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Inoculation effects of growth-promoting bacteria on corn root architecture: influence of nitrogen levels, bacterial populations, and plant genotypes

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ABSTRACT: Inoculating corn with diazotrophic bacteria as growth promoters has been demonstrated to be an efficient agricultural practice in Brazil, mainly due to the root stimulation they provide to plants. This study investigates the corn (*Zea mays* L.) root architecture in a greenhouse assay where *A. baldaniorum* Sp245 and *H. seropedicae* ZAE94 strains were inoculated and evaluated for 22 days under two N levels: 0.6 and 6 mmol L^{-1} of N. Short-term bioassays were conducted to assess the plant's response to the addition of indole-acetic acid, two bacterial populations, and two corn genotypes, utilizing image capture software *WinRhizo* Pro. The growth and distribution of tips, crossing, and length of fine roots were determined to be the most sensitive aspects to inoculation and indole-acetic acid induction. These responses were influenced by the genotype and the number of bacterial cells present. Biomass accumulation analyses quantified these modifications after a 22-day period. Additionally, the growth response was found to be more significant when applying the Hs-ZAE94 strain to plants fertilized with a higher dose of nitrogen (6.0 mmol L^{-1}), and this response was positively correlated with bioassay data. Selected strains used as an inoculant can improve root architecture and, consequently, the N use efficiency.

Keywords: plant-bacteria interaction, diazotrophs, phytohormones.

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INTRODUCTION

Corn (*Zea mays* L.) forms a complex root system during growth and the structure and functionality also change to supply the plant with water and nutrients (Hochholdinger et al., 2018). Growth initiates with the primary root (PR) elongation and the recurrent branching along the main axis from lateral roots (LR). The root architecture (RA) is based on the LR formation and subsequent formation of seminar roots (SR), which is the last one, the second embryonic root type formed after the primary root (Hochholdinger and Tuberosa, 2009). Corn forms a variable number of SR that emerge from the scutellar node about a week after germination (Feldman, 1994). The root branching occurs through the formation of the LR, allowing them to expand laterally into the soil, and these lateral growth divides into several new ones, providing developmental plasticity (Motte and Beeckman, 2019), changing soil conditions and nutrient acquisition (Motte et al., 2019). In cereals, communication at the root–soil interface is facilitated by a structure called rhizosheath, which contains tightly bound soil particles associated with root-hair-bearing roots and rhizobacteria (Mccully, 1995).

The root shape depends on PR elongation and the number and length of the LRs during growth. Regulation of RA involves the action of plant hormones and auxins and cytokinins are the central molecules involved in this process (Dubrovsky and Forbe, 2012; Orman-Ligeza et al., 2013); being the auxins responsible for PR elongation and LR formation (Alarcón et al., 2019). Although several genes and pathways are involved in this hormone production in plants (Barbez et al., 2017), bacteria associated with roots can also play an important role in RA development (Cassán et al., 2014), and the magnitude of both sources of auxin is needed at low levels for positive stimuli.

Azospirillum genus was described in 1980 and started with two species, *Azospirillum lipoferum* and *A. brasilense* (Tarrand et al., 1978). Since its description, several publications using these two species described as plant-growth-promoting bacteria turned this genus to be the most studied and used species in agriculture over the last decades, after rhizobia. Several mechanisms that explain this growth promotion were described and associated with the application of different strains and plants (Cassán and Diaz-Zorita, 2016). The principal morphological modification of plants inoculated with selected strains is associated with producing a pool of phytohormones, especially auxins; this observation was first reported in 1979 (Reynders and Vlassak, 1979).

Azospirillum is not an exception, associative bacteria that produce auxins are normally present in the rhizosphere (Spaepen et al., 2007), modifying the RA and stimulating water and nutrients acquisition (Cassán et al., 2014). *Herbaspirillum* genus was described six years later in association with different cereals and sugarcane (Baldani et al., 1986). *Herbaspirillum* spp. differ from the *Azospirillum* genus in several aspects: as a β proteobacterium, it is considered an aggressive colonizer of the root interior, establishing itself not only in the cortex and vascular tissues of roots but also systemically in the whole plant, being considered a plant endophyte by the inoculation experiments (Monteiro et al., 2012). Although colonization patterns can differ between these two genera/species and strains tested, both bacteria produce auxins and can modulate root development (Bastián et al., 1998).

Using scanner methodologies, it is possible to quantify these parameters during growth and evaluate the adaptations upon biotic and abiotic conditions applied to the corn plants. The *WinRHIZO* software is one of the available methods described by Bauhus and Messier (1999). Using this software, modification of RA can easily quantify the inoculation response using selected strains. Although root promotion is well described for *Azospirillum* (Cassán et al., 2014), it is less studied for other genera, such as *Herbaspirillum*, especially the RA modifications upon stressed conditions (Dias et al., 2021).

Nitrogen plays an essential role in root development (Liu and Von Wirén, 2017). The association of rhizosphere activity (RA) data during initial corn growth in controlled conditions can tremendously aid in plant growth promotion and serve as a robust tool for comparing new bacterial inoculants for cereals. In this study, we aimed to compare two diazotrophic bacterial genera, the *A. baldaniorum* species referred to as strain Ab-Sp245, a model strain formerly known as *A. brasilense* (Ferreira et al., 2020), and *H. seropedicae* species strain Hs-ZAE94, specifically selected for corn inoculation (Alves et al., 2021). We aimed to observe, over a period of 22 days after planting (DAP), how these two genera interacted with two distinct nitrogen levels, namely, 0.6- and 6.0 mmol L^{-1} of N. To determine the influence of cultivar or bacterial size on growth promotion, a bioassay was set up to compare the root architecture (RA) of two corn cultivars in response to the addition of exogenous auxin, i.e., indole-3-acetic acid (IAA), and their reaction to two bacterial concentrations (10^{-6} and 10^{-8} cells mL^{-1}) as well.

MATERIALS AND METHODS

Greenhouse experiment

The experiment was developed in a 2 × 3 factorial, with four repetitions, the first factor was composed of two nitrogen doses, 0.6 mmol L^{-1} (LN) and 6.0 mmol L^{-1} (HN) by the modified Hoagland and Arnold (1950) solution applied twice during the growth period. The second factor was the inoculation being: non-inoculated (NI = Control), inoculated *A. baldaniorum* (strain Sp245 = Ab-Sp245) and with *H. seropedicae* (strain ZAE94 = Hs-ZAE94). The experiment was conducted in an automatic greenhouse with humidity and temperature control for 22 days after planting (DAP) (Figure 1).

Inoculation and bacterial counting

The bacteria used were *A. baldaniorum* strain Sp245 (= BR11005, isolated from wheat roots planted in Rio Grande do Sul State, Brazil) and *H. seropedicae*, strain ZAE94 (= BR11417, isolated from surface-sterilized rice roots, Seropédica-RJ, Brazil). Both strains were acquired from the Centre of Biological Resources Johanna Döbereiner - CRB-JD (BR). Strain Ab-Sp245 can be considered a model bacterium for the studies involving auxin response and was the first diazotrophic bacterium with the genome sequenced by Zhulin and Wisniewsky-Dye (Wisniewski-Dyé et al., 2011). The strain Hs-ZAE94 was selected for corn based on the previous studies by Alves et al. (2015, 2020).

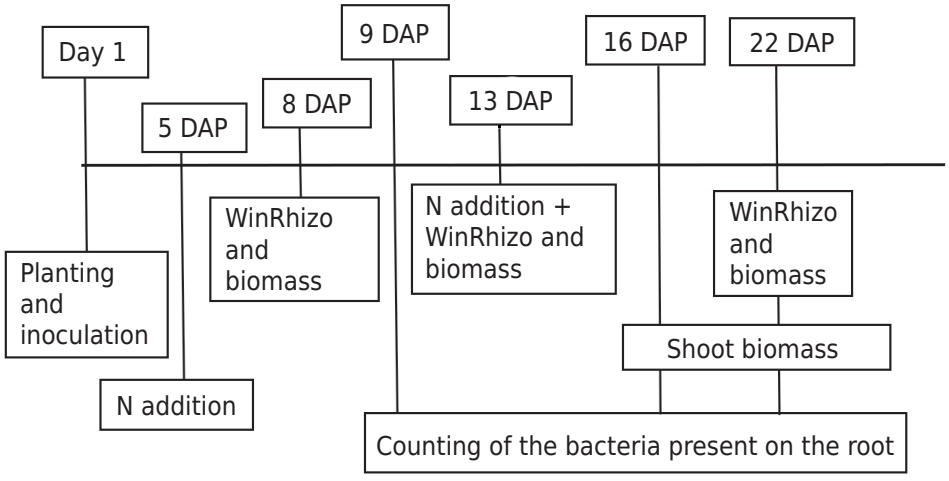


Figure 1. Timescale of the pot experiment. DAP: days after planting.

The procedure used in this evaluation is all described by Baldani et al. (2014). Initially, a single colony was obtained from the minimal medium NFb 3x the amount of bromothymol blue and inoculated in 5 mL of modified DYGS medium with malic acid pH 6.5. Bacteria were maintained at 30 °C in a rotary shaker at 175 rpm for 20 h. After that, 200 µL of cell suspension was inoculated in 150 mL of the NFB medium without an indicator. After cell growth, bacteria were counted using a Neubauer counting plate to equalize both populations to 10^8 cells mL^{-1} using saline solution to dilute the cells if necessary. The inoculant solutions were applied directly on the seeds at the time of planting at a dosage of 1 mL per seed, in the control treatment, phosphate buffer (pH 7.0).

At nine, 16, and 22 DAP, 1 g of roots was sampled for bacterial counting using the N-free semi-solid media NFb and JNFb for the strains Ab-Sp245 and Hs-ZAE94, respectively. Bacterial counting was performed using the most probable number technique (MPN) with the application of McCrady's Table using three replicates, as described by Baldani et al. (2014).

Pot experiment procedures and analysis

Seeds were superficially disinfested using NaOCl (0.5 %) plus Tween 20 (0.01 %) during 5 min agitating using a rotatory shaker at 165 rpm and washed three times using phosphate buffer 50 mmol L^{-1} pH 7.0 for 5 min each time at the same condition. Pots with 1, 2, and 3 kg capacity were used containing a sterile substrate, sand + vermiculite (2:1 v/v) autoclaved at 121 °C for 20 min and repeated this operation two 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^{2+} = 0.39; Mg^{2+} = 2.81; Al^{3+} = 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 10 mL each per pot of modified Hoagland solution with a pH of 5.8 (Hoagland and Arnold, 1950) using two N concentrations using ½ of the ionic force (Table 1).

Root architecture

Sampled roots immersed in 50 % ethanol were 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 Bauhus and Messier (1999). Roots were laid out in an acrylic container (0.30 × 0.40 m), with water at an approximate depth of 1 cm, and placed onto the scanner. Root length (RL - cm), projected (PA) and surface area (RS - cm^2), root volume (RV - cm^3), and the number of tips, forks, and crossings were recorded. Root length was classified as follows: very thin root length (L≤0.5 mm). After analysis, the roots were dried at 60 °C to obtain a constant root dry weight (RDW).

Bioassays

A bioassay was developed to evaluate the application of exotic IAA using four concentrations (10, 1, 0.1, 0.01, 0.001 nmol L^{-1}) with two corn hybrids, cultivar (cv) SHS5050 and Dekalb 7815. The second bioassay was made with two bacterial population densities, 10^6 and 10^8 cells mL^{-1}, of the strains Ab-Sp245 and Hs-ZAE94 using only SHS5050. Both strains were grown and multiplied previously. All bioassays were done with superficially disinfected seeds as described for the pot experiment.

The bioassays were pre-germinated on a sterile double layer using Germitest paper (28 × 38 cm) moistened with 40 mL of autoclaved distilled water for two days in BOD at 30 °C in the dark involved in plastic film. Afterward, the seedlings were selected according to the radicle length (± 1.5 cm) and were immersed for 1 h in the respective IAA concentrations or bacterial suspensions. Then, once again, they were placed to germinate

Table 1. Nutritive solution used in the growth of corn plant (Hoagland and Arnold, 1950)

Salt, acid or base	Nutrient	6 mmol	0.6 mmol
		mg L^{-1}	
Magnesium nitrate Calcium nitrate	$N\text{-}NO_3^-$	70.00	7.00
Ammonium sulfate	$N\text{-}NH_4^+$	14.00	1.40
Potassium phosphate dibasic	P	15.50	15.50
Potassium sulfate Potassium phosphate dibasic	K	117.00	117.00
Magnesium sulfate Ammonium sulfate Potassium sulfate	$S\text{-}SO_4^-$	63.00	112.60
Calcium sulfate		-	
Calcium nitrate	Ca	80.00	80.00
Calcium sulfate		-	
Magnesium nitrate Magnesium sulfate	Mg	24.00	24.00
Iron EDDHA	Fe	3.00	3.00
Boric acid	B	0.50	0.50
Copper sulfate	Cu	0.02	0.02
Manganese sulfate	Mn	0.60	0.60
Zinc sulfate	Zn	0.05	0.05
Anhydrous Sodium Molybdate	Mo	0.008	0.008

under the same conditions described previously for 6 days, with the photoperiod cycle adjusted to 12 h of light. The trial was conducted in a completely randomized design with 12 replications. In the end, plant roots were scanned for morphology assessment using the software *WinRHIZO* Pro™. In the bioassay, the images recorded and evaluated with differences observed by the statistic evaluation were the number of tips, forks, and crossings, and the length classified as very thin ($0.0 < L < 0.5$ mm). The other parameters did not differ during this initial growth period.

Statistical analysis

Pot experiment was analyzed in a factorial design and the bioassays were evaluated by genotype and strain concentration individually. The statistical analysis was performed using the statistical programs SAEG 9.0 and SISVAR 5.1. Analysis of variance was performed for the assumptions of normality (Lilliefors test) and homogeneity of error variances (Cochran's test 1941), the means of the variables were submitted to analysis of variance, using the Scott- Knott test with $p<0.05$ for comparison between means. A regression analysis was performed for the bioassays using increased concentrations of the IAA.

RESULTS

Pot experiment

Bacterial counting evaluated during the experiment showed that both strains were established on corn roots with population size higher than 10^6 cell g fresh mass after 9, 16, and 22 DAP and differing from the uninoculated control (Figure 2). Nitrogen levels also modulate the bacterial numbers being higher in roots inoculated with Hs-ZAE94 after 16 days at a high N level (HN-6.0 mmol L^{-1} N). Ab-Sp245 maintained a lower population compared to Hs-ZAE94 22 DAP at HN, reducing it at LN (0.6 mmol L^{-1}).

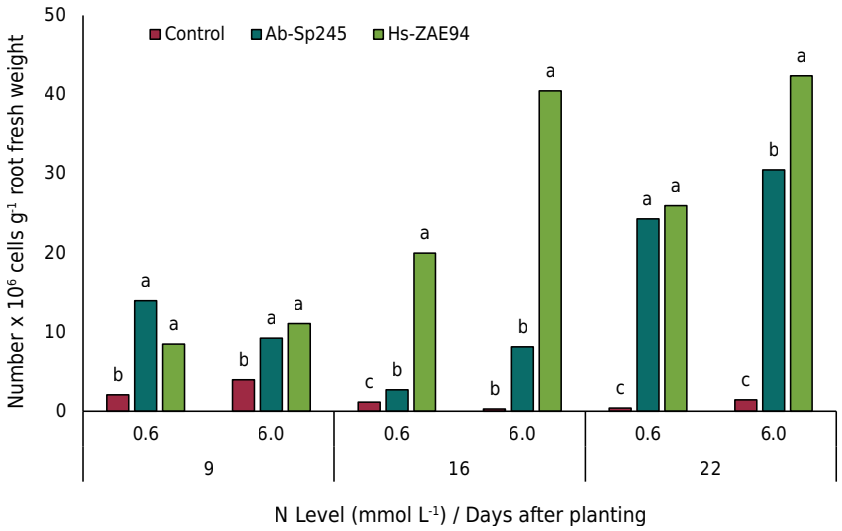


Figure 2. Bacterial counting using the Most Probable Number technique present in the root of corn inoculated with Ab-Sp 245 and Hs- ZAE 94 (n = 3) Lowercase letters differ by Scott-Knott at $p<0.05$.

After 22 days of growth, plants accumulate the mean value of 734 mg of shoot dry mass (SDM), although inoculation using Hs-ZAE94 improved the SDM by 17 % over the control, at LN, this value was not significant (Figure 3).

Applying ten times more N (6.0 mmol L^{-1} - HN), plants accumulated 1396 mg as a mean value, almost two times more SDM after this period (Figure 3a). Again, at HN an improvement was observed upon the inoculated plants with HS-ZAE94, resulting in 18 % more SDM than the control. Ab-Sp245 showed a reduced growth promotion compared to Hs-ZAE94. This data shows that growth promotion between the two strains occurred in HS-ZAE94 more efficiently in the N use than Ab-Sp245 during this initial growth phase (Figure 3), related to the high bacterial numbers of Hs-ZAE94, compared to Ab-Sp245 (Figure 2). Differences in SDM delay 22 days to appear when plants start to use the N sources of the substrate (Figure 3).

Root dry mass (RDM) of the corn plants was not modified by the high N level (6.0 mmol L^{-1}) observed after 22 DAP (Figure 3b). In this case, Ab-Sp245 reduced the RDM accumulation and HS-ZAE94 produced a similar mass compared to the uninoculated control (Figure 3b). It seems that corn inoculated with Hs-ZAE94 used the nutrients to improve SDM with a similar root mass. However, the architecture of the root can explain the differences observed and not sensed just using biomass data.

Root analysis revealed that inoculation altered the length of fine roots in both two strains tested (Figure 4). However, the extent of these modifications was found to be affected by the N level. Especially for Ab-Sp245, modifications were observed at a low N level (0.6 mmol L^{-1}), while for both strains, the fine roots were improved at the highest N level of 6.0 mmol L^{-1}.

Bioassays

Two bioassays were performed using two corn cultivars grown in the presence of external IAA solution diluted from 0.1 to 0.001 nmol L^{-1}. As expected, each cv. exhibited a similar response but in a different magnitude (Figure 5). A concentration of 1 nmol L^{-1} improved the number of tips (Figures 4a and 4b), forks (Figures 5c and 5d), and crossings (Figures 5d and 5e) in both cultivars tested, but in the Dekalb 7815, the dose-response is narrower than the one observed for SHS5050 (Figure 5). Also, the total length of fine roots (L ≤0.5 mm) showed the same pattern, differing the growth response in the root class

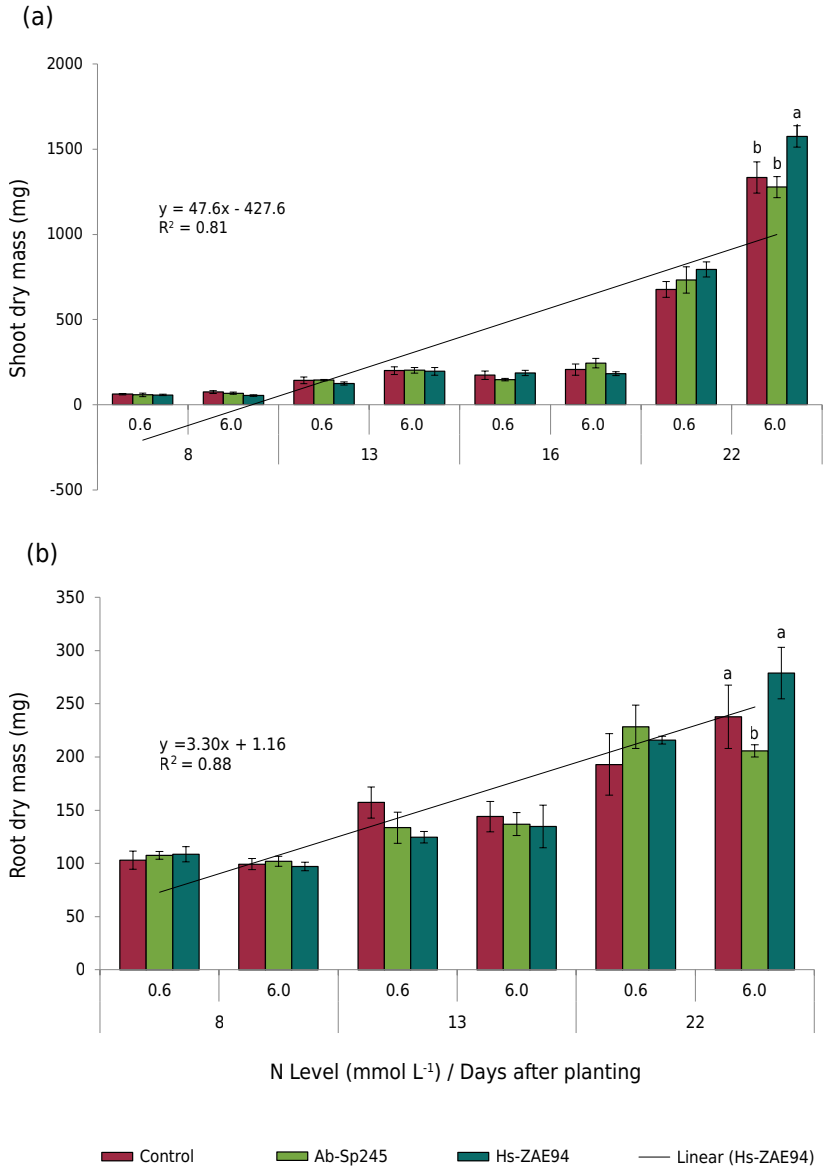


Figure 3. Corn dry mass accumulation of shoot (a) and root (b) grown under two N levels (0.6 and 6.0 mmol L^{-1}) for 22 days inoculated or not with Ab-Sp245 and Hs-ZAE94. Letters differ at $p<0.05$ by the Scott-Knott test (n = 4). Bars represent the standard error.

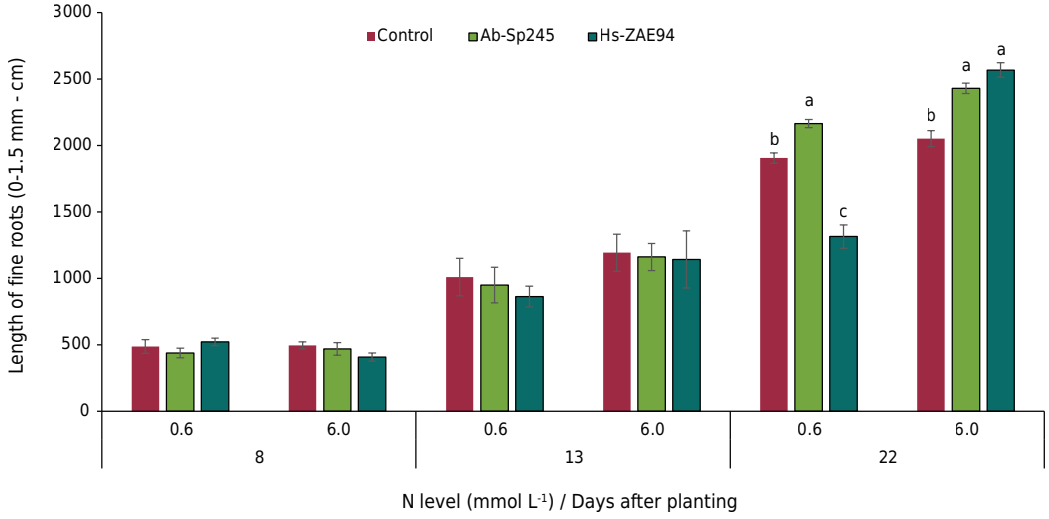


Figure 4. Estimation of the length of fine roots (root class <1.5 mm) of corn inoculated or not with Ab-Sp245 and Hs-ZAE94. Letters differ at $p<0.05$ by the Scott-Knott test (n = 4). Bars represent the standard error.

most important for nutrient and water acquisition (Figure 6). Cv. SHS5050, used in this study, presented a root growth response for the external IAA addition two times higher than observed for cv. Dekalb 7815 (Figure 6). In addition, cv. SHS5050 exhibited a wider range of positive responses than the cv. Dekalb, maintaining a higher response between 1 to 0.1 nmol L^{-1} IAA concentration for the number of tips, forks, and crossings (Figures 5a, 5c ad 5e) and improving the length of fine roots compared to cv. Dekalb (Figure 6).

The second comparison was done with bacterial population size (Figure 7). The two bacterial strains improved the root parameters evaluated after six days of growth in a different magnitude (Figure 8). Strain Ab-Sp245 improved root traits with a lower percentage of increment compared to Hs-ZAE94, in both cell numbers (10^6, and 10^8 cells mL^{-1}), and Ab-Sp245 reduced the increments response in the presence of higher cell numbers (Figure 7a). The opposite occurred in the plants inoculated with Hs-ZAE94, where increments were higher and observed in all traits evaluated, especially at the population of 10^8 cells mL^{-1} (Figure 7b), the same population size used in the pot assay. These differences can explain, in part, the growth response observed by the corn plants after 22 DAP (Figure 3), where population counts of both strains differ along harvest time (Figure 2).

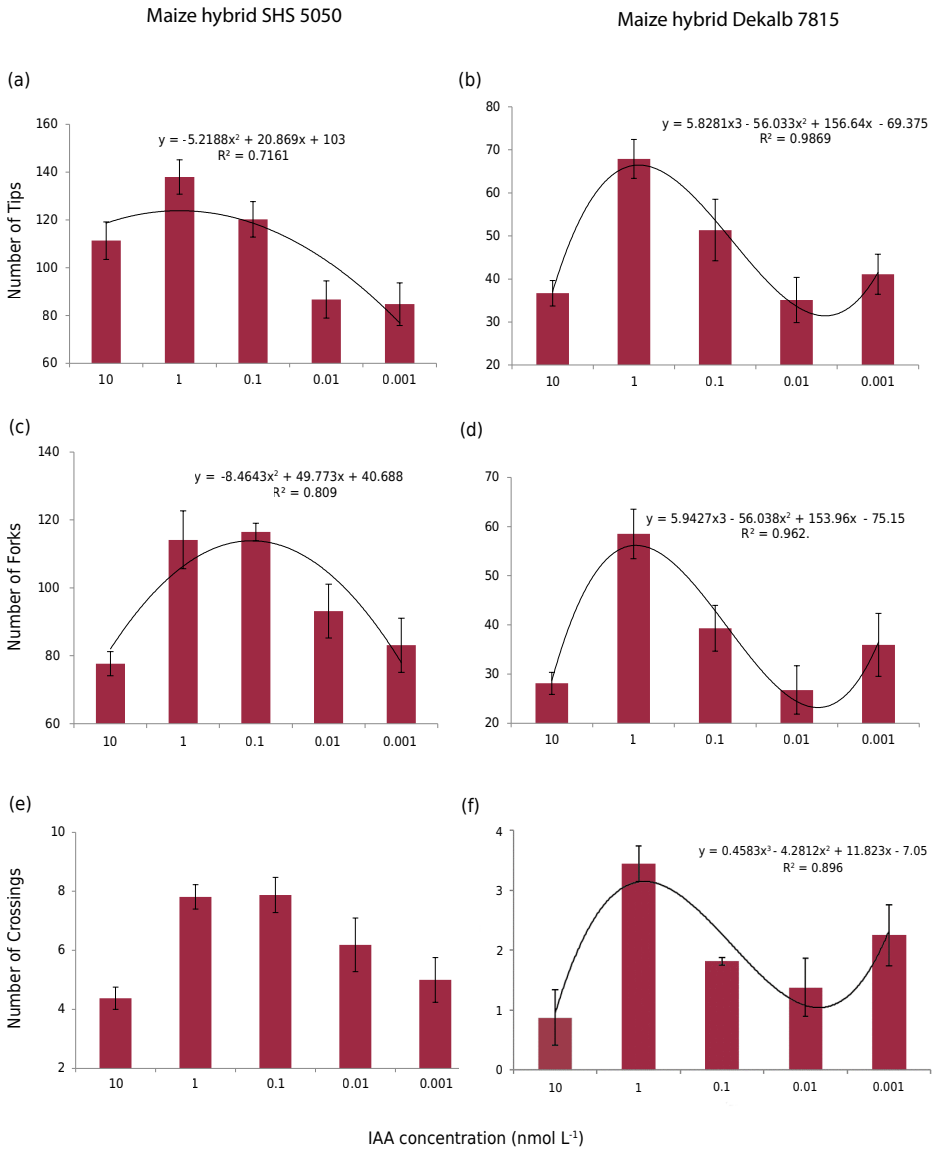


Figure 5. Root traits of two hybrid maize cultivars SHS 5050 and Dekalb 7815 grew in the presence of increased concentrations of IAA (10, 1, 0.1, 0.01, and 0.01 nmol L^{-1}) during six days (n = 12). Bars represent the standard error.

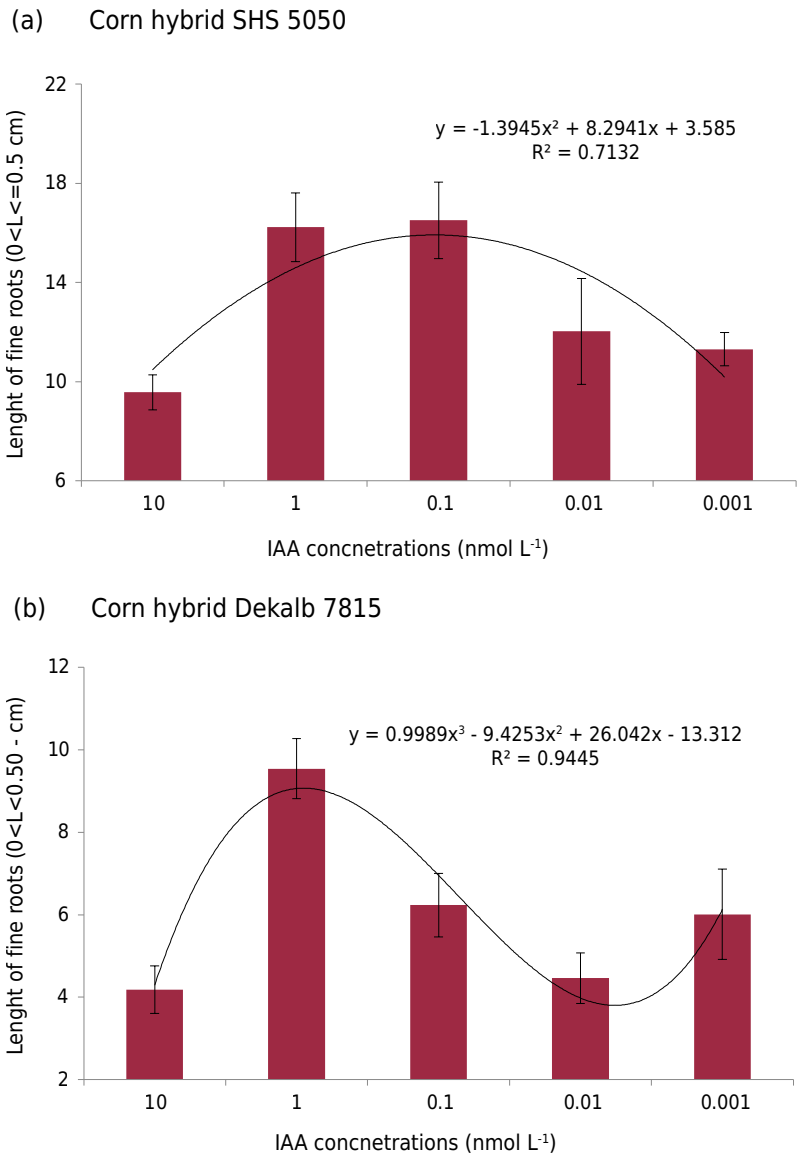


Figure 6. Evaluation of length of the class of fine roots (0< L ≤ 0.5 cm) of two corn cultivars SHS 5050 (a) and Dekalb 7815 (b) grown in the presence of increased concentrations of IAA (10, 1, 0.1, 0.01, and 0.01 nmol L^{-1}) during six days (n = 12).

DISCUSSION

Although both strains produce IAA in a culture medium, they differ in quantities produced, consequently leading to modifications of the root phenotype (Figures 5 and 6). Additionally, plant colonization (Figure 2) can affect growth performance (Figure 3). A previous study described that *A. baldaniorum* Ab-Sp245 produces 5 to 6 µg mL^{-1} IAA in a medium supplied with tryptophan (Ona et al., 2005). Under different stressed conditions, such as saline or oxidative stress, this strain produces varying amounts of IAA ranging from 0.4 to 6.2 µg mL^{-1} in a culture medium (Molina et al., 2018). On the other hand, *H. seropedicae* produces 7 ng mL^{-1} in a culture medium, especially in the free hormone fraction, as determined by GC-SIM analysis (Bastián et al., 1998). This level is 100 less compared to *A. baldaniorum*, as measured using colorimetric methods. It was found that the bacterium tested Hs-ZAE94 can reach a concentration of 11.97 mg L^{-1} when growing optimized for higher IAA (Scheidt et al., 2020). This indicates that depending on the growth conditions and medium tested, both bacteria can act as growth promoters and modify root architecture, as shown in figures 7 and 8.

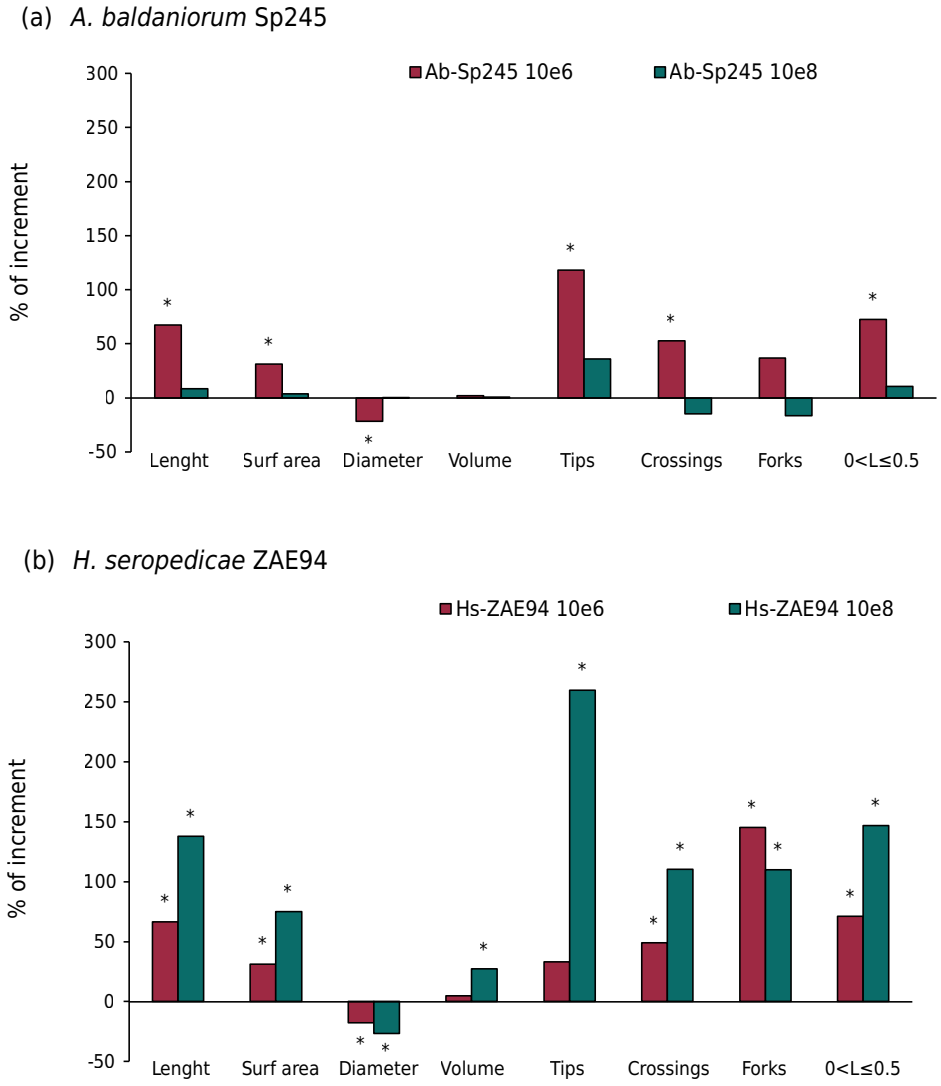


Figure 7. Measurement of corn root morphology increments using two population sizes (10^6 and 10^8 cells mL^{-1}) of Ab-Sp245 (a) and Hs-ZAE94 (b) measured six days after inoculation (n = 12). * Differ at p≤0.05 comparing inoculated plants over the control.

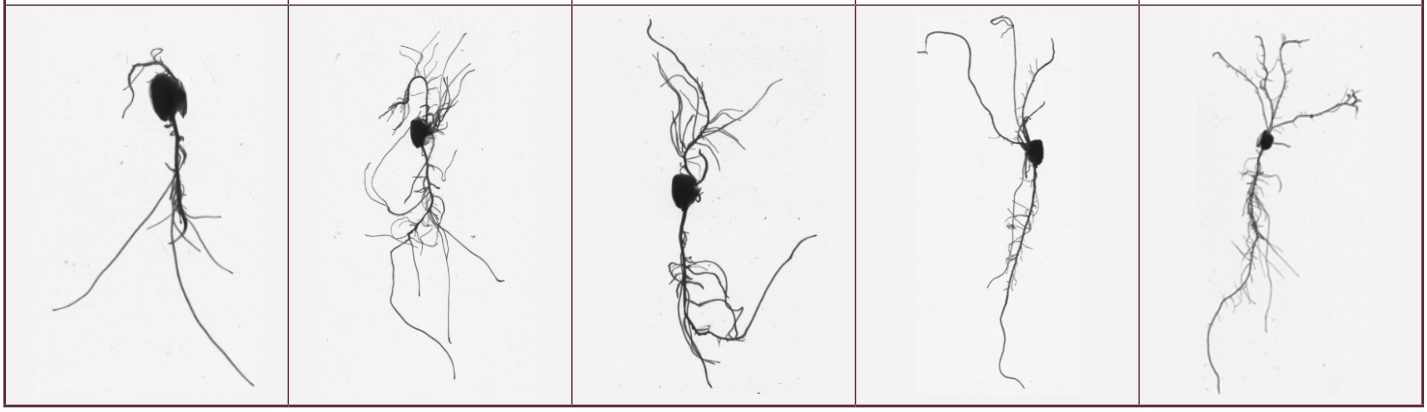
CONTROL	Ab-Sp245		Hs-ZAE94	
uninoculated	10^6 cells mL^{-1}	10^8 cells mL^{-1}	10^6 cells mL^{-1}	10^8 cells mL^{-1}
				

Figure 8. Corn bioassay cultivar SHS5050 inoculated with two bacterial concentrations and evaluated six days after inoculation. Root morphology was measured using the software *WinRHIZO* Pro™.

Genus *Azospirillum* has been the focus of research since 1999 regarding its ability to promote growth primarily through the production of indole-3-acetic acid (IAA) (Dobbelaere et al., 1999). The IAA is the most abundant naturally occurring auxin and is involved in plant development coordination (Abel and Theologis, 2010). The Sp245 strain, now known as the *A. baldaniorum* species, is the most extensively studied bacterium in terms of its auxin response to inoculated plants, particularly corn and other grasses (Steenhoudt and Vanderleyden, 2000), with at least three pathways described (Puyvelde et al., 2011), of which two are tryptophan dependent. Fine-tuning IAA levels is critical to promoting root development and other key plant developmental processes (Di et al., 2016). The IAA can be considered a communication signal to initiate plant interaction, and bacterial size can significantly impact growth promotion (Puyvelde et al., 2011), as observed in figures 2 and 3.

Another critical point to consider is the interaction between the inoculated bacterium and the natural population already present in the seed. This can be done through microbiome analysis for example, which allows us to understand how the natural population interacts with the inoculated strain. The research conducted by Carril et al. (2021) demonstrated that when *H. seropedicae* was used as an inoculant for wheat plants, it could alter the metabolic landscape within the endosphere. This, in turn, influenced the functionality of the endophytic community. These findings are observed in this study. Furthermore, the interaction between the bacterial species and corn cultivar tested was found to be different, as observed in figures 5 and 6. This highlights the importance of coordinating the modulation of indole-3-acetic acid (IAA) turnover and degradation by the bacteria with the specific plant cultivar to optimize the potential benefits. It is well described that the ideal internal balance of one plant cultivar will interact with the added inoculant and the bacterial degradation of IAA. Not only is its production, but it is also often required for full plant growth promotion and root development (Leveau and Lindow, 2005).

Root architecture modifies during plant development, and root plasticity is the key to acclimating plants in unfavorable environments, including N stress. How corn coordinated the RA associated with N level and inoculation treatments is not easy to follow and remains unclear (Kong et al., 2014) and involves the plant genotype (Dechorgnat et al., 2018). This study shows that bacteria used as an inoculant can also act in the presence of two N levels, high and low, and root biomass was not modified by the bacteria applied for 22 days and only at high N level, inoculation differs the root biomass (Figure 3b). However, RA shifted depending on the N level and bacteria tested (Figure 4), and this fine-tuning modified the shoot biomass accumulation (Figure 3a).

It is well known that RA is also dependent on the availability of macro and micronutrients, however, the two most important ones are N and P. The availability of these two elements modifies the growth response of SR and LR. Under high-N conditions, the LR is strongly inhibited, whereas, at low N, LR elongation is enhanced (Bellini et al., 2014; Kiba and Krapp, 2016; Liu and Von Wirén, 2017). Similarly, low LR branching density improves corn growth with low nitrogen (Zhan and Lynch, 2015) and water (Zhan et al., 2015), but high LR branching density improves phosphorus acquisition (Jia et al., 2018). The role of root architecture in plant development and acclimation to unfavorable environments, including N stress, is well established.

This study demonstrates that inoculated bacteria can act under various N levels, alter root architecture, and modify shoot biomass accumulation. Nitrogen serves as a central element in amino acids and proteins, and the bacterial strain, plant cultivar, and N source influence its uptake and assimilation. The tripartite interaction of these factors needs to be more clearly understood to optimize yields and N use efficiency. Finally, it is important to recognize that environmental factors play a significant role in managing plant growth and development, and these must be considered in conjunction with the interaction of bacterial strains, plant cultivars, and N sources.

CONCLUSION

Architecture of corn roots is altered by bacterial species/strains, with the extent of growth response being determined by the corn genotype. In particular, the association of *H. seropedicae* ZAE94, in combination with high levels of nitrogen, results in increased accumulation of both root and shoot biomass in maize plants after 22 days of growth. Population density of bacterial strain used as a seed inoculation also plays a role in the magnitude of root response, with Hs-ZAE94 eliciting a higher response compared to *A. baldaniorum* Sp245 at a density of 10^8 cells mL^{-1}. These modifications could also be observed using a six-day-old plant bioassay, but biomass analysis in a pot experiment is required for accurate measurement, which takes 22 days. Utilizing different strains for inoculation has the potential to alter the nitrogen use efficiency of corn plants, offering a promising approach to enhance plant growth and mitigate environmental N losses.

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Formal analysis: Thamires Ferreira Rodrigues da Silva (lead).

Funding acquisition: Veronica Massena Reis (lead).

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