

Fertigation and *Azospirillum* in the nutrition of *Agave angustifolia* vitroplants in a nursery

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Abstract

Humanity seeks to increase the productivity and quality of crops that provide basic or economic benefits. The work aimed to evaluate the development of *Agave angustifolia* vitroplants fertigated with different nutrient aliquots and inoculated with *Azospirillum brasilense* from 2023 to 2024. In an experiment with a 3 × 2 × 3 factorial experimental design, the factors were: 1) initial plant size (small, medium, and large); 2) inoculation with the bacterium *A. brasilense* and without inoculation; and 3) concentrations of the Steiner nutrient solution (SN) (0, 75 and 100%). After 20 months, morphological characteristics and the amount of nutrients in the leaves were evaluated. Plants with a larger initial size maintained differences in growth compared to medium and small plants. The plants fertigated with 100% SN had a height of 51 cm (18.6%), a rosette diameter of 85.8 cm (25.8%) and a stem diameter of 9.6 cm (65.5%). Their largest leaf was 66 cm (13.7%), and their leaves contained 6 987 ppm N (9.6%), 1 775 ppm P (11%), and 3 093 ppm K (-25.8%) compared to plants irrigated only with water. The plants inoculated with the bacterium had more leaves, a larger rosette diameter, greater leaf length, greater root volume and greater stem and root dry weight than non-inoculated plants. The micropropagated *A. angustifolia* plants that were fertigated in a nursery and in parallel, inoculated with *A. brasilense* reached adequate sizes for transplantation in the field.

Keywords:

nutrient analysis, plant growth-promoting bacteria, nutrient content, plant micropropagation, nutrient solution.



Introduction

In the state of Oaxaca, 17 700 ha are cultivated with agaves (SIAP, 2019; Olvera Vargas *et al.*, 2022). *A. angustifolia* is of great economic importance, as it occupies 80% of the plantation area and in 2023, 86.31% of the raw material for mezcal production came from this species (Enríquez-del Valle *et al.*, 2023). Plantations of this species have increased over the recent decade, demanding quality plants that ensure their survival and productivity in the field (Jarquín-Rosales *et al.*, 2022).

Each plant species and genotype require an optimal nutritional supply for its normal growth and development, thereby ensuring production that guarantees the growing demand for raw material for the industry (García and Oberschelp, 2015); one option is to apply inorganic fertilizers (Sánchez-Mendoza *et al.*, 2022). Zuñiga-Estrada *et al.* (2018) showed that *Agave tequilana* plants that received fertigation were superior to unfertilized plants in terms of height, number of leaves, and dry matter production.

Likewise, *A. angustifolia* plants that received 100% fertilization via irrigation in a nursery for three months reached a larger size and developed more leaves and leaf area than plants with a lower nutritional supply (Enríquez-del Valle *et al.*, 2023; Luna-Luna *et al.*, 2017). On the other hand, Domínguez-Duarte *et al.* (2019) point out that plant growth-promoting (PGP) microorganisms are a set of different species that help plants to show more development and productivity. Among these are the genera *Rhizobium*, *Pseudomonas* and *Azospirillum* (Bashan and Holguin, 1998).

Their mechanisms of action are phosphate solubilization, nitrogen fixation or hormone generation (Glick, 2014); they have a direct impact on plant metabolism by increasing the absorption of water and minerals, optimizing root development (Lavenus *et al.*, 2013); they increase the activity of enzymes in the plant or facilitate other beneficial microorganisms to exert a better effect on plants (Shu *et al.*, 2016). There is evidence of the advantages offered by plant growth-promoting bacteria (PGPB). In this regard, Okon and Vanderleyden (1997) inoculated corn plants with the bacterium *Azospirillum*, which resulted in larger tassels and higher productivity than in non-inoculated plants.

Likewise, Jarquín-Rosales *et al.* (2023) reported that *A. angustifolia* plants obtained from seed germination, fertigated with SN-100% and inoculated with *A. brasilense* had greater height, greater number and sizes of leaves, a larger stem diameter and greater dry weights of the aerial part, root and leaf area compared to non-inoculated plants. The study aimed to determine the morphological characteristics and leaf nutrient concentration of *A. angustifolia* vitroplants in the nursery stage that were fertigated with different concentrations of Steiner nutrient solution and inoculated with *Azospirillum brasilense*.

Materials and methods

The research was conducted at the nursery of the Technological Institute of the Valley of Oaxaca, in Santa Cruz Xoxocotlán, Oaxaca, Mexico, from 2023 to 2024. Micropropagated and acclimatized *A. angustifolia* plants, which had been in the nursery for six months, were used. The plants were separated into three size categories depending on the length of the largest leaf: 1) small (<12 cm), 2) medium (12 to 16 cm), and 3) large (>16 cm). They were established individually in 10 dm³ polyethylene pots containing a 1:2:1 v/v mixture of peat moss, sand, and forest soil, with a pH of 7.64, and an electrical conductivity of 1.11 dS m⁻¹.

To apply different irrigation methods, plants in each size category were separated into three groups to receive: 1) water; 2) fertigation with the Steiner (1984) nutrient solution (SN) diluted to 50%; and 3) fertigation with 100% SN. The amount of nutrients in mg L⁻¹ of the 100% SN was: 102.6 N, 30.6 P, 220.1 K, 182.3 Ca, 49 Mg and 110.9 S. Each plant was fertigated with 200 ml of SN once a week. Subsequently, plants in each size category and irrigation type were separated into two subgroups, and the first subgroup was inoculated with *Azospirillum brasilense*, applying 1 000 000 colony-forming units (CFU) per plant each month; by contrast, the second subgroup was not inoculated.

The bacteria were obtained from the biological fertilizer AzoFer Plus®. The experiment was organized according to a completely randomized design with a 3 × 3 × 2 factorial arrangement. At

20 months after the experiment, the following traits were evaluated in six plants of each treatment: number of leaves unfolded (NH), stem height (SH, cm), root length (RL, cm), length and width of the largest leaf (LH, AH, cm), rosette diameter (RD, cm) and stem diameter (SD, mm).

A Steren® digital vernier was used for measurements. Subsequently, the leaves, stem (piña) and roots were separated; root volume (RV, ml) and stem volume (PV, ml) were determined by immersion in a known volume of water in a 2 000 ml graduated cylinder, and the volume of water displaced was recorded. Leaf fresh weight (LFW, g), stem fresh weight (PFW, g) and root fresh weight (RFW, g) were determined using a Torrey Pcr-20® digital scale; subsequently, the vegetative parts of the plant (leaves, stem and root) were individually placed in paper bags and dehydrated over four days in a Memmert drying oven, model 100-800, at a temperature of 75 °C until a constant weight was obtained, and the dry weights of leaves, stem and root (LDW, SDW and RDW, g) were recorded.

To determine the nutrient content, five dried leaves were taken from the center of the rosette and pulverized in a Surtek® manual mill; the analysis was carried out in the Plant Nutrition Laboratory of the Postgraduate Program in Edaphology of the College of Postgraduates, Montecillo Campus, State of Mexico. N was determined following the method of Alcántar and Sandoval (1999) using the Kjeldahl procedure. The concentrations of P, K, Ca, Mg, Fe, Cu, B and Na were determined using ICP-OES spectrometry equipment.

The homogeneity of variances and the normality of the data were verified using the Shapiro-Wilk and Bartlett tests ($\alpha= 0.05$), respectively. Data that did not meet these assumptions were transformed to e^x for analysis of variance. For the test of means (Tukey, 0.05), the untransformed data were used. The statistical analyses were performed using SAS (SAS Institute, 2011).

Results and discussion

Analyses of variance (Table 1) indicate that the morphological characteristics and nutrient contents in leaf tissues of *A. angustifolia* plants were influenced by the initial plant size, the amount of nutrient supply in fertigation, and the inoculation with *A. brasilense* they received for 20 months.

Table 1. Analysis of variance of morphological characteristics of *Agave angustifolia* vitroplants that were fertigated for 20 months in a nursery with different concentrations of nutrients and inoculated with *Azospirillum brasilense*.

SV	DF	SD	NS	HEI	NL	RL	ADW	RDW	LA
TDZ	2	2.7**	5.1**	4.99**	7.21**	18.56**	1.03*	1.883**	0.06ns
BAP	2	0.2ns	0.2ns	0.01ns	0.13ns	0.06*	0.02ns	0.014ns	0.01ns
pH	2	0.4*	0.1ns	0.01ns	0.07ns	0.01ns	0.05ns	0.004ns	0.06ns
TDZxBAP	4	0.1ns	0.1ns	0.03*	0.39*	0.04*	0.08ns	0.013ns	0.14ns
TDZxpH	4	0.2ns	0.1ns	0.04*	0.32*	0.01ns	0.04ns	0.004ns	0.14ns
BAPxpH	4	0.1ns	0.2*	0.03*	0.08ns	0.01ns	0.15ns	0.025ns	0.19ns
TDZxBAPxpH	8	0.1ns	0.1ns	0.01ns	0.13ns	0.01ns	0.03ns	0.025*	0.04*
Error	297	0.1	0.1	0.01	0.08	0.01			

SV= sources of variation; DF= degrees of freedom; NL= number of leaves; PH= plant height; RD= rosette diameter; LL= leaf length; SD= stem diameter; RL= root length; ADW= aerial dry weight (leaves + stem); RDW= root dry weight; LA= leaf area; *= significant F-values ($p \leq 0.05$); **= highly significant F-values ($p \leq 0.01$); ns= non-significant F-values ($p > 0.05$); π = transformed variables.

For the plant size factor, the evidence shows that plants that were initially large had the highest values for the size of the aerial part (stem and leaves) and roots, surpassing the plants of medium and small initial sizes by 5 and 15%, respectively (Table 2). Plants with a small initial size continued to be the smallest. For the fertilization factor, the plants that received the lowest nutrient supply were the smallest across all the variables evaluated, and the plants reached larger sizes as they received greater nutrient supply.

Table 2. Morphological characteristics of plants as a function of levels of main factors.

Variable	Plant size			Fertilization			Inoculation	
	Small	Medium	Large	0	50	100	Without	With
NL	46 c	48.1 b	49.8 a	43.6 c	48.5 b	51.8 a	46.9 b	49.1 a
PH (cm)	8.4 c	9 b	9.8 a	7.9 c	8.9 b	10.5 a	9 b	9.2 a
RD (mm)	76.9 b	76.9 b	80.2 a	68.2 c	76.3 b	85.8 a	75.7 b	77.8 a
LL (cm)	61.2 c	63.7 b	66.2 a	58.3 c	63.2 b	66.7 a	63.2 b	64.2 a
SD (cm)	7 c	7.6 b	8.3 a	5.8 c	7.5 b	9.6 a	7.5 b	7.7 a
RL (cm)	31.4 c	35.2 b	38.4 a	29.5 c	34 b	41.5 a	32.8 b	37.2 a
RV (cm ³)	232 c	265.4 b	305.4 a	182.7 c	249.3 b	370.8 a	251.5 b	283.8 a
PV (cm ³)	131 c	143.6 b	154.2 a	101.4 c	142.4 b	185 a	133.8 b	152.1 a
PDW (g)	41 c	48 b	54 a	35 c	47 b	61 a	46 b	49 a
RDW (g)	90 c	110 b	120 a	80 c	110 b	140 a	100 b	120 a
LDW (g)	440 a	490 a	520 a	430 b	470 b	550 a	480 a	490 a

NL= number of leaves; PH= plant height; RD= rosette diameter; LL= leaf length; SD= stem diameter; RL= root length; RV= root volume; PV= pineapple volume; PDW= pineapple dry weight; RDW= root dry weight; LDW= leaf dry weight. Values with the same letter within each column and by factor are not significantly different (Tukey, $\alpha= 0.05$).

The plants that received nutrient solution at 100% concentration of nutrients, and the plants irrigated only with water had 51 and 43 leaves, a rosette diameter of 85.8 and 68.2 cm, a leaf length of 66 and 58 cm, a stem diameter of 9.6 and 5.8 cm, and dry weights of stem (pineapple), root and leaves of 61 and 35 g, 140 and 80 g, 550 and 430 g, respectively, magnitudes that were significantly different (Tukey, 0.05) in each case. The plants inoculated with *Azospirillum brasilense* reached a larger size and greater dry weights than non-inoculated plants in most of the evaluated stem, leaf and root variables (Table 2).

The supply of nutrients in quantity and during critical periods is an important factor, among others: water supply, temperature range, lighting (intensity and photoperiod), which influence the magnitude of plant growth (Muñoz-Flores *et al.*, 2015), that of having less than optimal supply limits growth and productivity. Pérez-Santiago *et al.* (2014) in *Agave americana*, described that there is a positive relationship between the magnitude of vegetative growth and nutrient supply to plants during the nursery stage and this relationship determines the quality, vigor in growth and morphology of plants.

In the present work, fertigated plants developed more leaves and were, on average, larger than those developed by non-fertigated plants. This could be reflected in differences in photosynthetic capacity (Jarquín-Rosales *et al.*, 2023). Cruz-García *et al.*, (2019) demonstrated the positive effect of nutrient supply on *Agave americana* var. *Oaxacensis* plants fertigated with 100% nutrient solution in a nursery, which exhibited greater vegetative growth compared to non-fertigated plants.

Data reported by Ríos-Ramírez *et al.* (2021) show the positive effect of nutrient supply, since *A. angustifolia* plants that during seven months in a nursery were fertigated with nutrient solution and plants irrigated only with water had 453 and 282 g of leaf dry matter, 103 and 75 g of root dry matter and 14 957 and 7 225 ppm N and 6 719 and 3 017 ppm P in their leaves. The results obtained from this evaluation are consistent with those reported by Enríquez-del Valle *et al.* (2018), that highlight that, in various crop species, plants that receive adequate nutritional supply, mainly nitrogen, increase their photosynthetic activity and the magnitude of biomass accumulation.

Likewise, Yescas-Arreola *et al.* (2016) reported that *A. americana* var. *Oaxacensis* plants that in nursery were fertigated with SN-100% solution, developed a greater quantity and size of leaves, in comparison with plants irrigated only with water. The leaves of the plants in the various treatments reached sizes, measured in length and width of the largest leaf, appropriate for the subsequent stage which is establishment in the field. In addition, plants that received a higher nutritional supply had better morphological characteristics.

Nutritional contents in leaves

The analyses of variance (Table 3), indicates that, the nutritional content of the leaf tissues of *A. angustifolia* plants showed differences attributable to the treatments. As the nutrient supply was increased by fertigation, the plants had a higher concentration of N, P, K, as well as Ca, Cu, B and Na in their leaf tissues, since the plants fertigated with 100% SN and the plants irrigated only with water had 6 987 and 6 291 ppm N, 1 775 and 1 599 ppm P, 3 093 and 4 170 ppm K and 3 075 and 2 257 ppm Ca in their leaves, amounts that were significantly (Tukey, 0.05) different (Table 4).

Table 3. Analysis of variance of nutrient contents in leaf tissues of *Agave angustifolia* plants that were fertigated for 20 months in a nursery with different nutrient concentrations and inoculated with *Azospirillum brasilense*.

SV	Siz	Fer	Inoc	SxF	SxI	FxI	SxFxI	Error
N [#]	0.014**	0.004**	0.001ns	**	**	**	**	0.0003
P [#]	2.8ns	45.1**	15.7ns	**	**	**	**	5.94
K	54101.1ns	7572836.3**	708819.7ns	**	**	*	**	184658.8
Ca	21239313ns	1263723687**	91220425ns	**	ns	ns	**	14824338
Mg	835084.6**	2841411.7**	508857.2**	**	**	**	**	140817.9
Fe [#]	133.83**	336.89**	310.25**	**	**	**	**	2.32
Cu [#]	0.34*	1.03**	0.34ns	**	**	ns	**	0.1
B [#]	3.48**	9.41**	2.51**	**	**	**	**	0.08
Na [#]	37.42**	117.07**	10.66ns	**	ns	*	**	4.81

SV= sources of variation; Siz= size; Fer= fertilization; Inoc= inoculation; SxF= size × fertilization interaction; SxI= size × inoculation interaction; FxI= fertilization × inoculation interaction; SxFxI= size × fertilization × inoculation interaction; T= total; DF= degrees of freedom; N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; Fe= iron; Cu= copper; B= boron; Na= sodium; π= transformed variables; ns= non-significant F-values (p > 0.05); * = significant F-values (p ≤ 0.05) and, ** = highly significant F-values (p ≤ 0.01).

Table 4. Nutrient contents (ppm) in leaves of *Agave angustifolia* Haw plants as a function of levels of main factors.

Var	Plant size			Fertilization			Inoculation	
	Small	Medium	Large	0	50	100	Without	With
N	6 162.5 b	6 270.8 b	7 320.8 a	6 291.7 b	6 475 b	6 987.5 a	6 486.1 a	6 683.3 a
P	1 591 a	1 635 a	1 665 a	1 517 b	1 599 b	1 775 a	1 595 a	1 665 a
K	3 668 a	3 748 a	3 752 a	3 093 b	3 905 a	4 170 a	3 623 a	3 822 a
Ca	2 919 a	3 002 a	3 107 a	2 257 c	3 067 b	3 705 a	2 897 b	3 122 a
Mg	3 874 b	4 192 a	4 202 a	3 707 b	4 187 a	4 374 a	4 005 a	4 173 a
Fe	734 b	730 b	1 036 a	965 a	993 a	544 b	701 b	966 a
Cu	12.9 b	12.65 b	14.49 a	11.45 b	14.18 a	14.43 a	12.75 b	13.94 a
B	14.83 c	16.71 b	21.31 a	13.37 c	15.02 b	24.46 a	15.92 b	19.31 a
Na	280.6 b	287.7 b	352.3 a	232.5 c	306.3 b	381.9 a	292 a	321 a

Var= variable; N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; Fe= iron; Cu= copper; B= boron; Na= sodium. Values with the same letter within each column and by factor are not significantly different (Tukey, α= 0.05).

For the plant size factor, the highest concentrations of nitrogen, iron, copper and boron were recorded in plants of large initial size, which differs from plants of smaller sizes. The nutritional concentrations of P, K and Ca were statistically similar in the three plant sizes. In this regard, Cruz-García *et al.* (2019) indicate that, a higher N content present in the leaves, agave plants develop larger and heavier stems (pineapple).

The inoculated plants did not show differences (p # 0.05) in nutrient concentrations for nitrogen, phosphorus, potassium and sodium; however, they did have significantly more Ca, Fe, Cu and B (Tukey, 0.05) than the non-inoculated plants (Table 4). Jarquín-Rosales *et al.* (2023) reported

that *A. angustifolia* plants obtained from inflorescence bulbils and established for eight weeks in a nursery and they reported that some plants inoculated with the bacterium *Azospirillum brasilense* had significantly higher values in size, number of leaves, stem diameter, rosette diameter, leaf length, root volume, pineapple volume and fresh root weight than non-inoculated plants.

Conclusions

Micropropagated *Agave angustifolia* plants, classified into three initial size categories, that were established for 20 months in a nursery and received nutritional supply through fertigation at 100% concentration and inoculation of *Azospirillum brasilense* reached higher values in height, stem diameter, number of leaves, leaf length and dry weights of stem, root and leaves and accumulated higher concentrations of N, P, K, Ca, Mg, Cu and B in their leaf tissues than non-fertigated or non-inoculated plants. The plants that had a larger initial size continued to be larger than those with medium and small initial sizes. All plants in all the treatments were the appropriate size for establishment in the field.

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