-Sp245, seven and 24 DAP using the MPN technique (n = 3).

Source: Elaborated by the authors.

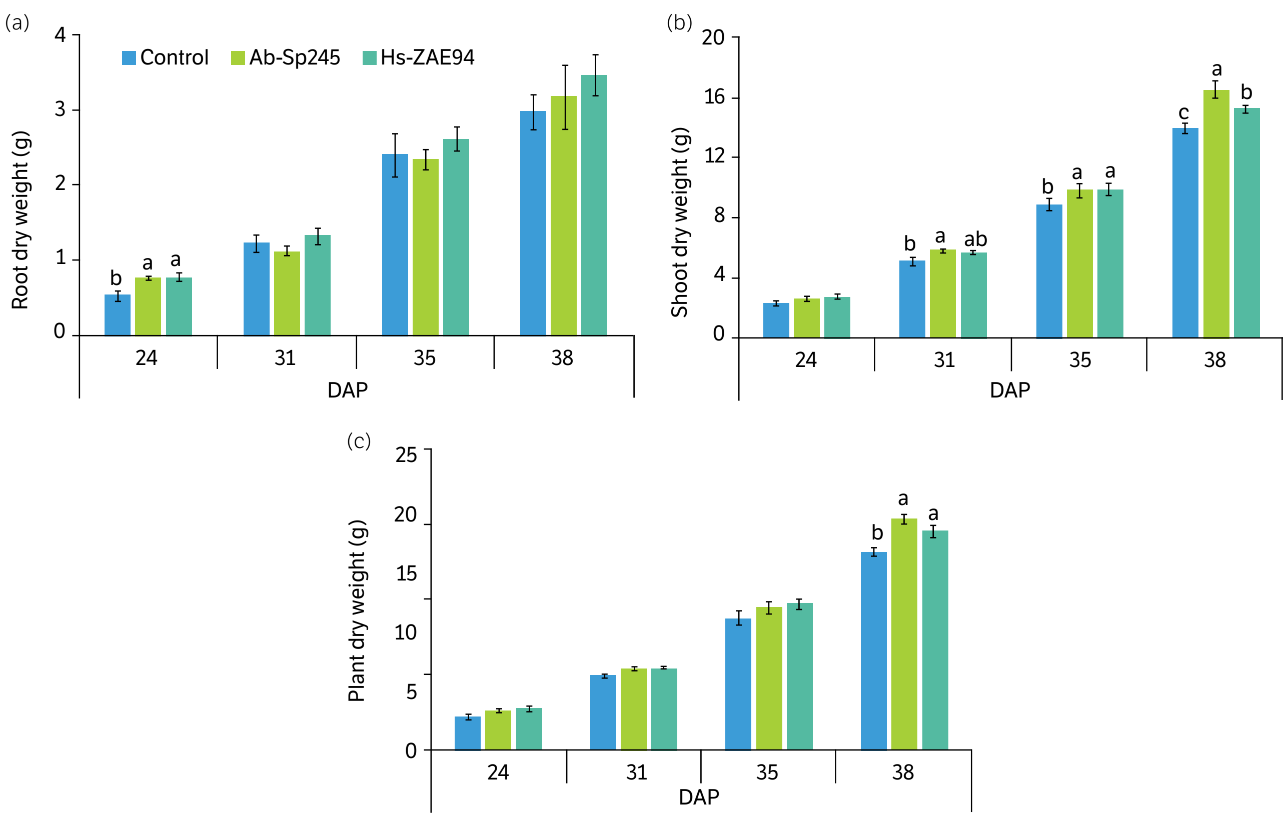

Figure 3. Biomass accumulation of maize inoculated with two diazotrophs evaluated at 24, 31, 35, and 38 DAP (a) Root dry weight; (b) Shoot dry weight; (c) Plant dry weight. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

The root morphology was compared between the different inoculation treatments for all four harvests. Inoculated plants showed significant differences in root length (Fig. 4a) and surface area (Fig. 4b) compared to the control. These differences were observed at 24, 35, and 38 DAP but not at 31 DAP. When maize roots were inoculated with Hs-ZAE94, there was an increase in root length. Specifically, the roots grew 52 m longer during the first period (from day 24 to day 31), followed by a further 56 m increase during the second period (31 – 35 DAP). In the third period (38 – 35 DAP), the roots grew additionally 32 m longer. Similarly, when Ab-Sp245 was used as an inoculant, there was a significant improvement as well in the parameter. Roots grew 40 m longer during the first period, followed by an additional 94 m longer in the second period. This increased root length was maintained during the final harvest (period 3 – Fig. 4a). The root surface showed the same pattern (Fig. 4b) as well as the projected area and root volume (Fig. 5). As the plants were not all the same, differences between harvests can vary depending on the individual plant used for root measurements. But, in any case, Ab-Sp245 initiated the root stimuli before Hs-ZAE94 (Fig. 4a). The number of tips, crossings, and forks differs between inoculation and control and between the tested strains (Fig. 6). Plants inoculated with Ab-Sp245 reached the maximum value at 35 DAP, but Hs-ZAE94 continued to grow.

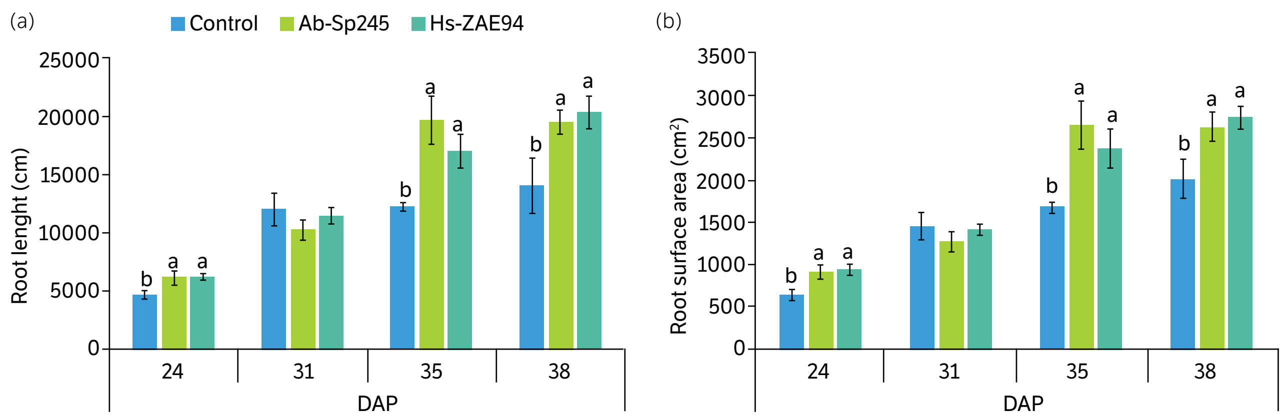

Figure 4. Root length (a) and surface area (b) of maize plants inoculated with two diazotrophs and evaluated at 24, 31, 35, and 38 DAP. (a) Root length; (b) Root surface area. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

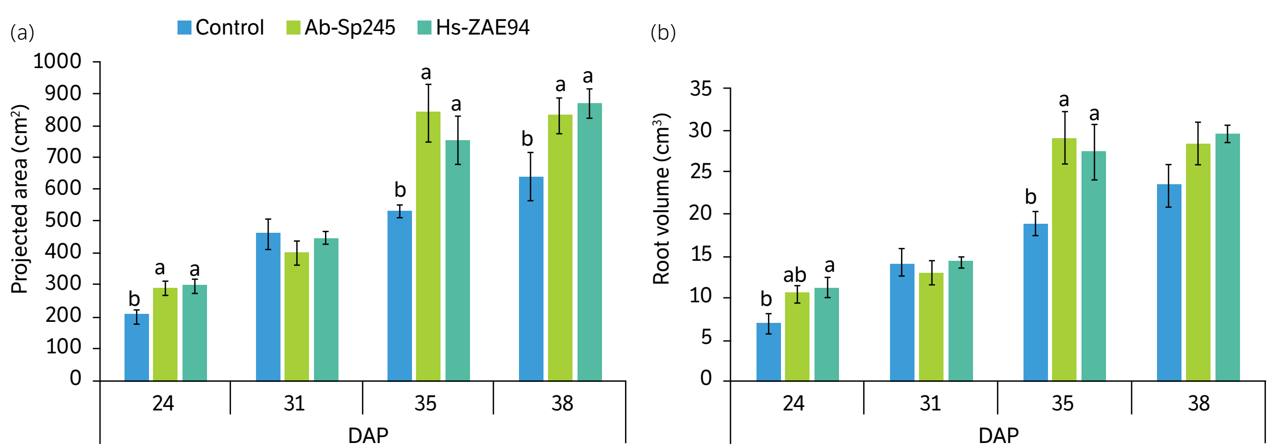

Figure 5. Root projected area (a) and volume (b) of maize inoculated with two diazotrophs (a) Projected area; (b) Root volume. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

Nitrogen accumulation of maize plants inoculated with Hs-ZAE94 was higher at 31 DAP compared to the other treatments (Fig. 7a). With Ab-Sp245, the higher total nitrogen was achieved at 38 DAP, plausibly a consequence of the root growth effect of the inoculated plants observed three days before (Figs. 5, 6). NRa activity was higher in plants inoculated with Ab-Sp245 at 35 DAP (Fig. 7b). At 38 DAP the activity was reduced, but in this case, Hs-ZAE94 showed a higher NRa activity compared to the other two treatments. This reduction in NRa throughout the harvests may indicate that NO_3^- was made available after the split fertilization was rapidly metabolized.

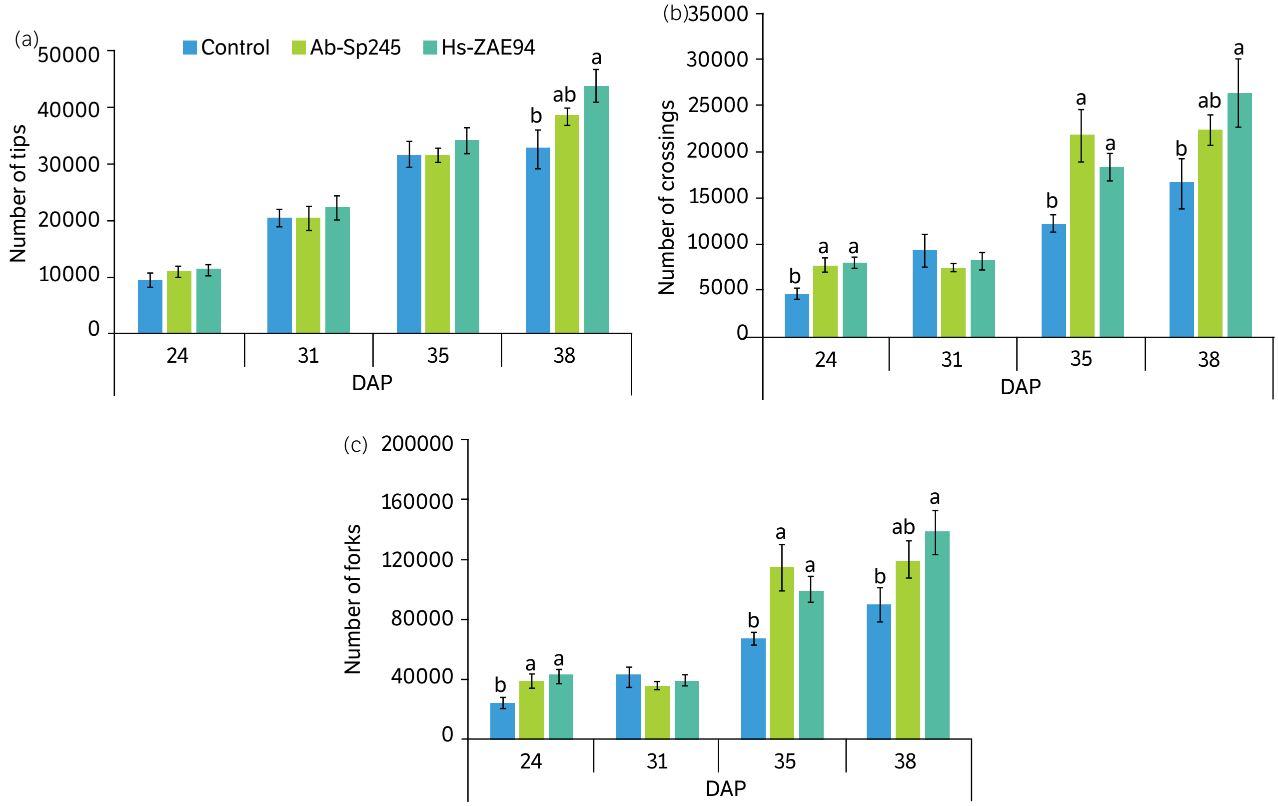

Figure 6. Morphological traits of maize roots inoculated with two diazotrophs and evaluated during 38 DAP: (a) Number of tips; (b) Crossings; (c) forks. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

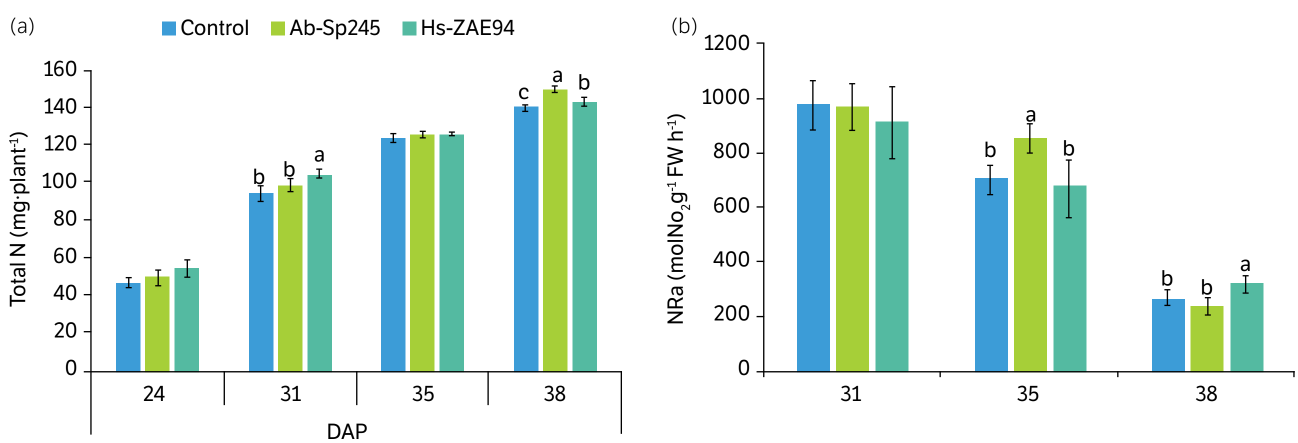

Figure 7. Total N (a) and Nitrate reductase activity (NRa) (b) of maize plants inoculated with Ab-Sp245 and Hs-ZAE94 was evaluated at 31, 35, and 38 DAP. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

The differential positive treatment effects were measured by the metabolic plant profile (Fig. 8). Depending on the harvest time and strain, NO_3^-, NH_4^+, and N-amino accumulated in the shoot tissue at higher levels in plants inoculated with Hs-ZAE94 (31 DAP), reducing to lower levels in the subsequent harvests at 35 DAP, except for N-amino. The plants likely used the nitrogen reserves accumulated in the previous period to produce biomass (Figs. 8a, 8b). Conversely, soluble sugars were lower in plants inoculated with Hs-ZAE94 at 31 DAP compared to the control (Fig. 8d). The higher NO_3^- corresponded to the lower NRa of inoculated plants with Hs-ZAE94 and the opposite occurred with Ab-Sp245 at 31 DAP (Figs. 7b, 8a). When reducing the nitrogen metabolites, soluble sugar increased but did not differ between treatments at 38 DAP (Fig. 8d).

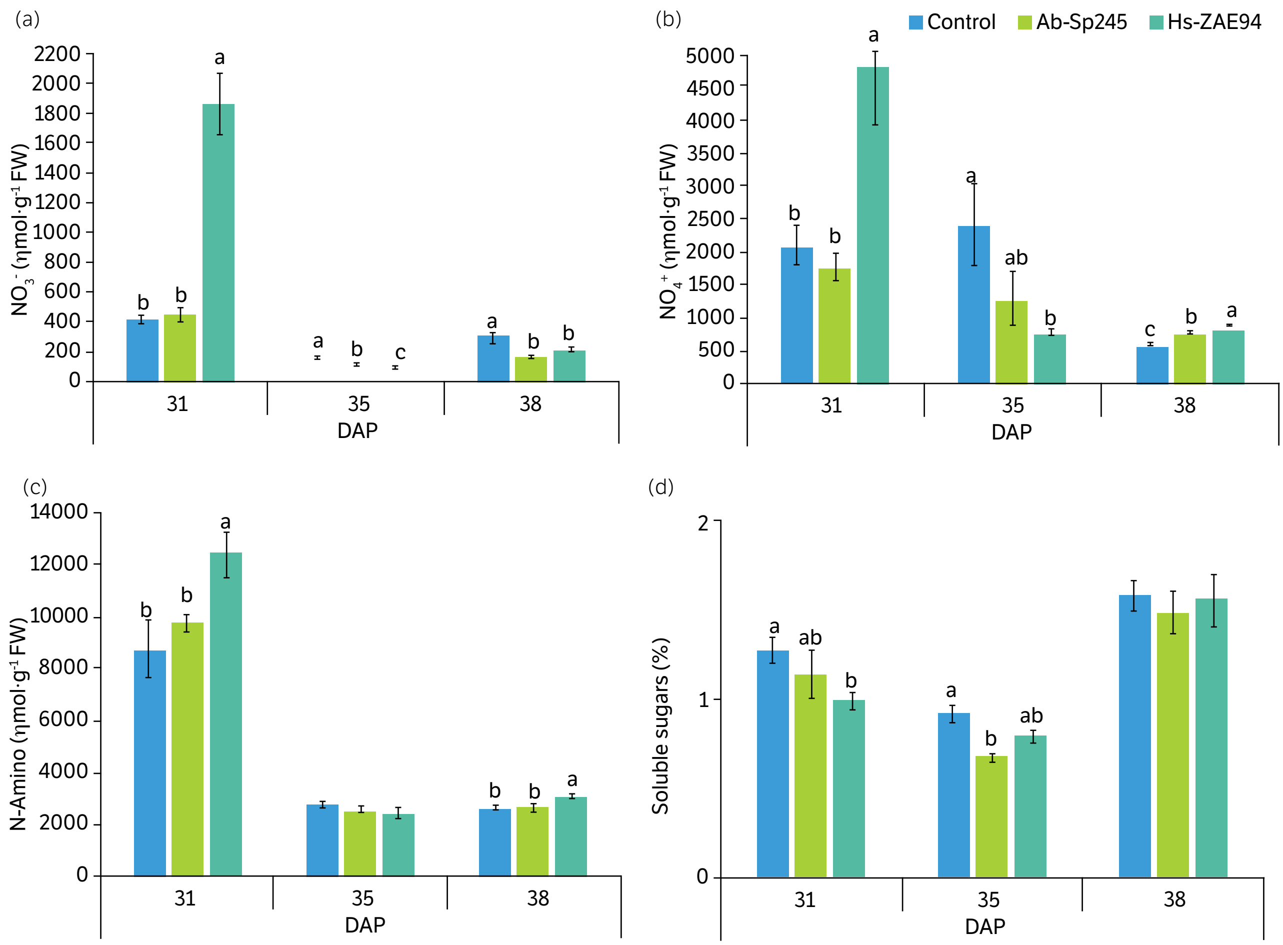

Figure 8. Soluble metabolites of maize plants inoculated with two diazotrophs: (a) Nitrate; (b) Ammonium; (c) N amino; (d) Soluble sugars. Columns represent the mean value, and the bars represent the standard error (n = 5). Letters differ at p≤0.05.

Source: Elaborated by the authors

Maize plants inoculated with two diazotrophs that colonize the roots in numbers higher than 10^5 cell·g FW (Fig. 2) were stimulated by improving roots (24 DAP) and shoot biomass at 31, 35, and 38 DAP (Fig. 3). Additionally, these plants displayed variation in root morphology (Figs. 4, 5, 6), accumulated higher levels of nitrogen (Fig. 7), and showcased improvements in NRa (Fig. 7) and soluble metabolites (Fig. 8). These responses were different for the two strains, contrasting with the control and varying depending on the harvest period. The data indicate that plants utilized bacterial inoculants to enhance nitrogen acquisition and facilitate higher biomass accumulation, as well as increased internal nitrogen levels in the plant tissue (Fig. 7). However, it is important to note that this occurred in a similar manner over the experimental period, albeit not simultaneously.

As indicated by the number of tips, crosses, and forks; the improvement of the root morphology can be linked to the strain used as an inoculant (Fig. 6). A contrasting trend was observed in the shoot dry weight (Fig. 3b). For instance, maize plants inoculated with Hs-ZAE94 showed decreased shoot weight after 38 DAP (Fig. 3b) but had a greater number of tips, crossings, and forks (Fig. 6), while the opposite effect was observed with plants inoculated with Ab-Sp245 (Figs. 3b and 6).

As several mechanisms are associated with the growth promotion of both strains, comparisons can be useful to quantify plant responses during the initial growth stages under controlled conditions, that allow root measurements in detail and associate them with the nitrogen uptake response. Internally, nitrogen metabolite levels were higher at the beginning (31 DAP), likely used to promote growth as measured at 35 DAP (Fig. 8). But plants inoculated with Hs-ZAE94 showed a different pattern, accumulating NO_3^-, NH_4^+, and N-amino at levels 4 times greater than the other treatments. Previously Dias et al. (2021), using a hydroponic system, observed a higher population of Hs-ZAE94 established in the roots of maize and compared this higher population with the growth-promoting effects on maize plants. In this study, Hs-ZAE94 also colonized the plants at an initially higher population size than Ab-Sp245 (Fig. 2). At 24 DAP, this bacterium colonization resulted in higher N-metabolite contents, and at 31 DAP plants accumulated higher nitrogen in the shoots (Fig. 7a) and lower NRa (Fig. 7b).

Azospirillum versus *Herbaspirillum* colonization patterns can partially explain the observed results, being the first one a rhizospheric bacterium, and *H. seropedicae* described as a true endophyte (Monteiro et al. 2012). In this study, one possibility is that the differential colonization can explain the higher bacterial numbers of Hs-ZAE94 observed in Fig. 2, and this colonization could explain the alteration of nitrate and ammonium levels in the shoots, especially at 31 DAP (Figs. 8a, 8b).

On the other hand, *Azospirillum brasilense*, the former species of *A. baldaniorum*, also modified the NRa, and expression of genes related to this enzyme as observed by Pereira-Defilippi et al (2017). These authors also inferred that the increments of the gene expression were associated with the root-enhanced morphology and in that case, maize plants were inoculated with the commercial product containing the mixed strains Abv5 and Abv 6. Based on these assumptions, both strains Hs-ZAE94 and Ab-Sp245 can act in a similar manner but in a different magnitude.

Bacteria of the genus *Azospirillum* are well known for their ability to promote plant growth. It has been found to induce the root response through the production of IAA (Puyvelde et al. 2011), which is a naturally occurring auxin class involved in coordinating plant development (Abel and Theologis 2010). *H. seropedicae* also produces auxins (Bastián et al. 1998) and has been found to have four pathways for their production (Pedrosa et al. 2011). The accelerated root development caused by these inoculated strains is crucial for crop growth as it allows plants to efficiently utilize the available nutrients in the soil, including nitrogen (Alves et al. 2020). Additionally, this enhanced root architecture can help to alleviate water stress, particularly in maize planted during the second crop season in Brazil (Alves et al., 2021) using the same two strains tested in this study. A correlation between the root dry weight and N accumulated in the shoots was high (0.94), indicating that the observed root traits improve nitrogen acquisition (Figs. 3, 7).

The observed root growth traits likely influenced nitrogen uptake (Fig. 7a), affecting the NO_3^-, NH_4^+, and N-amino as observed at 31 DAP (Figs. 8a, 8b, 8c), reducing the soluble sugars (Fig. 8d) as reported by earlier studies (Dias et al. 2021; Pereira-Defiliipi et al. 2017). In this study, Hs-ZAE94 differs from Ab-Sp245 (Fig. 8), showing that it is not only root development that can be attributed to the growth promotion effect.

Dias et al. (2021) compared these two strains using a hydroponic system with high and low nitrogen doses (3- and 0.3-mM N) showing that Ab-Sp245 improved root morphology and nitrogen use efficiency at 3 mM·N. In this study, nitrogen was not limited, and again Ab-Sp245 improved shoot dry weight (Fig. 3b), and total nitrogen accumulated at the final harvest (Fig. 7a), and this positive response was associated with higher NRa three days before measured in the shoots (35 DAP - Fig. 7b).

Herbaspirillum can be included in the same bacterial group, but this is a genus less-known bacterium (Alves et al., 2020) compared to the *Azospirillum* genus. *Azospirillum brasilense* is a species normally used as an inoculant by several countries, including Brazil, Argentina, Uruguay, South Africa, and others since the last century (Cassán and Diaz-Zorita, 2016). But *Herbaspirillum* has not been widely used as an inoculant. Under field conditions on an Oxisol in the Cerrado region, Martins et al. (2017) observed that Ab-Sp245 improved urea recovered by 72 kg $N \cdot ha^{-1}$ in the grains compared to 58 kg $N \cdot ha^{-1}$ by Hs-ZAE94. In an experiment with maize during the rainy season and high-water availability. Breda et al. (2020) the same two diazotrophic strains used in this study and they observed a difference in growth response between these two inoculation treatments. A high shoot dry weight was observed 35 days after emergence (DAE) for both inoculation treatments, but at 70 DAE, the strain Ab-Sp245 outperformed Hs-ZAE94 in terms of shoot dry weight without nitrogen addition. In this study plants inoculated with Hs-ZAE94 and treated with urea had higher grain yield and nitrogen accumulation compared to plants inoculated with Ab-Sp245 and the uninoculated control. Furthermore, they observed that nitrogen utilization was more efficient in the Hs-ZAE94 inoculated plants, as it was better converted into grain production (Breda et al. 2020).

Both bacteria strains improved maize growth and can be considered as a good choice for inoculants being *A. baldaniorum*, a former *A. brasilense* species, a well-known bacterium used since the last century under field conditions (Okon and Itzigsohn

1995) and applied to several crops including cereals (Hungria et al. 2010) or associated with rhizobia as a co-inoculant (Garcia et al. 2021) based on the visible root improvement. *H. seropedicae* is also a plant growth-promoting bacterium species and studies of its field application are restricted to cereals, sugarcane, and most of the cases under Brazilian conditions (Alves et al. 2020). Both technologies can improve nitrogen use efficiency and nutrient acquisition including water and maybe the association of both strains can be a useful choice for a future inoculant recommendation, but field experiments must be performed to prove this efficiency.

CONCLUSION

This study showed that both bacteria inoculants can improve root architecture during the initial maize growth period. *Azospirillum baldaniorum* Sp245 and *H. seropedicae* ZAE94 can produce several molecules that can act as plant growth regulators, and 24 days after planting the root morphology was modulated by both strains in a similar way, with similar root length and surface, projected area, root volume and the number of crossings. It is interesting to note that shoot dry weight differs between strains by the final harvest, being higher in plants inoculated with Ab-Sp245, compared to Hs-ZAE94 and the control. This efficiency of root development improved the nitrogen accumulated by the plants and the NRa. Hs-ZAE94 differs drastically from Ab-Sp245 in the allocation of the soluble fractions, resulting in plants with higher NO_3^- NH_4^+, and N-amino and lower soluble sugars, indicating that this endophyte modified the internal distribution of the nitrogen absorbed by the roots, even though the root morphology did not differ from the Ab-Sp245. This study shows that root morphology alone cannot explain the inoculation response of the maize plants, but also that the nitrogen use differs, being Ab-Sp245 associated with root traits and Hs-ZAE94 to the nitrogen balance and in consequence, differences in shoot dry weight could be detected after the initial 38 days of growth. Besides a few field studies were done comparing both strains, additional field trials would be required to determine whether the observed benefits of inoculation on seedling growth and nitrogen nutrition, will have a positive impact during the early crop growth stages, and/or whether the inoculation may have longer-lasting effects to improve crop growth and nutrition during the growing season, and final yields.

AUTHORS' CONTRIBUTION

Conception and design: Carvalho, A.D.; Alves, C.G.; Alves, B.J.R.; Santos, L.A.; Reis, V.M.; **Data curation:** Reis, V.M.; **Formal analysis:** Dias, A.C.; **Methodology:** Reis, V.M.; Santos, L.A.; **Project administration:** Reis, V.M.; **Resources:** Reis, V.M.; **Supervision:** Reis, V.M .;. Santos, L.A.; Alves, G.C.; **Writing original draft:** Dias, A. C.; Reis, V.M.; Santos, L.A.; Alves, B.J.

DATA AVAILABILITY STATEMENT

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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