# EVALUATION OF *Azospirillum brassilense* DOSE RESPONSE ON GROWTH AND YIELD OF MINITUBERS FROM APICAL ROOTED CUTTINGS GROWN IN COCO-PEAT

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## ABSTRACT
Potato (*Solanum tuberosum* L.) is one of the most valuable crops in the world, ranked as the 4th most important food crops after rice, wheat and maize. Currently, a variety of techniques for producing potato minituber have been investigated, including soilless aeroponic, hydroponic and deep-water culture systems. However, minitubers potato production in the greenhouse from apical rooted cutting grown coco-peat under a photoperiod and nutritional - shock system is a new and creative idea. The use of diazotrophic bacteria such as *Azospirillum brasilense* demonstrates a positive effect on nitrogen availability, a primordial element for the development of this plant. Therefore, this study aimed to evaluate the growth and minitubers yield in a potato cultivar inoculated with *A. brasilense* at different doses. The experiments were conducted in controlled greenhouse on concrete raising beds with dimensions of 10 m x 1 m x 0.3 m. The study revealed that the concentration of *A. brasilense* at different doses significantly affected the height growth, leaves growth, leaves area growth, the average number of tubers plant$^{-1}$, standard tuber and potato seed minituber yield. The treatment applied with *A. brasilense* at a concentration of 200 mL m$^{-2}$ after planting gave the highest yield of potato minitubers (277.8 tubers m$^{-2}$).
**Keywords: after *in vitro*, Atlantic, *Azospirillum brasilense,* diazotrophic bacteria, minitubers potato.**

## 1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most valuable crops in the world, ranked as the 4th most important food crops after rice, wheat and maize [1]. The potato area in the world reached 19.30 million hectares, with an average yield of 20.11 tons/ha, with an output of 388.2 million tons [2]. It consits of a high source of carbohydrates, proteins, vitamins, and minerals, so potato is currently consider a solution for feeding more than a billion people [3]. Therefore, enhancing the productivity of this root crop may be a key tool in fulfilling the nutritional requirement and the demand of minitubers for production practices.

Potato seed quality is an important factor determining yield. The long-term use of seed tubers produced in the field contributes to the accumulation of pathogens, especially viruses, leading to seed degradation and reduced yield and quality of potatoes [4]. Over the years, the use of basic potato seed minitubers in seed production has revolutionized the potato production industry by shortening the seed production cycle in the field and creating a large number of desease clean seed potatoes supplying for production. Basic potato seed minitubers are obtained from seed production on in vitro-derived seedling by different growing methods [5].

In order to improve yield, in addition to selection of varieties, it is necessary to apply adequate NPK fertilizers, manage crops, implement measures to improve soil quality, and use soil microorganisms effectively [6]. The overuse of chemical fertilizers in agricultural farming has negatively affected the natural environment, going against the concept of sustainable agriculture. To minimize the pressure of chemical fertilizer inputs in agricultural production, the use of soil microorganisms capable of fixing N or dissolving nutrients for plants is a smart choice [7].

Among the diazotrophic bacteria, we can mention the most important groups *Arthrobacter*, *Azobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Gluconacetobacter*, *Herbaspirillum*, *Pseudomonas,* and *Azospirillum [8, 9]*. *Azospirillum brasilense* is circumscribed in a special group of bacteria that have a high capacity to mobilize nitrogen contained in the soil in a non-labile way, in the environment, and the production of growthpromoting substances [10-12].

The study aimed to determine the influence of *A. brasilense* at different doses on the production of disease-free potato seed minitubers grown on coco-peat substrates, using hydroponic solutions and isolated under greenhouse conditions at Dalat, Vietnam.

## 2. RESEARCH METHODOLOGY

### 2.1. Plantlet production and growth conditions

Root-cutting seedlings of cultivars Atlantic were obtained from tissue culture plants. Seedlings were grown in pots with an average height of 6-7cm. Seedlings had 5-6 true leaves, roots reached the bottom of the pot (about 15 days after cutting), were disease-free with bacterial wilt and virus using the Elisa kit produced by the International Potato Research Center before conducting experiments.

### 2.2. Experiment conditions

The experiments were conducted in controlled greenhouse on concrete raising beds with dimensions of 10m x 1m x 0.3m. The growing substrate was clean coco-coir and watered with 80% moisture before planting 2 days. Potato seedlings with full roots (the roots reached the bottom of the pot - about 15 days after dipping), 6-7cm in height, 5-6 true leaves were planted in pots. Plant density was 100 plants $m^{-2}$ (distance 10 x 10 cm). The basis fertigation was (ppm) 102 N, 46.5 P, 253.5 K, 160 Ca, 36 Mg, 48 S, 4.0 Fe, 0.06 Cu, 0.22 Zn, 0.5 Mn and 0.26 B, pH = 6.0, EC = 1dS $m^{-1}$ (Novella et al., 2008) (Table 1). Nutrition will be mixed according to the defined formula. Mix into 2 tanks, tank A and tank B. The composition and amount of phase are as in Tables 1. When watering the nutrient solution, use 1 liter of solution A + 1 liter of solution B and 98 liters of water. Nutrients and water are provided through a drip irrigation system, using a drip line of the Netafim Company, Israel. The irrigation regime depends on the growth stage of the plant (Table 2). Measures to care for, prevent pests and diseases were carried out according to the procedure of Vegetable and Flower Research Center, Datlat, Vietnam. For seedlings inoculation, the mommercial product AgriStore (*A. brasilense* concentration: $2x10^{11}$ CFU $L^{-1}$) was used, supplied by the company Total Biotecnologia, Brazil.

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**Table 1. Nutrient solution**

| Chemical name | A Tank (100 L) | B Tank (100 L) |
|---|---|---|
| $Ca(NO_3)_2.4H_2O$ | 8,400 g | |
| Fe(EDTA) | 67 g | |
| $KNO_3$ | 2,900 g | |
| $K_2SO_4$ | | 2,300 g |
| $KH_2PO_4$ | | 1,500 g |
| MAP | | 450 g |
| $MgSO_4.7H_2O$ | | 2,300 g |
| $MnSO_4.H_2O$ | | 3.3 g |
| $CuSO_4.5H_2O$ | | 0.4 g |
| $Na_2MoO_4.2H_2O$ | | 2.5 g |
| $ZnSO_4.7H_2O$ | | 3.5 g |
| $H_3BO_3$ | | 1.48 g |

**Table 2. Nutritional irrigation regime for potato minnitubers production using root-cutting seedlings from tissue culture plants on coco-coir substrate**

| Growing duration | Irrigating times per day | Irrigation amount |
|---|---|---|
| 1-40 days after transplanting | 5 | 400L $ha^{-1}$ |
| 41-70 days after transplanting | 3 | 500L $ha^{-1}$ |

### 2.3. Experiment design

A study was conducted from 2020 at the Vegetable and Flower Research Center, Dalat, Vietnam. A experiment was set up as one - way factorial design (Completely Randomized Design), 3 replications to evaluate the effect of *A. brasilense* at different doses (T1: control, T2: Inoculation with 50 mL $1m^{-2}$, T3: Inoculation with 100 mL $1m^{-2}$, T4: Inoculation with 150 mL $1m^{-2}$, T5: Inoculation with 200 mL $1m^{-2}$, T6: Inoculation with 250 mL $1m^{-2}$, T7: Inoculation with 300 mL $1m^{-2}$) on growth performance and yield of first generation potato minitubers production of potato variety Atlantic [13].

### 2.4. Measurement of plant growth characteristics and seed tuber yield

Height growth (cm $week^{-1}$), leave growth (leave $week^{-1}$), leave area growth ($cm^2$ $week^{-1}$), number of tuber stolons $plant^{-1}$ (at 30 DAT), average number of tubers $plant^{-1}$, average weight of tubers (g), tuber yield (tubers $m^{-2}$) and standard tuber (Percentage of tubers over 3g) [14].

### 2.5. Data analysis

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

## 3. RESULTS AND DISCUSSION

### 3.1. Growth parameters

Experimental results showed that the growth rate of potato plant height was proportional to the concentration of *A. brasilense* (T) from 50 mL $m^{-2}$ to 300 mL $m^{-2}$, the plant height growth rate from 5.65 to 7.30cm $week^{-1}$, in which the highest concentration of *A. brasilense* is 300 mL $m^{-2}$. However, concentrations of *A. brasilense* from 200 mL $m^{-2}$ to 30 mL $m^{-2}$, the difference in height growth rate was not statistically significant (Table 3), the stem was smaller than the concentrations of *A. brasilense* from 150 Ml $m^{-2}$ to 200mL $m^{-2}$. The results of the correlation analysis show that the height growth rate of the plants is closely correlated with the concentrations of *A. brasilense* sprayed on the substrates, according to the equation $Y = -0.0003x^2 + 0.1276x - 6,6662$, correlation coefficient $R^2 = 0.98$.

**Table 3. Effect of *A. brasilense* doses on growth of cultivar Atlantic (mL $m^{-2}$)**

| Treatment | Height growth (cm $week^{-1}$) | Leaves growth (leaves $week^{-1}$) | leaves area growth ($cm^2$ $week^{-1}$) |
|---|---|---|---|
| T1 = 0 | 4.48f | 1.96d | 3.72d |
| T2 = 50 | 5.65d | 2.16c | 4.53c |
| T3 = 100 | 6.63c | 2.58b | 6.54b |
| T4 = 150 | 6.98b | 2.84ab | 8.34ab |
| T5 = 200 | 7.22a | 3.12a | 9.15a |
| T6 = 250 | 7.28a | 3.09a | 8.57ab |
| T7 = 300 | 7.30a | 2.91ab | 8.35ab |
| F-test | * | ** | ** |
| *CV %* | *5.12* | *5.03* | *4.86* |

*In the same average group. the values with the same accompanying characters do not have statistical significance $P < 0.05$; ns: none significant; $^*$ significant difference ($p < 0.05$); $^{**}$ significant difference ($p < 0.01$).*

The concentration of T in substrates affects not only the growth rate of plant height, number of leaves but also the growth of leaves area. From the concentration of T1 = 50Ml $m^{-2}$ to the level of T5 = 200 mL $m^{-2}$, the growth of leaves area was proportional to the increase of T level, the growth reached from 3.72 $cm^2$ $week^{-1}$ to 9.15 $cm^2$ $week^{-1}$, in which highest at T = 200 mL $m^{-2}$. Actual observations showed that the treatments using nitrogen levels from 0mL $m^{-2}$ to 150 mL $m^{-2}$, potato plants showed quite obvious nitrogen deficiency, leaves were light green, leaf area was the smallest at stage 40 - 50 days after planting. At the T levels in the substrates from 250 mL $m^{-2}$ to 300 mL $m^{-2}$, the leaves area growth rate started to decrease and also showed an excess of nitrogen, the leaves became darker green (Table 3).

The results of the regression analysis showed that the leaves growth rate was strongly correlated with the concentration of T in the growing medium according to the model $y = -0.0001x^2 + 0.0535x - 2.9136$, correlation coefficient $R^2 = 0.93$. Similarly, leaves area growth rate was strongly correlated with T concentration in growing medium according to the equation $Y = -0.0006x^2 + 0.2552x – 19.191$, correlation coefficient $R^2 = 0.93$.

**3.2. Yield components and yield**

After 30 day from planting, the Atlantic variety grew and developed well, without the appearance of pests and diseases. This is also the time when these potato varieties begin to form tubers.

The average number of tubers $plant^{-1}$ was a ranged of from 0.26 to 3.0 tubers $plant^{-1}$ [15], from 1.85 to 2.54 tubers $plant^{-1}$ [16]. However, Corrêa et al., (2008) [17] suggested that the average number of tubers would be from 7.0 to 8.31 tubers $plant^{-1}$ if there were appropriate technical measures to affect the tuber formation stage of potato plants. Cao and Tibbitts (1998) [18], when studying the response of potato plants to different nitrogen concentrations found that nitrogen changes at the stage of tuber stolon formation plays an important role in determining the number of tubers per plant.

The results showed that the average number of tubers $plant^{-1}$ had no statistically significant difference between the concentrations of T in the medium from T1 = 0 mL/$m^2$ to T7 = 300 mL/$m^2$. In general, the number of tubers $plant^{-1}$ was low, with an average of only 2.1 to 2.7 tubers $plant^{-1}$ (Table 4). However, the results of correlation analysis showed that the average number of tubers $plant^{-1}$ was correlated with the concentration of T in the medium according to the equation $Y = - 0.0001x^2 + 0.0558x - 2.518$, correlation coefficient $R^2 = 0.74$.

The average tuber fresh weight $plant^{-1}$ had a difference between the concentrations of T in the growing medium and gradually increased from 0 mL $m^{-2}$ to 200 mL $m^{-2}$, reaching an average of 10.15g to 16.84g and decreasing at 250 mL $m^{-2}$ and 300 mL $m^{-2}$. Similarly, the tuber yield $m^{-2}$ achieved gradually increased from 0 mL to 200 mL $m^{-2}$, reaching from 201.7 to 277.7 tubers $m^{-2}$, the highest at 200 mL $m^{-2}$ and gradually decreased at the levels of 250 mL $m^{-2}$ and 300 mL $m^{-2}$. This was completely consistent with the data of leaf area growth (Table 4). The results of the correlation analysis showed that the tuber yield $m^{-2}$ was correlated with the concentration of T in the growing medium according to the equation $Y = - 0.00165x^2 + 6.2248x - 313.03$, the correlation coefficient $R^2 = 0.90$.

The results in Table 4 showed that there was a statistically significant difference in the percentage of standard tubers. the percentage of standard tubers was from 66.5 to 91.3%, of which the highest in treatment T6 = 250 mL $m^{-2}$. The experimental results are completely consistent with the studies of Chil Chang *et al.,* (2006), Corrêa *et al.,* (2008), (2009) and Novella *et al.,* (2008) [17, 19, 20].

**Table 4. Effect of *A. brasilense* doses on yield components and yield of cultivar Atlantic (mL m$^{-2}$)**

| Treatment | Average $N_0$ tubers Plant$^{-1}$ | Average weight of tubers (g) | Tuber yield (tubers m$^{-2}$) | Standard tuber (%) |
|---|---|---|---|---|
| T1 = 0 | 2.1 | 10.15d | 201.7c | 66.5 |
| T2 = 50 | 2.3 | 14.34c | 231.3b | 71.8 |
| T3 = 100 | 2.7 | 16.23a | 271.6a | 82.8 |
| T4 = 150 | 2.7 | 16.76a | 274.5a | 88.6 |
| **T5 = 200** | **2.7** | **16.84a** | **277.8a** | **90.5** |
| T6 = 250 | 2.5 | 15.73b | 242.5b | 91.3 |
| T7 = 300 | 2.5 | 15.74b | 235.6b | 90.3 |
| F-test | NS | * | ** | - |
| CV % | 5.11 | 4.87 | 5.13 | - |

*In the same average group. the values with the same accompanying characters do not have statistical significance P < 0.05; ns: none significant; * significant difference (p < 0.05); ** significant difference (p < 0.01).*

Overall, the current study focused on *A. brasilense* doses so as to promote the ability of formation of potato seed minitubers using apical shoot cutting from in vitro plantlets grow on coco-peat substrates in controlled greenhouse. The results have determined the nutritional formula (mL m$^{-2}$): 102 N, 46.5 P, 253.5 K, 160 Ca, 36 Mg, 48 S, 4.0 Fe, 0.66 Cu, 0.22 Zn, 0.5 Mn, 0.26 B, pH = 6.0 , EC = 1dS m$^{-1}$, supplemented with *A. brasilense* irrigation at a concentration of 200 mL m$^{-2}$ after planting is a promising nutrient formula for the production of minitubers potato varieties from using apical shoot cutting from *in vitro* plantlets grow on coco-peat substrates.

## 4. CONCLUSIONS

Our results demonstrated the concentration of *A. brasilense* at different doses significantly affected the height growth, leaves growth, leaves area growth, the average number of tubers plant$^{-1}$ and potato seed minituber yield. The treatment applied with *A. brasilense* at a concentration of 200 mL m$^{-2}$ after planting gave the highest yield of potato minitubers (277.8 tubers m$^{-2}$).

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# ẢNH HƯỞNG CỦA NỒNG ĐỘ VI KHUẨN *Azospirillum brassilense* ĐẾN SINH TRƯỞNG VÀ NĂNG SUẤT KHOAI TÂY GIỐNG CỦ NHỎ TỪ CÂY GIỐNG SAU CẤY MÔ

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## TÓM TẮT

Khoai tây (*Solanum tuberosum* L.) là một trong những loại cây trồng có giá trị kinh tế cao trên thế giới, được xếp vào loại cây lương thực quan trọng thứ 4 sau lúa gạo, lúa mì và ngô. Việc sử dụng các vi khuẩn có khả năng cố định đạm như *Azospirillum brasilense* cho thấy tác động tích cực đến lượng đạm dễ tiêu trong đất, một nguyên tố cơ bản cho sự phát triển của loại cây này. Nghiên cứu này nhằm mục đích đánh giá sự tăng trưởng và năng suất củ nhỏ giống khoai tây Atlantic được ủ vi khuẩn *A. brasilense* ở các liều lượng khác nhau. Các thí nghiệm được tiến hành trong nhà màng có kiểm soát, trên luống bê tông có kích thước 10m x 1m x 0,3m. Nghiên cứu cho thấy nồng độ *A. brasilense* ở các liều lượng khác nhau ảnh hưởng rõ rệt đến tăng trưởng chiều cao, tăng trưởng lá, tăng trưởng diện tích lá, số củ trung bình trên cây và năng suất củ giống khoai tây. Nghiệm thức xử lý *A. brasilense* ở nồng độ 200 mL/$m^2$ sau khi trồng cho năng suất củ khoai tây nhỏ cao nhất (277,8 củ/$m^2$).

**Từ khóa: *Azospirillum brasilense*, giống khoai tây Atlantic, giống khoai tây củ nhỏ, sau nuôi cấy mô, vi khuẩn cố định đạm.**

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