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Inoculation methods of *Azospirillum brasilense* in lettuce and arugula in the hydroponic system[1]

Métodos de inoculação de *Azospirillum brasilense* em alface e rúcula em sistema hidropônico

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HIGHLIGHTS:
Inoculation of plant growth-promoting bacteria reduces NO_3^- accumulation in shoots.
Inoculation with A. brasilense *increased plant growth and nutrient accumulation in hydroponic arugula and lettuce.*
Foliar application and nutrient solution inoculations increase the quality of arugula and lettuce leaves.

ABSTRACT: Lettuce and arugula are the most cultivated leafy vegetables in hydroponic systems in the world, and the little information about *Azospirillum brasilense* effects in research in this system makes it necessary to verify the best method of inoculation of these bacteria. Therefore, this study aimed to verify the beneficial effects of inoculation methods of *A. brasilense* on plant growth and nutrition of lettuce and arugula grown in the hydroponic system. The study was conducted in a greenhouse with 30% shading. A randomized block design with five replicates in a 4 × 2 factorial scheme was used. Four *A. brasilense* inoculation methods (non-inoculated, foliar application, nutrient solution, and nutrient solution + foliar application) and two leafy vegetable species - arugula and lettuce - were evaluated. All inoculation methods improved plant height, shoot fresh and dry mass, root fresh and dry mass, fresh leaf yield, leaf chlorophyll index, accumulation of nitrogen, potassium, calcium, sulfur, phosphorus, iron, manganese, and copper and reduced nitrate accumulation in shoot and root of arugula and lettuce. Inoculation via nutrient solution + foliar application of *A. brasilense* is the most suitable for hydroponic arugula cultivation because it provides greater fresh leaf yield. All inoculation methods increased leaf yield in hydroponic lettuce. Hydroponic arugula and lettuce plants showed greater accumulation of nutrients and plant growth with less nitrate accumulation in the shoot under inoculation with *A. brasilense*.

Key words: *Lactuca sativa*, *Eruca sativa*, growth-promoting bacteria, reduction of nitrate accumulation

RESUMO: A alface e a rúcula são as hortaliças folhosas mais cultivadas em sistemas hidropônicos no mundo, e as poucas informações sobre os efeitos do *Azospirillum brasilense* em pesquisas neste sistema torna-se necessário verificar o melhor método de inoculação dessas bactérias. Portanto, o objetivo deste estudo foi verificar os efeitos benéficos de métodos de inoculação de *A. brasilense* em alface e rúcula no crescimento e nutrição das plantas em sistema hidropônico. O estudo foi conduzido em casa de vegetação com 30% de sombreamento. Foi usado o delineamento em blocos ao acaso com 5 repetições em esquema fatorial 4 × 2, com quatro métodos de inoculação de *A. brasilense* (não inoculado, foliar, solução nutritiva e solução nutritiva + inoculação foliar) e o segundo fator foi a aplicação em rúcula e alface. Todos os métodos de inoculação melhoraram a altura da planta, massa fresca e seca da parte aérea, massa fresca e seca da raiz, rendimento da planta, índice de clorofila foliar, acúmulo de nitrogênio, potássio, cálcio, enxofre, fósforo, ferro, manganês, cobre e redução do acúmulo de nitrato na parte aérea e raízes de rúcula e alface. A inoculação via solução nutriente + inoculação foliar de *A. brasilense* é a mais indicada para o cultivo hidropônico de rúcula, pois proporciona maior rendimento de folhas, e todos os métodos de inoculação foram capazes de aumentar o rendimento de folhas em alface hidropônica. Plantas hidropônicas de rúcula e alface apresentaram maior acúmulo de nutrientes e crescimento das plantas com menor acúmulo de nitrato na parte aérea sob inoculação com *A. brasilense*.

Palavras-chave: *Lactuca sativa*, *Eruca sativa*, bactérias promotoras de crescimento, redução do acúmulo de nitrato

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Introduction

Lettuce (*Lactuca sativa*) is the most consumed leafy vegetable in the world, for having desirable culinary characteristics for fresh consumption in the form of salads (Dalastra et al., 2020a). The arugula (*Eruca sativa* L.) is one of the most produced leafy vegetables in the hydroponic system in Brazil and the world. Its consumption and commercial value have increased in recent decades due to its peculiar "slightly spicy and flavorful leaves" (Cantarella et al., 2022). In the current scenario, using the hydroponic system to grow vegetables has enabled yield gains, versatility, precocity, and, most importantly, post-harvest improvements (Dalastra et al., 2020b).

The use of *A. brasilense* is an emerging technology to improve nutrient acquisition in vegetable plants. Inoculation with *A. brasilense* promotes plant growth by increasing the production of plant hormones, in addition to performing biological nitrogen fixation (Hungria et al., 2018). *A. brasilense* is related to nitrate reductase activity in leaves to reduce nitric N molecules, consequently reducing nitrate concentrations in leaves (Pereira-Defilippi et al., 2017).

Leaf inoculation with *A. brasilense* in hydroponic lettuce reduces NO_3^--shoot accumulation and increases nutrient accumulation and shoot and root growth (Moreira et al., 2022). Also, it reduces the concentration of NO_3^- and increases the concentration of nutrients and plant growth of hydroponic arugula (Oliveira et al., 2022). Inoculation via nutrient solution has not been conducted before in a hydroponic system; its efficiency may be greater than inoculation via foliar. Therefore, this study aimed to verify the beneficial effects of inoculation methods on plant growth and nutrition of *A. brasilense* on lettuce and arugula in a hydroponic system.

Material and Methods

The experiment on hydroponic lettuce with nutrient film technique (NFT) cultivation was conducted between June 18 and July 19, 2021, conducted under a protected environment with 30% shading at the São Paulo State University (UNESP), Ilha Solteira-SP (20° 25' 07" S, 51° 20' 31" W, and altitude of 376 m). The meteorological data was collected inside the protected environment with an automatic meteorological station of UNESP (Figure 1).

The experimental units in the NFT system were installed on individual benches six meters long and 10% slope. The cultivation channels were made of PVC with a rectangular section of 8 cm wide and 4 cm high and upper perforations to accommodate plants at every 25 cm. Each bench consisted of six cultivation channels apart 20 cm with an individual pumping system and reservoir of 310 L with a flow rate of 1 L min^{-1} and continuous flow.

The lettuce cultivar Angelina was used; it is a vigorous plant with excellent leaves formation, closed heads, compact and uniform in the field and in hydroponic system, moderate resistance to bacterial soft rot, intense and bright green leaves, planting safety in periods of climatic fluctuations (high adaptation to tropical growing conditions). The arugula cultivar Astro was used, characterized by early maturity, vigorous plants, wide leaves with less cut area, and high yields with a bunch of high visual quality (well-formed and broad leaves), according to Sakata (2023). The seedlings were produced in phenolic foam for 15 days and then transplanted into permanent benches of the NTF system, where they remained for 31 days until harvest.

The nutrient solution, composed of concentrated Hidrogood Fert fertilizers, was used at a dose of 0.666 g L^{-1} indicated for all stages of crop development, with the following nutrient concentrations (w/v): 10% N, 9% P, 28% K, 4.3% S, 3.3% Mg, 0.06% B, 0.01% Cu, 0.05% Mn, 0.07% Mo, and 0.02% Zn. Calcium nitrate (15.5% of N and 26.5% of Ca) at 0.495 g L^{-1} was used. Also, Hidrogood Fert Ferro EDDHA (6% Fe) was used at 0.020 g L^{-1}. Measurement and correction of conductivity and pH were performed daily in the morning. On this occasion, the EC was readjusted to the value determined for each cultivation bench with the replacement of fertilizers if necessary. The pH was kept between 5.5 and 6.5; phosphoric acid was used when the pH was above 6.5, and sodium hydroxide when the pH was below 5.5 (Figure 2).

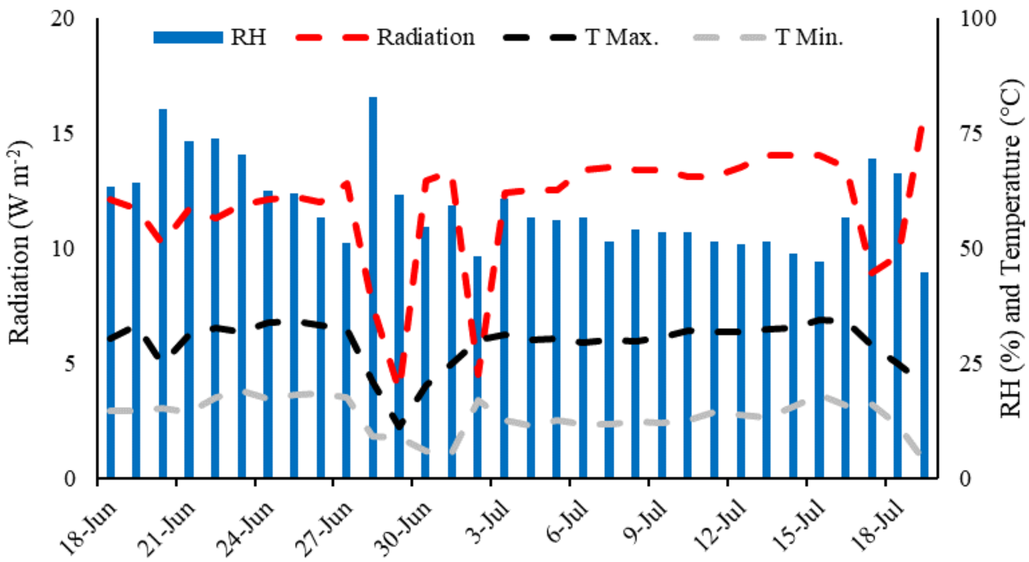


Figure 1. Relative air humidity (RH), maximum (Max. T) and minimum air temperature (Min. T), and radiation during experiment conduction

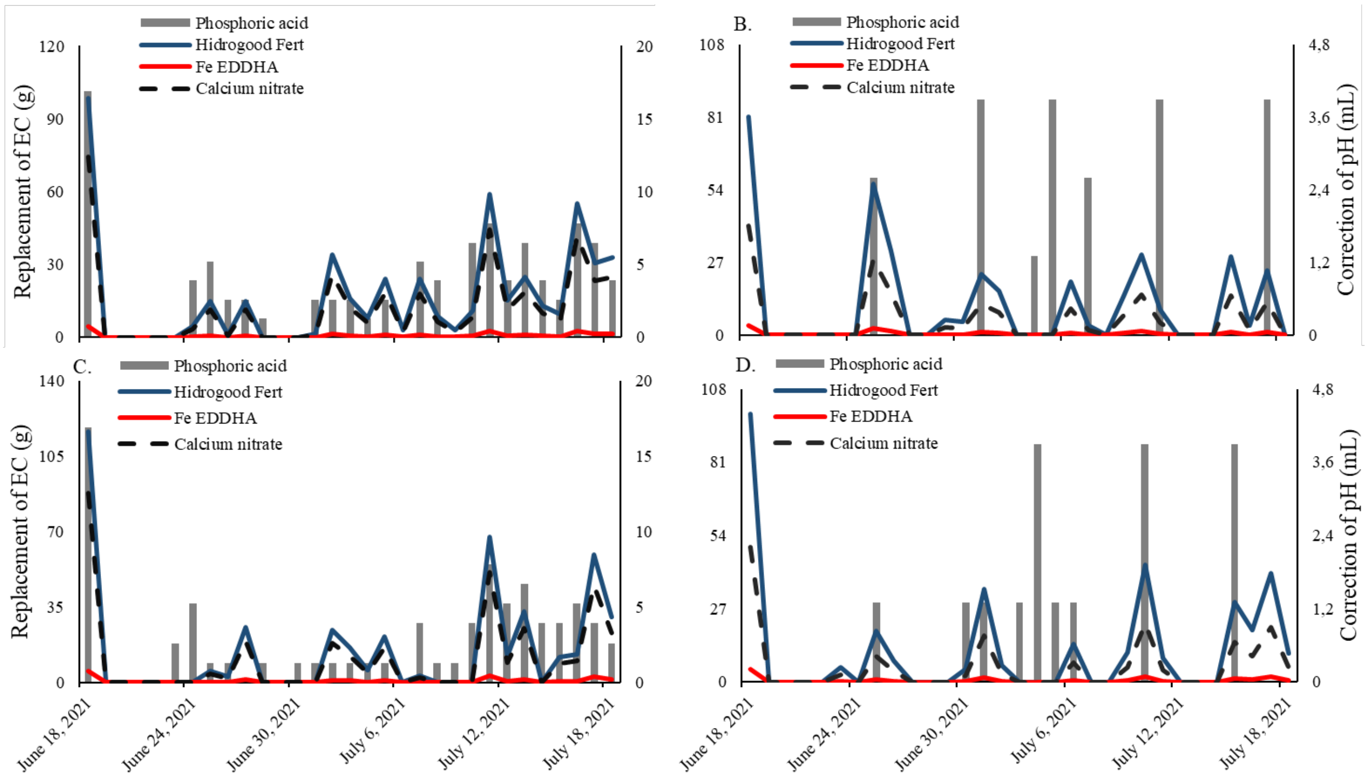


Figure 2. pH and electrical conductivity (EC) in hydroponic NFT system with arugula no-inoculation via nutritive solution (A), with inoculation via nutritive solution (C), with lettuce no-inoculation via nutritive solution (B), with inoculation via nutritive solution (D) in the period of the experiment

The experimental design was randomized blocks in a 4 x 2 factorial scheme with five replicates. The experimental unit was represented by eight plants. The first factor comprised *A. brasilense* inoculation methods: a) leaf inoculation (LI) at a dose of 300 mL ha^{-1} of inoculant and a spray volume of 250 L ha^{-1} (a backpack sprayer was used for the applications); b) inoculation via nutrient solution (NSI) at a dose of 0.1 mL L^{-1}, c) inoculation via nutrient solution + leaf inoculation (NSI+LI); d) non-inoculation (NI). A product containing *Azospirillum brasilense* - strains Ab-V5 and Ab-V6 - with guaranteed 2×10^8 colony forming units mL^{-1} was used. The second factor comprised two leafy vegetable species: a) Lettuce and b) Arugula.

The harvest was conducted 31 days after transplanting. Fresh matter and length of root and shoot were evaluated. Eight plants were placed on a table, and the shoot and root were separated; then the length of the shoot and root was measured, followed by weighing each part, obtaining the fresh matter. Leaves per plant were counted manually. The root volume was assessed using a volumetric cylinder. Leaf chlorophyll index was measured in two fully expanded leaves on each plant at 9 a.m. on a cloudless day using a chlorophyll meter (ClorofiLOG® - model CFL - 1030 Falker). Then, the material was sent for drying in an air-forced circulation oven at 60 °C for 72 hours to obtain the dry matter of root, shoot, and total dry matter. Fresh leaf yield was calculated based on Eq. 1.

$$LY = LFM \times PP \quad (1)$$

where:

LY - fresh leaf yield (kg m^{-2});
LFM - leaf fresh matter (kg $plant^{-1}$); and,
PP - plant population (plant m^{-2}).

After drying, weighing, and grinding plant materials in a Wiley-type mill, the content of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn in the lettuce and arugula shoot and root were determined according to the methodology of Malavolta et al. (1997). The determination of the concentration of nitrate (NO_3^-) and ammonium (NH_4^+) in the leaves and roots was carried out according to the methodology described by Silva (2009). The accumulation of nutrients in the shoot and root of plants was estimated based on Eq. 2.

$$NA = DM \times NC \quad (2)$$

where:

NA - nutrient accumulation (g m^{-2} or mg m^{-2});
DM - dry matter (kg m^{-2}); and,
NC - nutrient content (g kg^{-1} or mg kg^{-1}).

As the data of all variables presented normal distribution and homogeneous variances (by Shapiro-Wilk and Levene tests, respectively), they were submitted to the analysis of variance ($p \leq 0.05$). The means were compared by the Tukey test ($p \leq 0.05$). The software SISVAR (Ferreira, 2019) was used in the data analysis.

Results and Discussion

There was a significant interaction ($p \leq 0.01$) between the species cultivated in hydroponics and the inoculation methods of *A. brasilense* for the accumulation of NH_4^+, NO_3^-, and N in the shoot and root (Table 1).

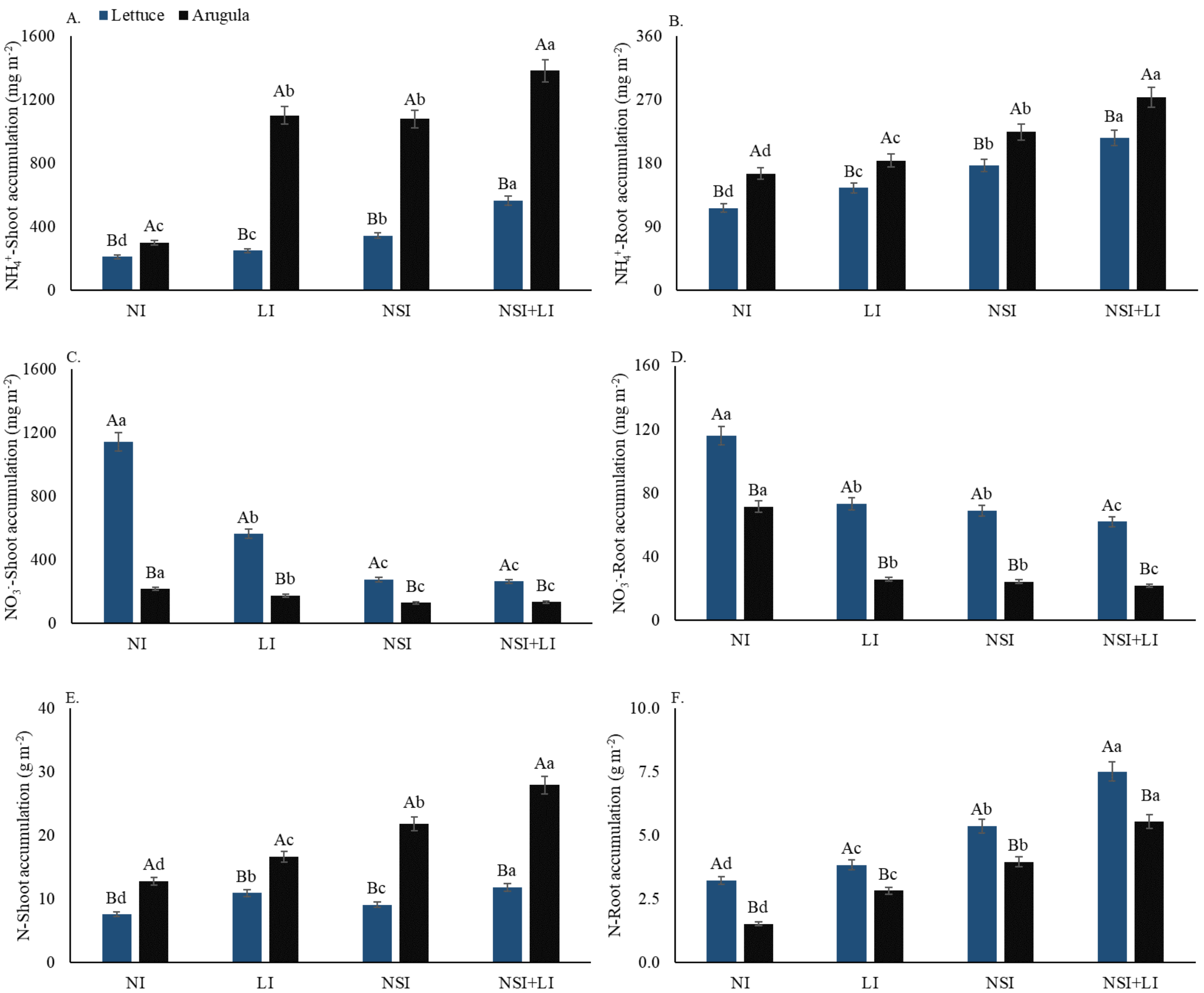
Table 1. Summary of analysis of variance for ammonium (NH_4^+), nitrate (NO_3^-), and nitrogen accumulation in the shoot and root of lettuce and arugula according to the *A. brasilense* inoculation methods

SV	Shoot			Root		
	NH_4^+	NO_3^-	N	NH_4^+	NO_3^-	N
	Probability					
Block	0.93^{ns}	0.32^{ns}	0.68^{ns}	0.33^{ns}	0.12^{ns}	0.22^{ns}
Species (S)	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Inoculation (I)	0.00**	0.00**	0.00**	0.00**	0.00**	0.05*
S*I	0.00**	0.00**	0.01**	0.00**	0.00**	0.01**
CV (%)	7.44	13.64	6.35	17.40	18.64	14.01

** - Significant at $p \leq 0.01$ by the F-test; * - Significant at $p \leq 0.05$ by the F-test; ^{ns} - not significant by the F-test; SV - Sources of variations, CV - Coefficient of variation

There was an increase in the accumulation of ammonium (NH_4^+) and nitrogen (N) in the shoot and root of lettuce and arugula plants; values were higher than in non-inoculated plants in all *A. brasilense* inoculation methods (Figures 3A, B, E, and F). A reduction in nitrate (NO_3^-) accumulation was found in the shoot and root of lettuce and arugula plants; the values were lower in all inoculation methods than in non-inoculated treatments (Figures 3C and 4D). There was higher NH_4^+ accumulation in the shoot and root of arugula plants than in lettuce plants in all treatments (Figures 3A and B). On the other hand, higher NO_3^- accumulation in the shoot and root of lettuce plants than in arugula plants was found in all treatments. Also, less accumulation of NO_3^- was observed in the shoot and root of both species in inoculated plants, regardless of the method, than in non-inoculated plants (Figures 3C and D).

The higher N accumulation in the shoot of arugula than in lettuce was observed in all inoculation methods. The



Bars represent the standard deviation of the means of the 40 plots. Uppercase letters indicate the difference between arugula and lettuce, and lowercase letters indicate differences between inoculation methods by the Tukey test at $p \leq 0.05$. NI - Non-inoculation, LI - Leaf inoculation, NSI - Nutrient solution inoculation

Figure 3. Ammonium accumulation in the shoot (A) and root (B), nitrate accumulation in the shoot (C) and root (D), and nitrogen accumulation in the shoot (E) and root (F) of lettuce and arugula plants according to the *A. brasilense* inoculation methods

combination of foliar inoculation with inoculation via nutrient solution provided the highest N accumulation in the shoot of arugula and lettuce (Figure 3E). In all inoculation methods, there was higher N accumulation in the root of lettuce than in arugula. The combination of foliar inoculation with inoculation via nutrient solution provided the highest N accumulation in the roots of the arugula and lettuce (Figure 3F).

The increase in N accumulation in lettuce and arugula plants with the *A. brasilense* inoculation via foliar and nutrient solution may occur due to the increase in the efficiency of absorption and use of fertilizers, as well as the occurrence of biological N fixation. The Ab-V5 and Ab-V6 strains of *A. brasilense* used in the study have similar nif and fix genes that confer their ability to fix atmospheric nitrogen (Hungria et al., 2018), which in symbiosis with plants increase the available nitrogen for plants to absorb and use in addition to reducing nitrogen fertilization costs (Galindo et al., 2018).

A greater accumulation of ammonium and nitrogen was observed in the roots of arugula and lettuce plants. Still, there was less nitrate accumulation in the shoot and root of plants of both species under the different inoculation methods. The *A. brasilense* increases nitrogen cycle enzyme activity, production of secondary metabolites and amino acids, and improves plant nutrition (Alvarez et al., 2019). Inoculation with *A. brasilense* stimulates increased concentrations of nitrate reductase enzyme activity in plant leaves, reducing nitrate molecules to nitrite (Pereira-Defilippi et al., 2017). Nitrate reduction occurs in the cytosol (through the activity of the nitrate reductase enzyme); the result is nitrite that enters the plastids in the roots or chloroplasts in leaves, reducing to ammonia (action of the nitrite reductase enzyme), which is fixed via glutamate synthase/glutamine synthase in amino acids, glutamine and glutamate which in turn serve as the substrate for transamination reactions for the production of amino acids used for protein ingestion (Donato et al., 2004).

There was a significant interaction ($p \le 0.01$) between the leafy vegetable species and *A. brasilense* inoculation methods for the accumulation of P, K, Ca, Mg, Zn, Cu, Mn, and Fe in the shoot (Table 2).

There was a higher accumulation of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the shoots of arugula and lettuce plants under all inoculation methods than in non-inoculation (Figure 4). A higher accumulation of P in the NSI and NSI+LI in arugula plants than in lettuce plants was found (Figure 4A). There was a higher accumulation of K in all inoculation methods in arugula plants than in lettuce plants (Figure 4B). It was verified higher accumulation of Ca in lettuce plants than in arugula plants under foliar inoculation. However, there was a greater accumulation of Ca in arugula plants concerning lettuce plants with NSI and NSI+LI (Figure 4C). A greater accumulation of Mg in the shoot of the arugula plants was observed concerning the lettuce under NSI and NSI+LI. There was no difference in the accumulation of Mg for the LI between the species (Figure 4D).

A. brasilense inoculation also increased P and protein concentrations in the tomato shoot seedlings (Mangmang et al., 2015). Foliar and seed inoculation with *A. brasilense* in wheat plants improved plant nutrition, increasing the absorption and Ca and Mg translocation and consequently increasing the concentration of these nutrients in the shoot (Besen et al., 2020). *A. brasilense* is a bacterium that provides greater efficiency in the use of fertilizers, improving plant nutrition and, consequently, the functioning of photosynthetic devices. Photosynthetic CO_2 assimilation rates are favored by adequate K and Mg nutrition, increasing intracellular CO_2 levels and leaf photosynthetic activity (Singh & Reddy, 2017). Due to the osmotic role of K in increasing leaf stomatal conductance (acting in guard cell regulation), K deficiency impairs plant photosynthesis (Jákli et al., 2017). Plant growth and metabolism require the translocation of carbohydrates from photosynthetically active tissues, sucrose is the main form of carbohydrate transport in plants, and K and Mg deficiency in phloem can impair the carbon efficiency transport far away into the plant, and the adequate supply of these two molecules increases photoprotection (Tränkner et al., 2018).

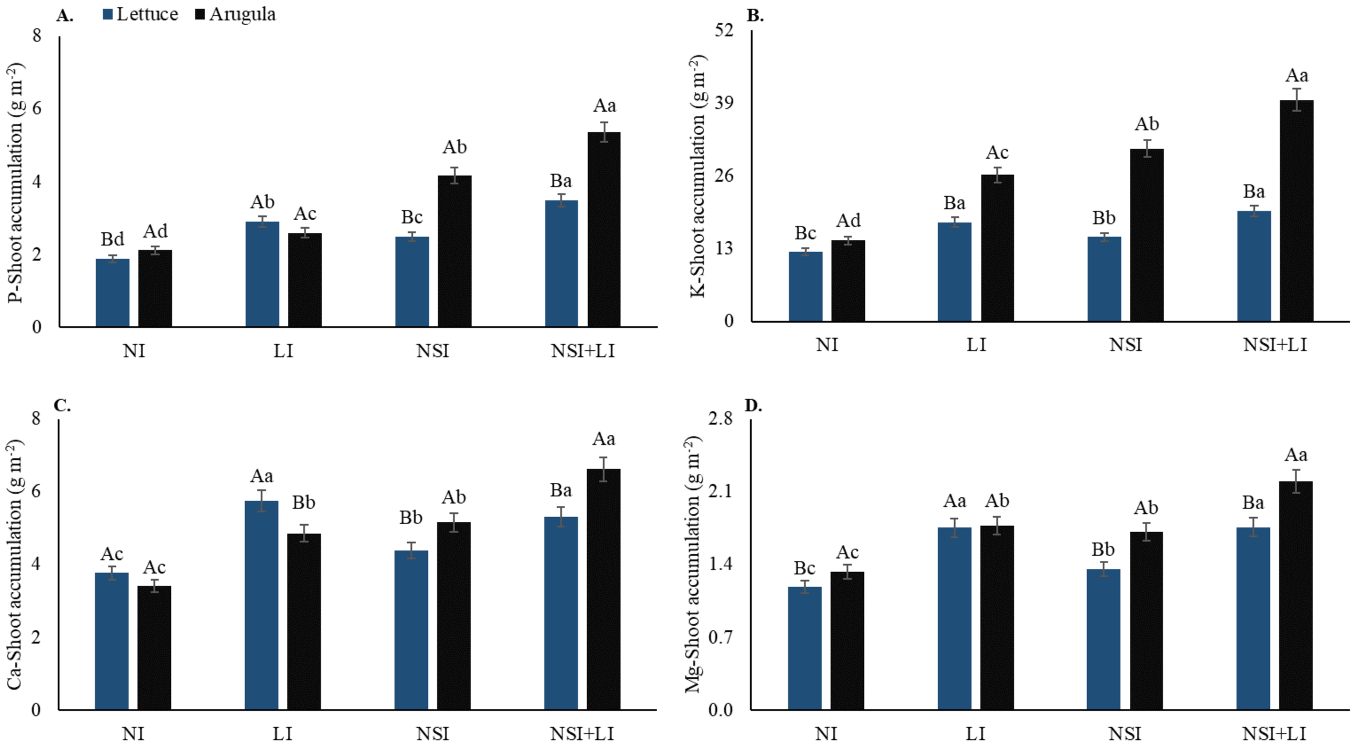
All *A. brasilense* inoculation methods provided greater accumulation of copper (Cu), iron (Fe), and manganese (Mn) in the shoot of arugula and lettuce plants (Figures 5A, B, and D). NSI and NSI+LI provided a higher accumulation of Zinc (Zn) concerning NI in lettuce and arugula plants. There was no difference between LI and NI for the accumulation of Zn in arugula plants; however, the foliar inoculation of *A. brasilense* favored the increase of Zn accumulation in lettuce plants (Figure 5C). The highest accumulation of Cu was observed in the shoot of the lettuce plants concerning the arugula plants (Figure 5A). Also, the highest accumulation of Fe in the shoot of the arugula plants concerning the lettuce plants was observed in all treatments (Figure 5B). The highest Zn accumulation was observed in the shoot of the lettuce plants concerning the arugula plants under LI and NSI (Figure 5C). The highest accumulation of Mn was observed in the shoot of the arugula plants concerning the lettuce plants with LI and NSI+LI; the opposite was observed for LI (Figure 5D).

There was an influence of the interaction ($p \le 0.01$) between the leafy vegetable species and *A. brasilense* inoculation

Table 2. Summary of analysis of variance for the accumulation of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) in the shoot of lettuce and arugula according to the *A. brasilense* inoculation methods

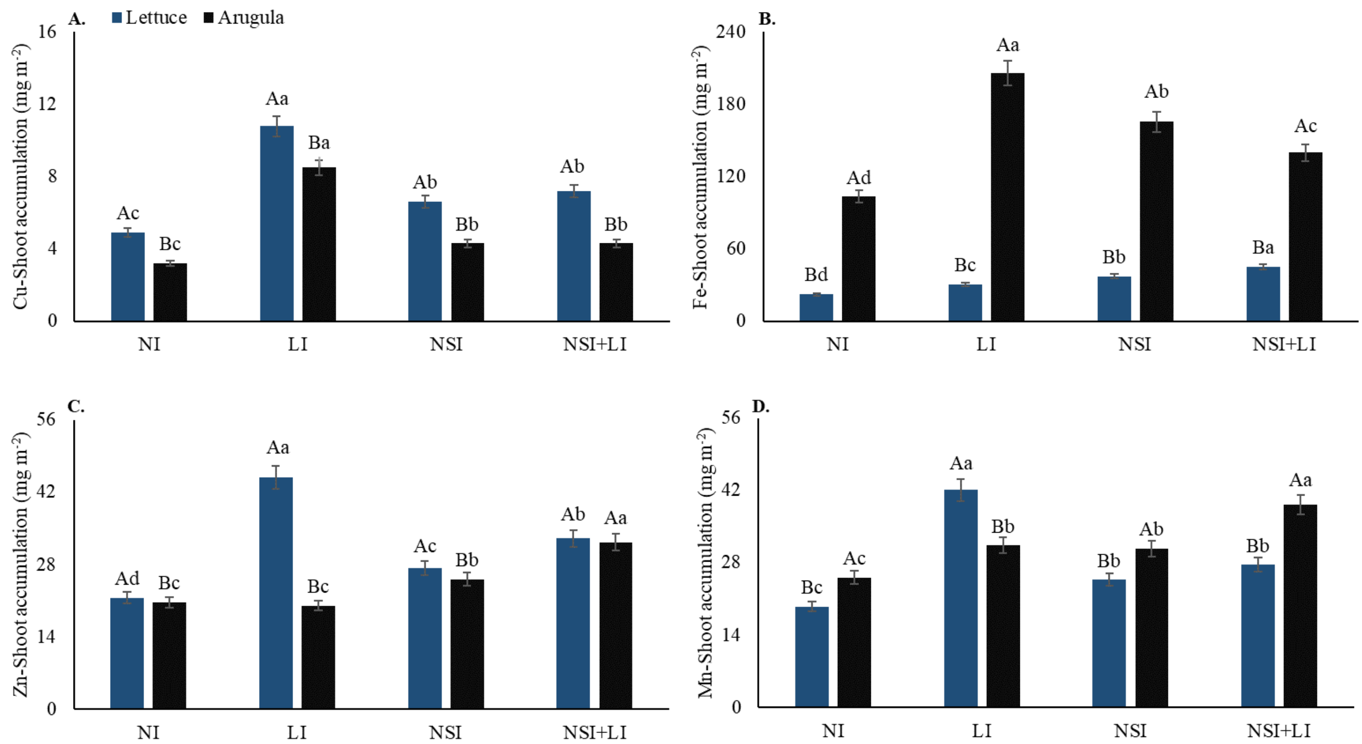
SV	P	K	Ca	Mg	Zn	Cu	Mn	Fe
	Probability							
Block	0.26^{ns}	0.16^{ns}	0.56^{ns}	0.22^{ns}	0.46^{ns}	0.13^{ns}	0.78^{ns}	0.37^{ns}
Species (S)	0.00**	0.00**	0.40^{ns}	0.00**	0.00**	0.00**	0.02*	0.03*
Inoculation (I)	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
S*I	0.00**	0.00**	0.02*	0.04*	0.00**	0.00**	0.00**	0.00**
CV (%)	7.97	9.12	11.91	9.72	8.84	8.75	9.89	17.57

** - Significant at $p \le 0.01$ by the F-test; * - Significant at $p \le 0.05$ by the F-test; ^{ns} - not significant by the F-test; SV - Sources of variations, CV - Coefficient of variation



Bars represent the standard deviation of the means of the 40 plots. Uppercase letters indicate the difference between arugula and lettuce, and lowercase letters indicate differences between inoculation methods by the Tukey test at p ≤ 0.05. NI - Non-inoculation, LI - Leaf inoculation, NSI - Nutrient solution inoculation

Figure 4. Accumulation of phosphorus (A), potassium (B), calcium (C), and magnesium (D) in the shoot of lettuce and arugula plants according to the *A. brasilense* inoculation methods



Bars represent the standard deviation of the means of the 40 plots. Uppercase letters indicate the difference between arugula and lettuce, and lowercase letters indicate differences between inoculation methods by the Tukey test at p ≤ 0.05. NI - Non-inoculation, LI - Leaf inoculation, NSI - Nutrient solution inoculation

Figure 5. Accumulation of copper (A), iron (B), zinc (C), and manganese (D) in the shoot of lettuce and arugula plants according to the *A. brasilense* inoculation methods

methods on root fresh matter, root dry matter, root length, and root volume (Table 3).

The inoculation with *A. brasilense* provided a higher root length of lettuce and arugula; however, the NSI had lower values than LI and NSI+LI. The roots of arugula plants were longer in the control treatment, foliar inoculation, and inoculation via nutrient solution than lettuce roots (Figure 6A). Root volume was higher in lettuce plants without inoculation, leaf inoculation, and inoculation via nutrient solution + foliar inoculation than in arugula plants. All inoculation methods

Table 3. Summary of analysis of variance for root fresh matter (RFM), root dry matter (RDM), root volume (RV), and root length (RL) of lettuce and arugula plants according to the *A. brasilense* inoculation methods

SV	RFM	RDM	RV	RL
	Probability			
Block	0.68[ns]	0.33[ns]	0.82[ns]	0.32[ns]
Species (S)	0.00**	0.01**	0.00**	0.00**
Inoculation (I)	0.00**	0.00**	0.00**	0.00**
S*I	0.00**	0.00**	0.01**	0.01**
CV (%)	5.91	9.72	9.09	6.01

** - Significant at $p \leq 0.01$ by the F-test; * - Significant at $p \leq 0.05$ by the F-test; [ns] - not significant by the F-test; SV - Sources of variations, CV - Coefficient of variation

with *A. brasilense* promoted higher lettuce and arugula root volume than non-inoculated treatment (Figure 6B). All inoculation methods provided higher fresh and dry matter of roots than non-inoculated treatment. NSI and NSI+LI provided a higher fresh and dry matter of roots in arugula plants than in lettuce plants. In NI and LI, there was a higher fresh and dry matter of roots in lettuce plants than in arugula (Figures 6C and D).

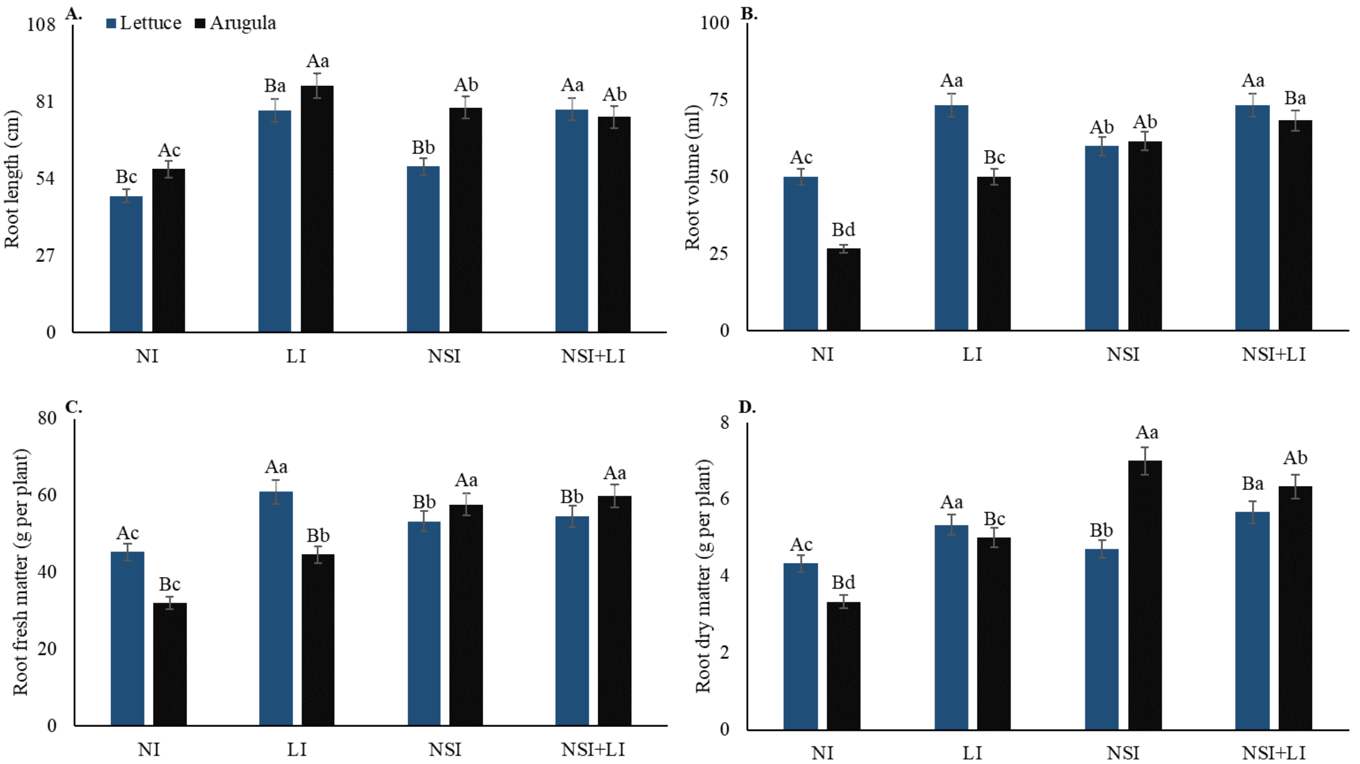
A. brasilense stimulates the production and release of auxins that favor greater growth of plant roots (Duca et al., 2014); increased root growth favors increased absorption of water, nutrients, and soil exploitation (Rondina et al., 2020). The interaction of *A. brasilense* with plant roots in the soil improves plant growth through several mechanisms, such as the production of plant hormones and secondary osmolytes, and also improves the activity of enzymes related to the nitrogen cycle (Oliveira et al., 2022).

The positive effect of inoculation via nutrient solution in a hydroponic system without soil is still unknown because, in the interaction between plant × soil × bacteria, the mechanisms of phytohormones release and their efficiency in promoting plant growth have not been described. In this way, the results of increased growth and root mass gain in arugula and lettuce in a soilless system have a new perspective when the inoculation method is performed in hydroponics. The inoculation of a biostimulant based on *Bacillus species* via nutrient solution in lettuce cultivation in soil was previously reported to be beneficial for increasing root and shoot growth of lettuce plants (Vetrano et al., 2020).

There was an influence of the interaction ($p \leq 0.01$) between the leafy vegetable species and *A. brasilense* inoculation methods on shoot fresh matter, shoot dry matter, shoot length, number of leaves, leaf chlorophyll index, and fresh leaf yield (Table 4).

The highest shoot length, number of leaves, and shoot dry matter were observed in arugula plants concerning lettuce plants in all treatments (Figures 7A, B, and D). On the other hand, shoot fresh matter and leaf yield were higher in lettuce plants than in arugula plants in all treatments (Figures 7C and F). All inoculation methods provided higher leaf chlorophyll index, number of leaves, shoot fresh matter, shoot dry matter, and fresh leaf yield of lettuce and arugula plants than non-inoculation treatment (Figure 7).

The number of leaves, shoot dry and fresh matter, fresh leaf yield, and leaf chlorophyll index were higher when inoculated with *A. brasilense* via foliar and nutrient solution alone and together for arugula and lettuce plants. The shoot length of arugula plants was greater under any inoculation method compared to the NI, but only the NSI provided a higher shoot length than all other treatments. Inoculation of *A. brasilense*



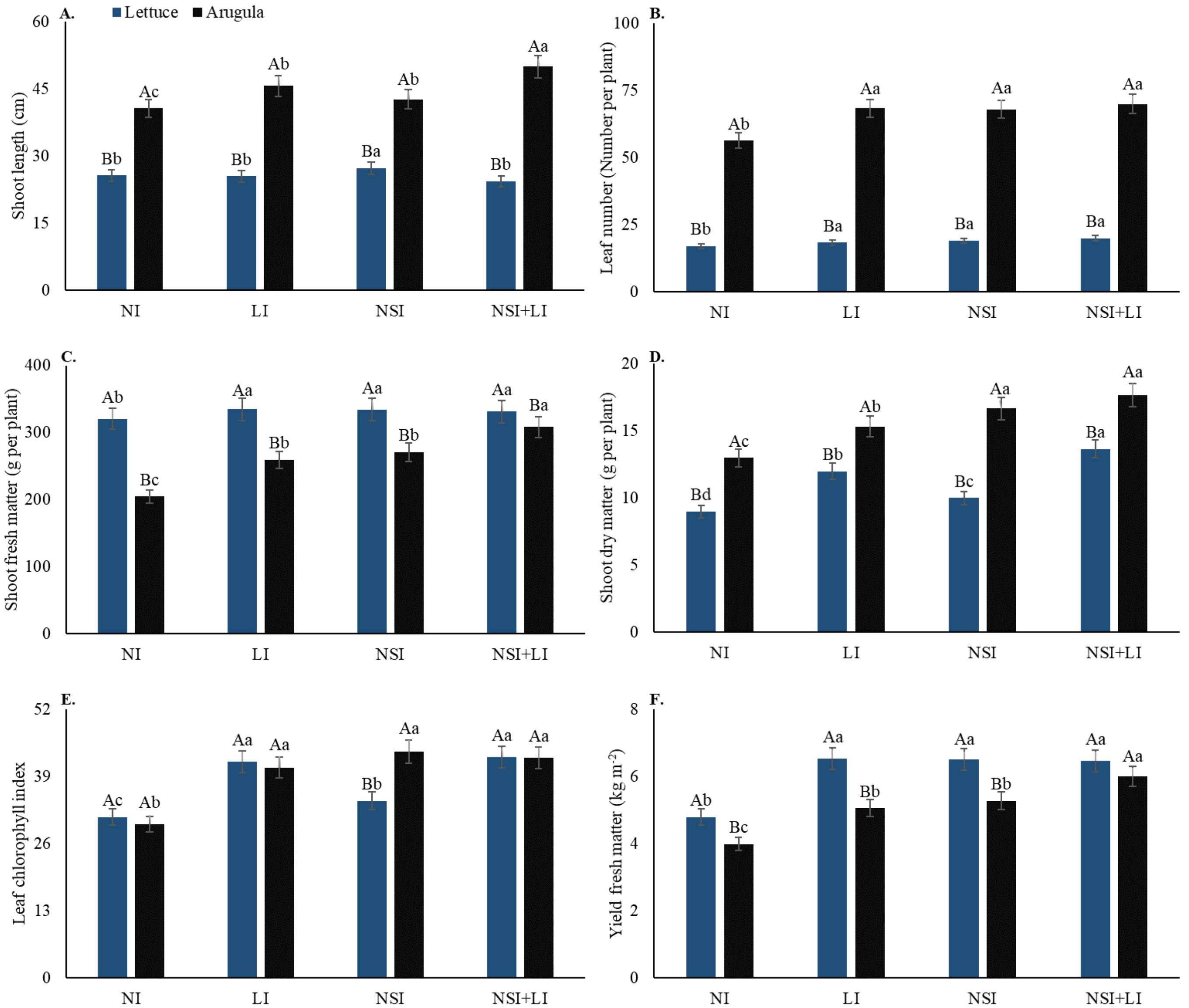
Bars represent the standard deviation of the means of the 40 plots. Uppercase letters indicate the difference between arugula and lettuce, and lowercase letters indicate differences between inoculation methods by the Tukey test at $p \leq 0.05$. NI - Non-inoculation, LI - Leaf inoculation, NSI - Nutrient solution inoculation

Figure 6. Length (A), volume (B), fresh matter (C), and dry matter (D) of the root of lettuce and arugula plants according to the *A. brasilense* inoculation methods

Table 4. Summary of analysis of shoot fresh matter (SFM), shoot dry matter (SDM), shoot length (SL), number of leaves (NL), leaf chlorophyll index (LCI), and fresh leaf yield (YIELD)

SV	SFM	SDM	SL	NL	LCI	YIELD
	Probability					
Block	0.27^{ns}	0.56^{ns}	0.41^{ns}	0.14^{ns}	0.70^{ns}	0.27^{ns}
Species (S)	0.00**	0.00**	0.00**	0.00**	0.06^{ns}	0.00**
Inoculation (I)	0.00**	0.00**	0.03*	0.00**	0.00**	0.00**
S*I	0.00**	0.01**	0.00**	0.00**	0.00**	0.00**
CV (%)	5.91	5.97	5.67	5.91	5.23	5.72

** - Significant at p ≤ 0.01 by the F-test; * - Significant at p ≤ 0.05 by the F-test; ^{ns} - not significant by the F-test; SV - Sources of variations; CV - Coefficient of variation



Bars represent the standard deviation of the means of the 40 plots. Uppercase letters indicate the difference between arugula and lettuce, and lowercase letters indicate differences between inoculation methods by the Tukey test at p ≤ 0.05. NI - Non-inoculation, LI - Leaf inoculation, NSI - Nutrient solution inoculation

Figure 7. Shoot length (A), leaf number (B), shoot fresh matter (C), shoot dry matter (D), leaf chlorophyll index (E), and fresh leaf yield (F) of lettuce and arugula plants according to the *A. brasilense* inoculation methods

via foliar inoculation in lettuce hydroponic increases leaf chlorophyll index; it results in higher photosynthetic efficiency and favors greater plant growth and matter accumulation in plant tissues (Moreira et al., 2022), as observed in arugula hydroponic under foliar inoculation with *A. brasilense* (Gato et al., 2023). The balance between the plant and the *A. brasilense* contributes to better plant growth and development since plant-associated microbial communities play a significant role in plant growth, plant nutrition, and the carbon and/or nitrogen cycles (Vacheron et al., 2015).

In general, all *A. brasilense* inoculation techniques in arugula and lettuce increased root and shoot growth, as well as in crop yield, in shoot accumulation of N, P, K, S, Ca, Mg, Fe, Cu, Mn, Zn, and even managed to reduce the accumulation of foliar nitrate, considered carcinogenic for humans and with effect on the flavor of lettuce leaves by increasing the bitterness of the leaves. However, inoculation via nutrient solution is easier than the foliar application, allowing labor allocation to other services within the farms.

Conclusions

1. All inoculation methods of *A. brasilense* are indicated for the hydroponic lettuce crop. Inoculation via foliar + nutrient solution is the most suitable for the hydroponic arugula crop to increase the fresh leaves yield of the plants. Inoculation with *A. brasilense* increases the growth, yield, and nutrient accumulation of lettuce and arugula plants.

2. Inoculation with *A. brasilense* reduces nitrate accumulation in the shoot of lettuce and arugula plants.

3. Arugula plants had a higher number of leaves and shoot dry matter than lettuce plants. However, the yield was higher in lettuce plants.

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