Effect of inoculation methods of *Azospirillum brasilense* on growth and yield of lettuce in the hydroponic system

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Ảnh hưởng của phương pháp xử lý *Azospirillum brasilense* tới sinh trưởng và năng suất giống xà lách trồng trong hệ thống thủy canh

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ABSTRACT

Lettuce (Lactuca sativa L.) is one of the most popular and economically critically leafy vegetable crops in the world. Nevertheless, the existing studies offered information limited on the Azospirillum brasilense e ects in this research system makes it necessary to verify the best method of inoculation for these bacteria. Currently, a variety of techniques for producing Lettuce have been investigated, including soilless aeroponic, hydroponic, and deep-water culture systems. Therefore, this study aimed to verify the beneficial e ects of inoculation methods of A. brasilense on plant growth and nutrition of two lettuce cultivars grown in the hydroponic system. The study was conducted in a greenhouse with 30% shading at Vietnam National University of Forestry – Dong Nai campus, Trang Bom district, Dong Nai province. A randomized block design with three 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 lettuce cultivars were evaluated. All inoculation methods improved plant height, shoot fresh and dry mass, fresh leaf yield, and accumulation of potassium, calcium, phosphorus, iron, manganese, and copper. Inoculation via nutrient solution + foliar application of A. brasilense is the most suitable for hydroponic lettuce GR-607 cultivation because it provides greater fresh leaf yield. All inoculation methods increased leaf yield in hydroponic lettuce.

Từ khóa:
Azospirillum brasilense, thủy canh, vi khuẩn thúc đẩy sinh trưởng, xà lách.

TÓM TẮT

Xà lách là loài rau ăn lá trồng phổ biến trong hệ thống thủy canh trên thế giới. Tuy nhiên, rất ít thông tin về ảnh hưởng của loài vi khuẩn Azospirillum brasilense tới sinh trưởng, năng suất của giống rau xà lách trồng trong hệ thống này. Do đó, mục đích của nghiên cứu này là nhằm xác định những ảnh hưởng có lợi của phương pháp xử lý vi khuẩn A. brasilense tới sinh trưởng và tích lũy dinh dưỡng cây xà lách trồng trong hệ thống thủy canh. Nghiên cứu này thực hiện trong điều kiện nhà màng với 70% ánh sáng tự nhiên tại huyện Trảng Bom, tỉnh Đồng Nai. Thí nghiệm được thiết kế theo khối ngẫu nhiên, 3 lần lặp lại, 2 yếu tố. Bốn phương pháp xử lý gồm: Không xử lý, xử lý bằng phương pháp phun sương, xử lý thông qua hòa vào dung dịch dinh dưỡng và kết hợp giữa phun sương và hòa vào dung dịch dinh dưỡng. Hai giống xà lách được sử dụng cho thí nghiệm này. Kết quả cho thấy tất cả phương pháp xử lý đều cải thiện chiều cao cây, trọng lượng tươi và khô, năng suất lá, sự tích lũy các chất dinh dưỡng như đạm, lân, kali, canxi, magie, đồng và sắt. Phương pháp xử lý kết hợp giữa phun sương và hòa vào dung dịch dinh dưỡng cho kết quả tốt nhất với giống xà lách GR-607 trồng trong hệ thống thủy canh.

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most economically important leafy vegetable crops in the world. In terms of nutrition, the value of lettuce is determined by those properties that are recognized as health-improving due to the high content of vitamin C, polyphenols, and fiber [1] . Iceberg lettuce is a minimally processed vegetable that is increasingly in demand for consumption in restaurants and fast foods [2]. It has also been recognized as an important functional food due to its high contents of vitamins, minerals, and biologically active compounds, such as photosynthetic pigments and phenols [3]. Its cultivation in a hydroponic system has obtained more attention for healthy and quality production in a short duration [4], using a nutrient solution and protected environments to promote nutrient use efficiency for greater plant growth, nutrient accumulation, and even yield [5]. The higher concentration of nutrients (electrical conductivity) and time of exposure to the nutrient solution can increase water use efficiency, yield, and precocity of hydroponic lettuce [6].

The interaction of soil-plant-microorganisms is an alternative strategy that could contribute to soil-plant health and productivity [7]. The PGPBs (Plant Growth Promoting Bacteria) has the ability to fight for colonization site in above and below soil parts of several crops and synthesis of phytohormones [8-10].

Among the diazotrophic bacteria, we can mention the most important groups *Arthrobacter*, *Azobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Gluconacetobacter*, *Herbaspirillum*, *Pseudomonas,* and *Azospirillum* [11, 12]. 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 [13].

Therefore, the current study aimed to verify the benefcial e ects of inoculation methods on plant growth and nutrition of *A. brasilense* on Lettuce in a hydroponic system at Vietnam National University of Forestry – Southern Campus.

2. RESEARCH METHODOLOGY

2.1. Plantlet production and growth conditions

The Yellow 1611 and GR-607 Lettuce varieties used in this study are strong growing plants, highly uniform, resistant to pests and diseases, have bright yellow green leaves, and have good quality. The seedlings were produced in phenolic foam for 15 days and then transplanted into permanent benches of the NTF (Nutrient Film Technique) system, where they remained for 31 days until harvest.

2.2. Experiment conditions

The experiment on hydroponic Lettuce with nutrient film technique (NFT) cultivation was conducted between June 18 and July 19, 2022, conducted under a protected environment with 30% shading in the greenhouse (with 4m in height) at the Vietnam National University of Forestry – Southern Campus. This is an ecological zone of tropical forest, with mean annual rainfall of 202 mm and relative humidity of 82% (Statistical yearbook of Dong Nai province, 2022). The area has a dry season from November to April and a rainy season from May to October. Mean annual temperature is 27^0C, with the highest temperatures occurring toward the end of April.

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 ow rate of 1 L min^{-1} and continuous ow.

Figure 1. Experimental design

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.

2.3. Experiment design

The experimental design was randomized blocks in a 4 x 2 factorial scheme with three replications. The experimental unit was represented by eight plants. The first factor comprised *A. brasilense* inoculation methods: (1) 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); (2) inoculation via nutrient solution (NSI) at a dose of 0.1 mL L^{-1}, (3) inoculation via nutrient solution + leaf inoculation (NSI+LI); (4) noninoculation (NI). The second factor comprised two lettuce cultivars: Yellow 1611 and GR-607

2.4. Measurement of plant growth characteristics and seed tuber yield

The harvest was conducted 31 days after transplanting. Fresh matter and length of shoot were evaluated. Eight plants were placed on a table, and the shoot was separated; then the length of the shoot was measured, followed by weighing each part, obtaining the fresh matter. Leaves per plant were counted manually. 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 shoot, and total dry matter. Fresh leaf yield was calculated based on Eq. 1 [14].

$$LY = LFM \times PP$$

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 contents of N, P, K, Ca, Mg, Cu, and Fe in the Lettuce shoot were determined according to the methodology of Malavolta *et al.* (1997). The accumulation of nutrients in the shoot and root of plants was estimated based on Eq. 2.

$$NA = DM \times NC$$

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}).

2.5. Data analysis

Data was analysed by analysis of variance (ANOVA), followed by the Ducan test using SAS software 9.1. Treatment differences were regarded as significant at $p<0.05$ or $p<0.01$.

3. RESULTS AND DISCUSSION

3.1. Nutrient accumulation

Experimental results showed that there was an increase in the accumulation of nitrogen (N) in the shoot of Lettuce plants; values were higher than in non-inoculated plants in all *A. brasilense* inoculation methods (Table 1).

The research results show that the higher N accumulation in the shoot of the GR-607 cultivar than the Yellow 1611 cultivar was observed in all inoculation methods. The combination of foliar inoculation with inoculation via nutrient solution provided the highest N accumulation in the shoot of Lettuce. In all inoculation methods, there was higher N accumulation in the root of the GR-607 cultivar than in the Yellow 1611 cultivar. The increase in N accumulation in GR-607 and Yellow 1611 cultivar 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 *A. brasilense* used in the study has similar nif and fix genes that confer their ability to fix atmospheric nitrogen [13], which in symbiosis with plants increase the available nitrogen for plants to absorb and use in addition to reducing nitrogen fertilization costs [15].

Recent studies illustrated that growth-promoting and diazotrophic bacteria have improved N acquisition by plants through biological N fixation and by increasing root hair growth through physiological changes in plants that have increased the production of plant growth hormones such as indole-3-acetic acid, cytokinins, gibberellins and ethylene, which could influence the ability of plant roots to penetrate into the soil for greater water and nutrient absorption [16].

The accumulation of P, K, Ca, Mg, Cu, and Fe in the shoot was significantly influenced by the interaction between the leafy vegetable species and *A. brasilense* inoculation methods ($p \leq 0.05$) (Table 1).

There was a higher accumulation of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the shoots of GR-607 and Yellow 1611 cultivars under all inoculation methods than in non-inoculation. A higher accumulation of P in the NSI and NSI+LI in the GR-607 cultivar than in the Yellow 1611 cultivar was found (Yellow 1611: 2.1 and 3.8 g m^{-2}; GR-607: 4.0 and 4.5 g m^{-2}). There was a higher accumulation of K in all inoculation methods in the GR-607 cultivar than in the Yellow 1611 cultivar. It was verified higher accumulation of Ca in the Yellow 1611 cultivar than in the GR-607 cultivar under foliar inoculation. However, there was a greater accumulation of Ca in the GR-607 cultivar than in the Yellow 1611 cultivar with NSI and NSI+LI. A greater accumulation of Mg in the shoot of the GR-607 cultivar was observed concerning the Yellow 1611 under NSI and NSI+LI. There was no di erence in the accumulation of Mg for the LI between the cultivars.

The results from the current study showed that *A. brasilense* inoculation also increased P and protein concentration in tomato seedling shoots [17]. *A. brasilense* treatment sprayed on leaves and seeds of wheat plants improved plant nutrition, increasing the ability to absorb and metabolize Ca and Mg and thus increasing the concentration of these nutrients in shoots [18]. *A. brasilense* is a bacterial species that has the ability to bring about greater efficiency in fertilizer use, improve plant nutrition and thus improve photosynthetic function. The rate of CO_2 accumulation in photosynthesis depends on adequate K and Mg nutrients, increasing intracellular CO_2 concentration and photosynthetic activity of leaves [19]. All *A. brasilense* inoculation methods provided greater accumulation of copper (Cu) and iron (Fe) in the shoot of two Lettuce cultivars. The

highest accumulation of Cu was observed in the shoot of the lettuce at the LI method. Furthermore, the highest accumulation of Fe in the shoot of the lettuce was observed in all treatments.

Table 1. **Effect of innoculation method and Lettuce cultivar on nutrient accumulation in the shoot of Lettuce**

Parameter	Cultivar	Inoculation method			
		NI	LI	NSI	NSI+LI
N ($g\ m^{-2}$)	Yellow 1611	8.0	9.0	8.5	11.2
	GR-607	10.3	11.0	18.0	19.5
		CV% = 6.5; P<0.05			
P ($g\ m^{-2}$)	Yellow 1611	1.8	2.4	2.1	3.8
	GR-607	2.0	2.2	4.0	4.5
		CV% = 7.9; P<0.05			
K ($g\ m^{-2}$)	Yellow 1611	13.0	19.3	14.2	19.5
	GR-607	13.2	26.2	27.3	39.0
		CV%= 9.15; P<0.05			
Ca ($g\ m^{-2}$)	Yellow 1611	3.7	5.7	4.5	5.2
	GR-607	3.2	4.6	5.1	6.3
		CV%= 12.4; P<0.05			
Mg ($g\ m^{-2}$)	Yellow 1611	1.1	1.7	1.4	1.8
	GR-607	1.3	1.7	1.6	2.2
		CV%= 8.0; P<0.05			
Cu ($mg\ m^{-2}$)	Yellow 1611	5.2	10.8	6.2	6.3
	GR-607	3.1	8.0	3.8	3.7
		CV%= 8.9; P<0.05			
Fe ($mg\ m^{-2}$)	Yellow 1611	15,0	28.0	40.8	41.2
	GR-607	20,8	34.3	41.0	60.0
		CV%= 8.7; P<0.05			

3.2. Yield components and yield

There was a significant interaction between Lettuce cultivars and *A. brasilense* inoculation methods on shoot fresh matter, shoot dry matter, shoot length, number of leaves, and fresh leaf yield ($p \leq 0.05$) (Table 2).

The highest shoot length, number of leaves, shoot fresh matter and fresh leaf yield were observed in the GR-607 cultivar concerning Yellow 1611 in all treatments (Table 2). On the other
hand, shoot dry matter was higher in Yellow 1611 than GR-607 in all treatments. All inoculation methods provided a higher number of leaves, shoot fresh matter, shoot dry matter, and fresh leaf yield of lettuce cultivars than non inoculation treatment. The number of leaves, shoot dry and fresh matter, and fresh leaf yield were higher when inoculated with *A. brasilense* via foliar and nutrient solution alone and together for two lettuce cultivars. The shoot length of the GR-607 cultivar was greater under any inoculation method compared to the NI, but only the NSI + LI provided a higher shoot length than all other treatments. Inoculation of *A. brasilense* via foliar inoculation in lettuce hydroponic increases leaf chlorophyll index [14]; it results in higher photosynthetic efficiency and favors greater plant growth and matter accumulation in plant tissues [20], significantly affecting the height growth, leaves growth, leaves area growth, the average number of tubers $plant^{-1}$ and potato seed minituber yield [21], as observed in arugula hydroponic under foliar inoculation with *A. brasilense* [22]. The balance between the plant and *A. brasilense*

contributes to better plant growth and development since plant-associated microbial communities play a signifcant role in plant growth, plant nutrition, and the carbon and/or nitrogen cycles [23].

Table 2. **Effect of innoculation method and lettuce cultivar on growth and yield of Lettuce**

Parameter	Cultivar	Inoculation method			
		NI	LI	NSI	NSI+LI
Shoot length (cm)	Yellow 1611	22.0	22.5	23.2	22.8
	GR-607	41.3	45.2	43.0	46.3
		CV% = 5.7; P<0.05			
Leaf number (Number per plant)	Yellow 1611	12.0	12.5	13.0	12.9
	GR-607	13.4	15.8	15.6	16.0
		CV% = 6.3; P<0.05			
Shoot fresh matter (g per plant)	Yellow 1611	83.0	84.5	86.0	85.6
	GR-607	82.3	90.5	91.2	93.0
		CV%= 5.9; P<0.05			
Shoot dry matter (g per plant)	Yellow 1611	9.5	12.3	11.3	12.4
	GR-607	8.5	9.1	9.8	10.9
		CV%= 5.9; P<0.05			
Yield fresh matter (kg m^{-2})	Yellow 1611	1.8	1.9	2.0	2.3
	GR-607	2.1	2.7	2.8	3.1
		CV%= 5.7; P<0.05			

Overall, the study focused on the effect of innoculation method and lettuce cultivar on nutrient accumulation in the shoot, growth and yield of Lettuce in a controlled greenhouse. The results have determined that all *A. brasilense* inoculation techniques in lettuce increased shoot growth, as well as in crop yield, in shoot accumulation of N, P, K, S, Ca, Mg, Fe, and Cu. However, inoculation via nutrient solution is easier than the foliar application, allowing labor allocation to other services within the farms.

4. CONCLUSIONS

The results of this study revealed 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 GR-607 cultivar to increase the fresh leaves yield of the plants. Inoculation with *A. brasilense* increases the growth, yield, and nutrient accumulation of lettuce.

These findings will be the basis for building a Lettuce cultivation process in hydroponic systems, enhancing nutrient accumulation and absorption, minimizing chemical inputs, and approaching circular agriculture.

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