Ciência Rural, Santa Maria, v.53:3, e20210863, 2023
http://doi.org/10.1590/0103-8478cr20210863
ISSNe 1678-4596
BIOLOGY

Agave angustifolia bulbil growth in different substrates, with doses of fertigation and inoculation with *Azospirillum brasilense*

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ABSTRACT: *There is little information about the production of agave plants in the nursery, so this research assessed the growth in the nursery of Agave angustifolia plants originating from inflorescence bulbils, subjected to different substrates, fertigation doses, and inoculation with Azospirillum brasilense. The bulbils were established for two months in a 50% peat-50% perlite substrate. One hundred and eighty plants were individually transferred to 3.8 dm³ pots for evaluation in an experiment with three factors: 1) type of irrigation: 1.1) water, or 1.2) fertigation with Steiner's nutrient solution (NS), NS-50%, or 1.3) NS-100%; 2) inoculation with Azospirillum brasilense, 2.1) inoculated plants, 2.2) non-inoculated plants; 3) substrate, mixtures of bovine manure (BM) + sand (S) in different proportions, Mix1: 75% BM + 25% S; Mix2: 25% BM + 75% S; Mix3: 50% BM + 50% S. Morphological characteristics were quantified for one year. The largest plants were those that were in substrates with 50% BM, and were fertirrigated with NS-100% and inoculated with Azospirillum brasiliense. The smallest plants were those established a in a substrate with the lowest manure content, irrigated with only water and without inoculation, which was respectively 71.6 and 54.10 cm high, 21 and 15.6 leaves, 76.6 and 62.2 mm in stem diameter, 84.4 and 59.8 cm in rosette diameter.*
Key words: *agave, Azospirillum brasilense, fertigation, growth, substrate mixtures.*

Crescimento de bulbos de *Agave angustifolia* em diferentes substratos, com doses de fertirrigação e inoculação com *Azospirillum brasilense*

RESUMO: *Existem poucas informações sobre a produção de agave em viveiro, portanto o objetivo deste trabalho foi avaliar o crescimento em viveiro de Agave angustifolia provenientes de bulbilhos de inflorescência, submetidos a diferentes substratos, doses de fertirrigação e inoculação com Azospirillum brasilense. Os bulbilhos foram estabelecidos por dois meses em substrato 50% turfa-50% perlita. Cento e oitenta plantas foram transferidas individualmente para vasos de 3,8 dm3 para avaliação em um experimento com três fatores: 1) tipo de irrigação: 1.1) água ou 1.2) fertirrigação com solução nutritiva de Steiner (NS), NS-50% ou 1.3) NS-100%; 2) inoculação com Azospirillum brasilense, 2.1) plantas inoculadas, 2.2) plantas não inoculadas; 3) substrato, misturas de esterco bovino (BM) + areia (S) em diferentes proporções, Mix1: 75% BM + 25% S; Mix2: 25% BM + 75% S; Mix3: 50% BM + 50% S. As características morfológicas foram quantificadas por um ano. As maiores plantas foram aquelas que estavam em substrato com 50% de BM, foram fertirrigadas com NS-100% e inoculadas com Azospirillum brasiliense, enquanto as menores foram aquelas estabelecidas em substrato com menor quantidade de esterco, irrigado apenas com água e sem inoculação, que tinham respectivamente, 71,6 e 54,10 cm de altura, 21 e 15,6 folhas, 76,6 e 62,2 mm de diâmetro do caule, 84,4 e 59,8 cm de diâmetro de roseta.*
Palavras-chave: *agave, Azospirillum brasilense, crescimento, fertirrigação, misturas de substratos.*

INTRODUCTION

Agave angustifolia is a crassulaceous acid metabolism species adapted to shallow soils, slight or pronounced slopes, and arid and semiarid climates (NAVA-CRUZ et al., 2014). This species can reproduce asexually through rhizome shoots or bulbils from inflorescences. A plant can produce 6 to 8 rhizome shoots in its lifetime (MACOSSAY & CASTILLO, 1986). In the case of bulbils, it has been reported that an *Agave angustifolia* Haw. the plant produces 500 to 1,735 bulbils, highly heterogeneous in size, ranging from 1 to 15 cm. Although, the rhizome shoots are used more often to

Received 12.07.21 Approved 03.21.22 Returned by the author 06.05.22
CR-2021-0863.R1
Editors: Leandro Souza da Silva Alessandro Dal'Col Lúcio

establish definitive *Agave angustifolia* plantations, bulbils selected by size categories have advantages: all the small plants have the same physiological age. Classified by size, plantations from bulbils are more uniform, and bulbils develop roots more rapidly than rhizome shoots (ENRÍQUEZ-DEL VALLE, 2008). In the state of Oaxaca, México, *Agave angustifolia* is highly important economically because it is a raw material for the production of mezcal and is widely cultivated in the districts of Sola de Vega, Miahuatlán, Yautepec, Ocotlán, Ejutla, Zimatlán and Tlacolula, which together are called the mezcal region. From 2006 to 2013, an average of 13, 572 ha were planted with agaves, of this area 70% was *Agave angustifolia*, at a rate of two thousand plants ha^{-1}. Considering that a crop requires seven to nine years to reach harvest, it is necessary to produce 2.6 million plants to replenish the harvest area every year (RÍOS-RAMÍREZ et al., 2017). Only eight nurseries are reported in the state. The most of plants established in plantations are propagated by the farmers themself, and specialized production of plants from rhizome shoots is incipient or non-existent. Although, the nursery is part of the agave-mezcal chain, this activity is scarce in the state (PALMA et al., 2016). In the nursery, water and essential nutrients are necessary for optimal plant growth, currently, there are techniques such as fertigation that allow the efficient use of water and fertilizers, because it allows constant application in the necessary quantities in each stage of growing (CADAHIA, 2000; URRESTARAZU, 2004; VALERA et al., 2014). It has been reported that in nurseries nutrient supply to *A. angustifolia, A. potatorum,* and *A. americana* var. oaxacensis plants is an important factor, affecting growth in terms of both height and accumulation of biomass, leaf area, and number of extended leaves (ENRÍQUEZ-DEL VALLE et al., 2013; ENRÍQUEZ-DEL VALLE et al., 2016). The substrate is a very important factor in the production of seedlings (ABAD, 1995), they must have physical and chemical characteristics that allow greater water retention, nutrient absorption, root development; and consequently, have an effect on seedlings quality for transplanting (ABAD et al., 2004; ANSORENA, 1994; CADAHIA, 2000). Some organic materials such as bovine manure are used in substrates that provide some essential nutrients for plants (ÁLVAREZ-SÁNCHEZ et al., 2006; COSTA et al., 2015; SHAJI, et al., 2021). However, its use must be in combination with other materials that allow its structure to be improved by increasing permeability, cation exchange capacity, aggregate stability and decreasing bulk density (ABAD, 2004). Sand is an option to mix with bovine manure, which is a material that can be obtained easily and at low cost, for this reason it is feasible to use it to improve its characteristics. In micropropagated agave plants, it has been demonstrated that the type of substrate in the nursery acclimatization stage is important for good development of the plants (ENRÍQUEZ-DEL VALLE et al., 2009; ENRÍQUEZ-DEL VALLE et al., 2018). Like nutrient supply and substrate type, the microorganisms associated with the rhizosphere are an important factor for plant growth (ORTIZ-CASTRO, 2013; ORTIZ-CASTRO & LÓPEZ-BUCIO, 2013). Conversely, there are microorganisms that are reported in the rhizosphere of plants and among them, there are some bacteria that provide beneficial effects for the plant, which are called Plant Growth Promoting Rhizobacteria (AHEMAD et al., 2014; MEHNAZ, 2015; MA et al., 2018). The genera *Pseudomonas, Bacillus, Azotobacter,* and *Azospirillum* are some of the most studied rhizobacteria (MEHNAZ, 2015). The main mechanisms by which *Azospirillum* improves plant growth is by colonizing the roots, and producing phytohormones, mainly indole acetic acid, as well as nitrogen fixation (NGUYEN et al., 2019). There is research that demonstrated the benefits of this bacterium. Young bitter mesquite trees (*Prosopis spp*) 210 days after inoculation in the nursery with *Azospirillum brasilense*, developed 0.4 and 1.2 g root and shoots dry weight, and leaves had 400 µg more chlorophyll *a*, and 120 µg chlorophyll *b* g^{-1} of fresh leaf weight, relative to non-inoculated trees, which had 0.2 and 0.9 g root and shoot dry weight, respectively (GONZALEZ et al., 2018). Also, maize plants inoculated with this bacterium achieved greater length and total root volume, shoot dry matter, root dry matter, and total biomass, as well as a higher content of total soluble proteins than non-inoculated plants (ZEFFA et al., 2019).

For these reasons, this research was to evaluate the growth of plants obtained from inflorescence bulbils, which under nursery conditions were subjected to various doses of fertigation, substrate and inoculation with *Azospirillum brasilense*.

MATERIALS AND METHODS

Plant material

Bulbils from *Agave angustifolia* inflorescences were collected in November 2018 in the municipality of Zaachila (16° 96'01.196" N, 96° 75'79.45" W, 1,518 m altitude), Oaxaca, México. The bulbils were transported to the laboratory of the Instituto Tecnológico del Valle de Oaxaca (17° 02' N, 96° 44' W and altitude 1,530 m), in Santa Cruz Xoxocotlán.

Bulbil development

The bulbils were taken to the nursery and established in polystyrene foam trays 26.5 cm wide, 53 cm long and 6 cm high, divided into 50 conical cavities, which had an upper diameter of 4.5 cm, a lower diameter of 3 cm and a volume of 90 cm^3. The substrate was a mixture of peat moss and perlite in a 1:1 proportion. The trays with bulbils were placed inside an 8 m wide, 12 m long and 3.3 m high metal structure with white translucid polyethylene cover. Prevailing temperatures were 9 °C at night and 26.6 °C during the day. Water-only irrigation was provided once every two days for two months.

Of the total number of plants, 180 of these exhibiting uniform size and no physical damage were selected. These were transplanted individually to high-density polyethylene pots 17 cm in diameter, 17 cm high, and 1,858.67 cm^3, which contained one of three substrate mixtures (Mix) of bovine manure (BM) and sand (S) in different proportions: Mix1: 75% BM + 25% S, Mix2: 25% BM + 75% S, and Mix3: 50% BM + 50% S. Samples of each substrate were taken to analyze their physical and chemical characteristics. All the plants were placed inside a metal structure greenhouse with a white translucid polyethylene cover. A total of 60 plants in each type of substrate were separated into three groups for the application of different types of irrigation. Irrigation type 1) was potable water only, and types 2 and 3 were STEINER (1984) nutritive solution (NS) at different dilutions: 2) NS-50% and 3) NS-100%). Steiner nutritive solution at 100% contains (mg L^{-1}) 166.42 N, 30.68 P, 276.44 K, 182.34 Ca, 49.09 Mg, 111.15 S, 1.25 Fe, 0.21 Mn, 0.025 Zn, 0.076 B, 0.005 Cu and 0.021 Mo. Irrigation was applied once a week for one year. Each group of plants that received an irrigation type in each substrate was separated into two subgroups to evaluate the effect of the inoculations with *Azospirillum brasilense* bacteria: 1) non-inoculated plants and 2) plants inoculated monthly with 1,000,000 colony-forming units (CFU) per plant. The bacteria were obtained in the presentation MaxiFer® from the company Biofabrica siglo XXI S.A. de C.V.

The experiment was established according to a completely randomized design with a 2×3×3 factorial arrangement of treatments, where the factor substrate had three levels, the factor irrigation type had three levels, and the factor inoculation with bacteria had two levels, making a total of 18 treatments with ten replicates. The experimental unit was one plant. At the beginning of the experiment and then every two months during the year, the following variables were measured in 10 plants of each treatment: the number of extended leaves, plant height (cm), rosette diameter (cm), length and width of the longest leaf (cm, with a metal Arly® ruler), and stem diameter (mm, with a Titan® digital vernier). With the data of each variable, at the beginning and end of the experiment, relative growth rates (RGR) were calculated in height (RGRH), the number of leaves (RGRNL), stem diameter (RGRSD), rosette diameter (RGRRD), length of the largest leaf (RGRLLL), and width of the largest leaf (RGRWLL), using the formula $RGR = (Final\ size - initial\ size)/365$.

Destructive sampling

After 12 months, four plants per treatment were harvested. The leaves, stem without leaves (piña) and the roots of these plants were separated and weighed on an analytical scale (Shimadzu model AY224) with a 500 g capacity and precision of 0.1 mg. Root, piña, and leaf volumes (cm^3) were determined by immersion in a test tube with a known volume of water. The leaf area was determined with an HP Scanjet 4890 scanner and the software ImageJ. The roots, piña, and leaves of each plant were placed separately in paper bags and dried at 70 °C for 72 h in a convection oven (Memmert®, model 100-800). After drying, root, piña and leaves, dry weight was quantified on the analytical scale.

Statistical analyses

Variance homogeneity and normality of the data were verified with the Bartlett and Shapiro-Wilk tests, respectively. The data that did not comply with these assumptions were those of the length of the largest leaf, were transformed with an exponential and later subjected to an analysis of variance. For comparison of means (Tukey, 0.05), the data were used without transformation. In all cases for the statistical analysis, the Statistical Analysis System (SAS, 2004) software was used.

RESULTS AND DISCUSSION

At the beginning of the experiments, the plants had an average height of 12.31 cm, an average of 6.8 extended leaves, average stem diameter of 22.5 mm, and the largest leaf was on average 10.67 cm long and 2.26 cm wide. During the entire period of evaluation in all the treatments the plants had active growth, but at different relative growth rates by the effect of the conditions of the substrate, nutrient supply through irrigation, and inoculation. The amount of organic fertilizer incorporated into the substrate had a direct relationship with number of nutrients of the substrate (Table 1), this result agrees with SHAJI et al. (2021). The substrate with 75% bovine manure + 25% sand (Mix1) had a higher quantity of macro and micronutrients and greater electric conductivity (15 dS m^{-1}) than the substrate with less bovine manure (Mix2). The quantity of nutrients in the substrate had a direct relationship with the relative growth rates and the final plant size. The application of organic fertilizer increases the bacterial population of the soil that helps to mineralize the soil nutrients and facilitates their absorption by the plant, causing greater growth and yield of the plants (NGUYEN et al., 2019; SHAJI et al. 2021). The analyses of variance (Tables 2 and 3) showed that the levels of the factor irrigation type had significantly different effects ($P \leq 0.001$) on height, number of leaves, rosette diameter, the width of the largest leaf, the relative growth rate in height (RGRH), relative growth rate of rosette diameter (RGRRD), leaf area, piña volume, leaf, and piña fresh weight and leaves dry weight. The additional growth of the plants due to the supply of nutrients in the irrigation is an indicator that the substrate did not provide enough nutrients for the plants to express their optimal growth.

The levels of the factor inoculation with *A. brasilense* had highly significantly different effects ($P \leq 0.001$) on the number of leaves, width of the largest leaf, RGRH, RGRRD, and significant different effects ($P \leq 0.05$) on the relative growth rate the of number of leaves (RGRNL), leaf area, leaves volume, piña volume, and root dry weight. These increases in the different evaluated variables are due to the benefits of *A. brasilense* such as the ability to fix nitrogen and the production of indoleacetic acid, which promote greater plant growth (NGYEN, et al., 2019).

The levels of factor substrate (Mix) had significant different effects ($P \leq 0.001$) on height, rosette diameter, width of the largest leaf, RGRH, RGRRD, and significant different effects ($P \leq 0.05$) on the variables number of leaves, stem diameter, length, and width of the largest leaf, RGRL, leaves volume, piña volume, leaves, and piña fresh weight, leaves and piña dry weight. These increases may be due to the differences in the physicochemical characteristics that the different mixtures of substrates had, due to the content of bovine manure that modified the nutritional content and electric conductivity of the different mixtures evaluated (Table 1). The interaction one (NSxAb) between the type of irrigation and inoculation with *Azospirillum brasilense (Ab)*, had significant effects ($P \leq 0.05$) on the variables number of leaves, stem diameter, RGRL, and root dry weight. The interaction two (NSxMix) between irrigation type and substrate type had highly significant effects ($P \leq 0.001$) on plant height, rosette diameter, width of the largest leaf, RGRH and RGRRD, leaves, dry weight, leaf area, and leaf volume, and significant effect ($P \leq 0.05$) on piña fresh weight, leaf dry weight, piña

Table 1 - Physiochemical characteristics of the substrate mixtures. Oaxaca Mexico, 2018/19.

Substrate	N	P	K	Ca	Mg	Fe	Zn
	-----------------	-----------------	-----------------	mg kg^{-1}	-----------------	-----------------	-----------------
Mix1: 75% BM + 25% S	1738.00	41.64	4119.60	6329.50	1983.20	1.81	3.24
Mix2: 25% BM + 75% S	960.50	11.85	559.90	1906.10	429.30	1.75	1.47
Mix3: 50% BM + 50% S	1360.50	34.60	1816.60	3756.90	1108.80	1.20	2.85
	Cu mg kg^{-1}	Mn mg kg^{-1}	R C/N	EC dS m^{-1}	pH	OM %	OC %
Mix1: 75% BM + 25% S	0.39	4.10	11.59	15.00	8.12	3.48	2.02
Mix2: 25% BM + 75% S	0.22	2.54	11.59	8.20	8.08	1.92	1.11
Mix3: 50% BM + 50% S	0.22	2.75	11.59	10.10	7.40	2.72	1.58

Mix: substrate, BM: bovine manure, S: sand, N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Fe: iron, Zn: zinc, Cu: copper, Mn: manganese, R: ratio, C: carbon, EC: electrical conductivity, pH: potential hydrogen, OM: organic matter, OC: organic carbon.

Table 2 - Summary of 10 analyses of variance in size characteristics of *A. angustifolia* plants originating from bulbils that during 12 months in the nursery were established in different substrates, nutrient supply through irrigation and inoculation with *A. brasilense.* Oaxaca Mexico, 2018/19.

Sources of variation	DF	---Mean squares---				
		Height	Number of leaves	Stem diameter	Rosette diameter	Length of the largest leaf
Type of irrigation	2	690.822**	86.422**	55.858^{ns}	395.297**	8.162e55^{ns}
A. brasilense	1	86.632^{ns}	92.011**	106.711*	26.569^{ns}	2.184e55^{ns}
Mix	2	439.447**	13.678*	129.633*	448.172**	1.933e55*
NSxAb	2	10.134^{ns}	11.344*	153.286*	36.342^{ns}	2.092e+55^{ns}
NSxMix	4	804.515**	3.144^{ns}	114.667*	667.629**	8.087e+55*
AbxMix	2	47.864^{ns}	4.678^{ns}	48.211^{ns}	26.101^{ns}	2.263e+55^{ns}
NSxAbxMix	4	154.420^{ns}	4.111^{ns}	254.161**	56.687^{ns}	2.140e+55^{ns}
Error	162	14.209	2.078	19.061	22.340	2.896e+55
Total	179					

Source of variation	DF	---Mean squares (Relative growth rate)---				
		Width of the largest leaf	Height	Number of leaves	Stem diameter	Rosette diameter
Type of irrigation	2	2.374**	0.001522**	0.00017*	0.000055^{ns}	0.002177**
A. brasilense	1	0.004^{ns}	0.001115*	0.00082**	0.000753^{ns}	0.000031^{ns}
Mix	2	0.570*	0.001278**	0.00013*	0.001533*	0.003291**
NSxAb	2	0.052^{ns}	0.000120^{ns}	0.00009*	0.002519**	0.000059^{ns}
NSxMix	4	0.669**	0.001318**	0.00004^{ns}	0.000065*	0.005149**
AbxMix	2	0.097^{ns}	0.000176^{ns}	0.00002^{ns}	0.000038^{ns}	0.000354^{ns}
NSxAbxMix	4	0.087^{ns}	0.000509*	0.00009*	0.002409**	0.000355^{ns}
Error	162	0.058	0.000113	0.00002	0.000201	0.0001947
Total	179					

DF: degrees of freedom, Mix: substrate, NS: nutritive Solution, Ab: *A. brasilense*, ns: non-significant value of F ($P > 0.05$), *: significant F value ($P \leq 0.05$), **: highly significant F value ($P \leq 0.01$).

volume, RGRH, RGRSD, stem diameter and width of the largest leaf. The interaction three (AbxMix) between Ab and Mix only had significant effects ($P \leq 0.05$) on leaf volume, piña volume, and piña fresh weight. Interaction four (NSxAbxMix) between irrigation type, Ab and Mix had highly significant effects ($P \leq 0.001$) on RGRSD and significant effects ($P \leq 0.05$) on RGRH, RGRL, leaf area, and leaf dry weight. The significant effects of the various interactions on some plant growth characteristics showed that, in addition to the significant different effects of the levels of a particular independent variable, it is possible that variations in the magnitude of the response occur due to changes in the levels of another independent variable.

When data were ordered in function of nutrient supply in the irrigation water, plant size was positively related to the amount of nutrients they received in the irrigation water, in such a way that the plants fertigated with NS-100 were larger than the plants that received only water (Figure 1). In each of these cases, the values were significantly different (Tukey, 0.05) (Tables 4, 5 and 6). ENRÍQUEZ-DEL VALLE et al. (2016) reported similar results with micropropagated-acclimatized *Agave potatorum* plants that, for five months in a nursery, received different doses of nutrient supply. The plants that received 100% Steiner solution had 1.73 times more dry weight, three times more leaf area, and 1.73 times more leaves than plants fertilized with 1% Steiner solution. In our study, the *A. angustifolia* plants fertigated with NS-100% Steiner formulation had a growth rate in height of 4.25 cm month^{-1}. These

Table 3 - Summary of 10 analyses of variance of destructive sampling of *A. angustifolia* plants originating from bulbils that during 12 months in the nursery were established on different substrates, fertigation doses and inoculation with *A. brasilense*. Oaxaca Mexico, 2018/19.

Source of variation	DF	---Mean squares---				
		Leaf area	Root volume	Leaf volume	Piña volume	Root fresh weight
Type of irrigation	2	852078.24**	566.17ns	515923.64*	1950.72**	0.29ns
A. brasilense	1	988541.26*	1020.01ns	377580.50*	342.35ns	2532.35*
Mix	2	97331.02ns	669.19ns	224179.04*	633.09*	306.79ns
NSxAb	2	99951.14ns	2970.06ns	40907.79ns	137.06ns	869.01ns
NSxMix	4	936716.80**	3024.58ns	258698.10**	468.70*	470.15ns
AbxMix	2	64576.75ns	62.35ns	1264497.38*	1020.18*	50.04ns
NSxAbxMix	4	320254.98*	3608.89ns	73045.60ns	274.33ns	1702.12ns
Error	123	76925.39	1339.21	37108.05	154.92	548.71
Total	143					
Source of variation	DF	---Mean squares---				
		Leaf fresh weight	Piña fresh weight	Root dry weight	Leaf dry weight	Piña dry weight
Type of irrigation	2	430946.06**	1842.26**	553.55*	9303.30**	77.06ns
A.brasilense	1	368797.35*	242.00ns	815.07*	1326.55ns	87.21ns
Mix	2	169705.43*	1233.60*	46.75ns	2594.093*	154.73*
NSxAb	2	38080.39ns	37.04ns	489.85*	569.14ns	38.547ns
NSxMix	4	210976.24**	491.81*	125.71ns	2364.65*	29.21ns
AbxMix	2	70035.60ns	534.13*	115.14ns	408.9ns	43.93ns
NSxAbxMix	4	70956.08ns	143.92ns	140.82ns	1388.52*	58.01ns
Error	123	28045.87	159.88	108.35	400.29	28.31
Total	143					

DF: degree of freedom, Mix: substrate, NS: nutritive Solution, Ab: *A. brasilense*, ns: non-significant value of F (P > 0.05), *: significant F value (P ≤ 0.05), **: highly significant F value (P ≤ 0.01).

data coincide with those of ZÚÑIGA-ESTRADA et al. (2018), who reported that *Agave tequilana* plants showed more growth, in the field when they received deep fertilization and fertigation. At the end of 77 months, the plants reached a height of 153.3 cm and had a larger number of leaves, more dry matter, and total soluble solids than the control plants, which grew to 129 cm. YESCAS-ARREOLA et al. (2016) reported that *Agave americana* var oaxacensis nursery-grown plants that were fertigated with NS-100% developed a greater number of leaves and larger than other plants irrigated with only water.

When the data were ordered in function of the factor inoculation with *Azospirillum brasilense*, the inoculated plants grew more than the non-inoculated plants. In most of the characteristics, a promoting effect, but only significant (Tukey, 0.05), of inoculation is observed on the formation of new leaves, the relative growth rate in height, and RGR of new leaves (Tables 4, 5, and 6). BUSTOS-BARRERA et al. (2017) reported that *Solanum lycopersicum* L. plants inoculated with bacteria of the genus *Azospirillum* produced 12 kg m^{-2} of fruits, which was 67.3% higher than the yield of non-inoculated plants. Moreover, ZEFFA et al. (2019) reported that maize plants inoculated with *Azospirillum brasilense* showed better root growth, higher concentration of total soluble proteins, more efficient nitrogen use, and higher yield than non-inoculated plants.

When the data were ordered in the function of levels of substrate mix, we observed that the amount of manure was an important

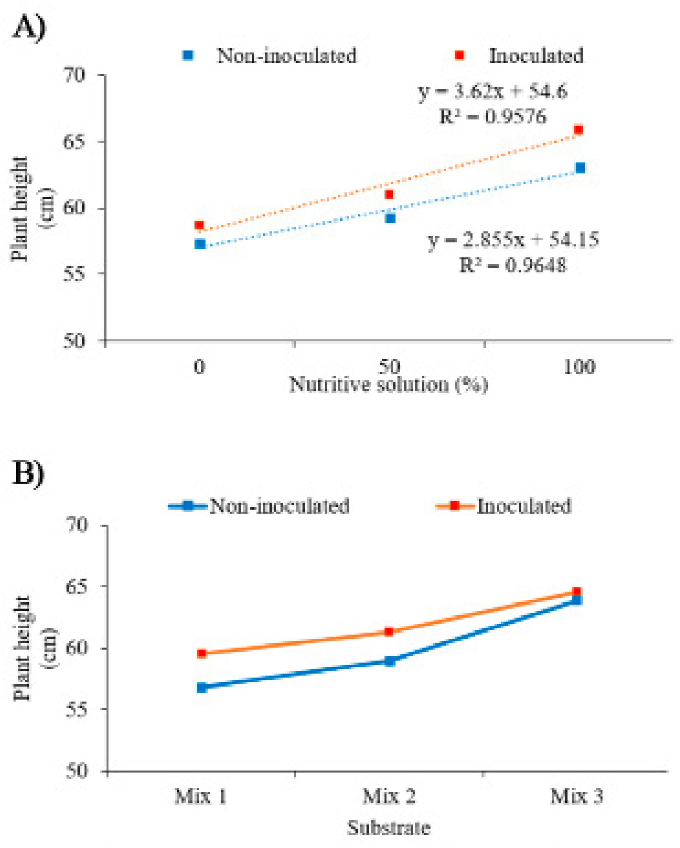

Figure 1 – Plant height of A. angustifolia plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with A. brasilense. Oaxaca Mexico, 2018/19. Mix1: 75% BM + 25% S, Mix2: 25% BM + 75% S, Mix3: 50% BM + 50% S.

condition that affected the magnitude of plant growth. The plants established in substrate with 50% sand and 50% bovine manure (Mix3) reached a larger size than plants established in the substrate with 25% BM and 75% sand (Mix2) (Figure 1B). The plants in the substrate with 50% manure tended to have significantly larger values in most of the characteristics (Tukey, 0.05): in leaf and piña volume, leaf fresh weight, piña fresh weight, leaf dry weight, piña dry weight (Tables 4, 5, and 6). *Agave angustifolia* plants grown from inflorescence bulbils, established for 12 months in a nursery with the mixture that contained 50% sand + 50% bovine manure, fertigated with NS-100%, and inoculated, were outstanding compared with plants that were established in substrate with 25% bovine manure and 75% sand, irrigated with water and non-inoculated (Table 7).

Diverse studies have shown the importance of substrate characteristics. For example, Dutch-type cucumbers (*Cucumis sativus* L.) established in a mixture of coconut fiber + 20% commercial organic fertilizer were larger and yielded more fruits (15.57 kg m^{-2}) than plants grown in a substrate with 70% palm fiber + 30% sawdust, which produced 10.37 kg m^{-2} (MENESES-FERNANDEZ et al., 2018). Also, *Agave americana* var. Oaxaquensis plants growing in the nursery and established in a substrate of 66% peat moss and 33.3%

Table 4 - Characteristics of *A. angustifolia* plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with *A. brasilense.* Oaxaca Mexico, 2018/19.

Factor	Height (cm)	Number of Leaves	Stem Diameter (mm)	Rosette Diameter (cm)	Length of largest leaf (cm)
--------Type of irrigation--------					
H_2O	57.72^c	16.33^c	63.35^b	65.45^b	50.50^b
NS-50	60.36^b	17.50^b	64.48a^b	67.95^b	52.31a
NS-100	64.46a	18.73^a	66.06^a	72.60^a	56.48^a
--------Inoculation--------					
Non-inoculated	59.86^b	16.51^b	63.54^b	69.21^a	52.55^b
Inoculated	61.83^a	18.53^a	65.72^a	68.12^a	54.98^a
--------Substrate--------					
Mix1: 75% BM + 25% S	59.33^b	18.07^a	65.80^a	67.11^b	51.38^b
Mix2: 25% BM + 75% S	59.23^b	16.77^b	62.23^b	65.82^b	52.91^b
Mix3: 50% BM + 50% S	63.97^a	17.73^a	65.87^a	73.06^a	56.98^a

NS: nutritive solution, Mix: substrate, BM: bovine manure, S: sand. In each column, at levels of each factor, values with the same letter are not significantly different (Tukey, 0.05).

sand and fertigated with NS-100% grew to a larger size than plants in substrates with a higher proportion of sand (33.3% peat moss + 66.6% sand) and were not fertigated (CRUZ- GARCÍA et al., 2019).

When analyzing the total combinations of factors and levels, the treatments with the highest amount of nutrients applied by fertigation (50 and 100%) had the highest values in the plant growth

Table 5 - Growth characteristics of *A. angustifolia* plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with *A. brasilense*. Oaxaca Mexico, 2018/19.

Factor	Width of the largest leaf (cm)	--------Relative growth rate--------			
		Height (cm day^{-1})	Number of leaves (NL day^{-1})	Stem diameter (mm day^{-1})	Rosette diameter (cm day^{-1})
--------Type of irrigation--------					
H_2O	4.71^c	0.12^b	0.028^b	0.12^a	0.15^b
NS-50	4.95^b	0.13^a	0.031^a	0.12^a	0.16^b
NS-100	5.27^a	0.14^a	0.033^a	0.12^a	0.17^a
--------Inoculation--------					
Non-inoculated	4.98^a	0.13^b	0.027^b	0.11^a	0.16^a
Inoculated	4.97^a	0.14^a	0.033^a	0.12^a	0.16^a
--------Substrate--------					
Mix1: 75% BM + 25% S	4.91^b	0.13^b	0.033^a	0.12^a	0.16^b
Mix2: 25% BM + 75% S	4.89^b	0.13^b	0.028^b	0.11^b	0.15^b
Mix3: 50% BM + 50% S	5.14^a	0.14^a	0.031^a	0.12^a	0.17^a

NS: nutritive solution, Mix: substrate, BM: bovine manure, S: sand. In each column, at levels of each factor, values with the same letter are not significantly different (Tukey, 0.05).

Table 6 - Characteristics of the destructive sampling of *A. angustifolia* plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with *A. brasilense.* Oaxaca Mexico, 2018/19.

Factor	Leaf area (cm^2)	Volume (cm^3)			Fresh Weight Root (g)
		Root	leaf	Piña	
Type of irrigation					
H_2O	1750.6[b]	85.3[a]	891.7[b]	46.0[b]	68.3[a]
NS-50	1965.8[a]	81.9[a]	1062[a]	56.0[a]	68.5[a]
NS-100	2126.1[a]	75.7[a]	1183.5[a]	64.0[a]	68.5[b]
Inoculation					
Non-inoculated	1830.3[b]	77.2[a]	973.3[b]	53.2[a]	62.6[b]
Inoculated	2064.7[a]	84.7[a]	1118.2[a]	57.5[a]	74.4[a]
Substrate					
Mix1: 75% BM + 25% S	1967.3[a]	81.3[a]	1046.5[ab]	55.0[ab]	69.0[a]
Mix2: 25% BM + 75% S	1876.3[a]	86.0[a]	948.8[b]	50.4[b]	71.7[a]
Mix3: 50% BM + 50% S	1998.90[a]	75.5[a]	1142.0[a]	60.7[a]	64.7[a]
Factor	Fresh weight (g)		Dry Weight (g)		
	Leaf	Piña	Root	Leaf	Piña
Type of irrigation					
H_2O	804.2[c]	47.0[b]	26.0[b]	93.5[c]	14.2[a]
NS-50	949.6[b]	57.3[a]	26.8[b]	108.9[b]	14.7[a]
NS-100	1071.9[a]	64.4[a]	34.7[a]	132.6[a]	17.5[a]
Inoculation					
Non-inoculated	870.3[b]	54.4[a]	25.8[b]	107.4[a]	14.3[a]
Inoculated	1013.5[a]	58.1[a]	32.5[a]	116.0[a]	16.4[a]
Substrate					
Mix1: 75% BM + 25% S	938.6[ab]	54.7[b]	28.4[a]	110.9[ab]	14.7[ab]
Mix2: 25% BM + 75% S	859.5[b]	50.0[c]	28.3[a]	101.7[b]	13.4[b]
Mix3: 50% BM + 50% S	1027.6[a]	64.0[a]	30.8[a]	122.5[a]	18.3[a]

NS: nutritive solution, Mix: substrate, BM: bovine manure, S: sand. In each column, at levels of each factor, values with the same letter are not significantly different (Tukey, 0.05).

variables, compared to those that did not have application. of nutrient solution, water only (Tables 7 and 8). These data coincide with those of ZÚÑIGA-ESTRADA et al. (2018) carried in the cultivation of *Agave tequilana* Weber when applied different concentrations of nutrient solution. Results of the relative growth rates of the variables evaluated, had the same behavior, the values increased when the nutrient content applied by fertigation was higher, that is, plants that received fertigation at 100% nutrient concentration reached larger sizes compared to plants that were only irrigated with water (Table 7). In the case of the destructive variables, only the leaf area was the variable that did not have the same behavior of presenting a higher value according to the nutrient content in the applied nutrient solution (Table 8).

CONCLUSION

Agave angustifolia obtained from inflorescence bulbils and established in a substrate mixture of 50% bovine manure + 50% sand, where

Table 7 - Characteristics of *A. angustifolia* plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with *A. brasilense.* Oaxaca Mexico, 2018/19.

T	Height (cm)	Number of Leaves	Stem Diameter (mm)	Rosette Diameter (cm)	Length of largest leaf (cm)	width of the largest leaf (cm)
1	60.2^{cde}	17.0^{cde}	66.3^{bcde}	76.1^{abc}	51.3^{bcde}	4.9^{bc}
2	56.2^{cde}	15.4^{e}	59.6^{ef}	65.8^{cdefg}	49.3^{cde}	4.9^{bc}
3	55.6^{de}	15.2^{e}	61.0^{def}	64.9^{cdefg}	49.0^{cde}	4.8^{bc}
4	60.0^{cde}	18.0^{abcd}	68.8^{abcd}	67.4^{cdrfg}	52.9^{bcde}	4.7^{bc}
5	57.4^{cde}	15.8^{e}	60.6^{def}	61.9^{g}	51.4^{bcde}	4.7^{bc}
6	58.4^{cde}	16.2^{de}	62.0^{cdef}	61.6^{g}	52.0^{bcde}	4.6^{bc}
7	54.1^{e}	15.6^{e}	62.8^{bcdef}	59.8^{g}	46.1^{e}	4.6^{c}
8	58.8^{cde}	$16.0d^{e}$	63.2^{bcdef}	63.2^{efg}	52.0^{bcde}	5.0^{bc}
9	64.8^{bc}	17.8^{abcd}	71.6^{abc}	75.6^{abce}	59.0^{ab}	5.0^{bc}
10	63.3^{bcd}	20.0^{abc}	72.7^{ab}	67.8^{cdefg}	55.6^{abcd}	5.1^{bc}
11	57.9^{cde}	17.6^{bcde}	60.4^{def}	62.4^{fg}	52.3^{bcde}	4.7^{bc}
12	61.7^{cde}	18.4^{abcd}	57.0^{f}	73.8^{abcde}	58.0^{ab}	5.1^{bc}
13	56.2^{cde}	17.6^{bcde}	63.8^{bcdef}	61.0^{g}	46.9^{de}	5.0^{bc}
14	61.7^{cde}	16.6^{de}	59.4^{ef}	73.2^{bcdef}	55.6^{abc}	5.1^{bc}
15	71.2^{ab}	17.4^{bcde}	64.2^{bcdef}	83.2^{ab}	63.8^{a}	5.8^{a}
16	60.7^{cde}	20.6^{ab}	62.2^{cdef}	65.4^{cdefg}	52.7^{bcde}	5.1^{bc}
17	63.4^{bcd}	19.2^{abcd}	70.2^{abcd}	68.4^{cdefg}	56.9^{abc}	5.1^{bc}
18	73.61^{a}	21.0^{a}	76.6^{a}	84.4^{a}	63.0^{a}	5.7^{a}
---Relative growth rate---						
T	Height (cm day^{-1})	Number of leaves (NL day^{-1})	Stem Diameter (mm day^{-1})	Rosette Diameter (cm day^{-1})	Length of largest leaf (cm day^{-1})	width of the largest leaf (cm day^{-1})
1	0.13^{bcde}	0.03^{bcde}	0.13^{abcd}	0.18^{abc}	0.11^{cdef}	0.007^{abc}
2	0.12^{de}	0.02^{de}	0.10^{de}	0.15^{bcdef}	0.11^{def}	0.007^{abc}
3	0.12^{de}	0.02^{e}	0.11^{bcde}	0.15^{bcdef}	0.11^{def}	0.006^{bc}
4	0.13^{cde}	0.03^{abcd}	0.13^{abcde}	0.16^{bcdef}	0.11^{bcdef}	0.006^{bc}
5	0.13^{cde}	0.03^{cde}	0.11^{cde}	0.14^{ef}	0.11^{bcdef}	0.007^{abc}
6	0.13^{cde}	0.03^{bcde}	0.11^{bcde}	0.15^{def}	0.12^{bcdef}	0.007^{abc}
7	0.11^{e}	0.03^{cde}	0.12^{bcde}	0.13^{f}	0.10^{f}	0.006^{c}
8	0.13^{bcde}	0.03^{cde}	0.12^{bcde}	0.15^{bcdef}	0.12^{abcde}	0.008^{abc}
9	0.14^{bcd}	0.03^{abcde}	0.14^{abcd}	0.18^{ab}	0.13^{abc}	0.008^{abc}
10	0.14^{abc}	0.04^{ab}	0.14^{ab}	0.16^{bcdef}	0.13^{abcd}	0.008^{abc}
11	0.12^{cde}	0.03^{abcd}	0.10^{de}	0.15^{def}	0.12^{bcdef}	0.006^{bc}
12	0.14^{bcde}	0.03^{abcd}	0.10^{de}	0.18^{abcd}	0.13^{abc}	0.008^{abc}
13	0.12^{e}	0.03^{abcde}	0.11^{bcde}	0.14^{ef}	0.10^{fe}	0.007^{abc}
14	0.13^{bcde}	0.03^{cde}	0.10^{e}	0.17^{abcd}	0.12^{abcde}	0.008^{abc}
15	0.15^{ab}	0.03^{bcde}	0.10^{de}	0.20^{a}	0.14^{ab}	0.009^{ab}
16	0.13^{bcde}	0.04^{abc}	0.11^{bcde}	0.15^{cdef}	0.11^{bcdef}	0.007^{abc}
17	0.14^{bcde}	0.03^{abcde}	0.13^{abcde}	0.16^{bcdef}	0.13^{abcd}	0.008^{abc}
18	0.17^{a}	0.04^{a}	0.15^{a}	0.20^{a}	0.14^{a}	0.010^{a}

T: treatment, NS: nutritive solution, Ab0: non-inoculated, Ab1: inoculated, Mix1: 75% BM + 25% S, Mix2: 25% BM + 75% S, Mix3: 50% BM + 50% S, 1: NS-0+Mix1+Ab0, 2: NS-0+Mix2+Ab0, 3: NS-0+Mix3+Ab0, 4: NS-0+Mix1+Ab1, 5: NS-0+Mix2+Ab1, 6: NS-0+Mix3+Ab1, 7: NS-50+Mix1+Ab0, 8: NS-50+Mix2+Ab0, 9: NS-50+Mix3+Ab0, 10: NS-50+Mix1+Ab1, 11: NS-50+Mix2+Ab1, 12: NS-50+Mix3+Ab0, 13: NS-100+Mix1+Ab0, 14: NS-100+Mix2+Ab0, 15: NS-100+Mix3+Ab0, 16: NS-100+Mix1+Ab1, 17: NS-100+Mix2+Ab1, 18: NS-100+Mix3+Ab1. In each column, at levels of each factor, values with the same letter are not significantly different (Tukey, 0.05).

Table 8 - Characteristics of the destructive sampling of *A. angustifolia* plants that during 12 months in the nursery were established in different substrates, fertigation doses and inoculation with *A. brasilense.* Oaxaca Mexico, 2018/19.

T	Leaf área (cm^2)	----------Volume (cm^3)----------			Fresh Weight Root (g)
		Root	leaf	Piña	
1	2143.7^{abcd}	122.5^{a}	1005.0^{bcd}	42.5^{cd}	81.0^{a}
2	1717.9^{bcde}	117.5^{a}	825.0^{d}	45.0^{bcd}	85.3^{a}
3	1793.2^{bcde}	58.0^{a}	870.0^{cd}	41.0^{d}	61.8^{a}
4	2073.6^{abcde}	81.3^{a}	1100^{abcd}	52.5^{abcd}	66.0^{a}
5	1699.2^{cde}	72.5^{a}	772.5^{d}	45.0^{bcd}	68.8^{a}
6	1665.7^{cde}	75.0^{a}	885.0^{cd}	46.3^{bcd}	69.3^{a}
7	1533.9^{de}	55.0^{a}	810.0^{d}	52.3^{abcd}	46.0^{a}
8	1828.5^{abcde}	72.5^{a}	862.5^{d}	50.0^{bcd}	49.8^{a}
9	2219.1^{abcd}	85.0^{a}	1187.5^{abcd}	65.8^{abcd}	74.8^{a}
10	2510.4^{ab}	101.3^{a}	1362.5^{abc}	67.8^{abcd}	89.0^{a}
11	2106.1^{abcd}	112.5^{a}	1095.0^{abcd}	51.3^{abcd}	90.0^{a}
12	1895.4^{abcde}	65.00^{a}	1054.8^{abcd}	48.8^{bcd}	61.5^{a}
13	1278.6^{e}	43.00^{a}	762.5^{d}	45.0^{bcd}	39.8^{a}
14	1827.5^{abcde}	51.3^{a}	987.5^{bcd}	61.3^{abcd}	58.3^{a}
15	2454.3^{abc}	90.0^{a}	1450.0^{ab}	75.8^{ab}	66.3^{a}
16	1748.9^{bcde}	70.0^{a}	1131.3^{abcd}	73.8^{abc}	70.3^{a}
17	2128.3^{abcd}	90.0^{a}	1150.0^{abcd}	50.0^{abcd}	78.3^{a}
18	2604.4^{a}	95.0^{a}	1512.5^{a}	82.5^{a}	76.5^{a}
	----------Fresh weight (g)----------		----------Dry Weight (g)----------		
	Leaf	Piña	Root	Leaf	Piña
1	919.5^{bcd}	42.8^{c}	27.7^{ab}	116.7^{bcd}	11.5^{a}
2	736.0^{d}	43.8^{c}	31.3^{ab}	87.6^{cd}	13.7^{a}
3	788.3^{d}	46.5^{c}	23.2^{ab}	103.8^{bcd}	12.4^{a}
4	967.5^{bc}	52.0^{abc}	27.7^{ab}	99.7^{cd}	13.0^{a}
5	705.3^{d}	43.3^{c}	22.4^{ab}	80.9^{cd}	13.6^{a}
6	817.0^{cd}	51.8^{abc}	24.0^{ab}	96.3^{cd}	21.4^{a}
7	711.8^{d}	53.0^{abc}	16.5^{b}	85.0^{cd}	13.2^{a}
8	799.5^{cd}	49.3^{bc}	21.6^{ab}	96.1^{cd}	12.6^{a}
9	1045.0^{abcd}	68.3^{abc}	31.2^{ab}	127.6^{abcd}	19.3^{a}
10	1225.0^{abc}	69.8^{abc}	38.2^{ab}	132.8^{abc}	18.2^{a}
11	980.3^{bcd}	51.5^{abc}	24.4^{ab}	107.3^{bcd}	11.9^{a}
12	936.3^{bcd}	52.3^{abc}	28.7^{ab}	104.8^{bcd}	12.8^{a}
13	680.0^{d}	48.3^{bc}	23.0^{ab}	80.0^{d}	11.9^{a}
14	880.3^{bcd}	58.0^{abc}	25.9^{ab}	115.0^{bcd}	14.8^{a}
15	1272.8^{ab}	79.8^{ab}	31.7^{ab}	154.8^{ab}	19.7^{a}
16	1019.8^{abcd}	64.0^{abc}	36.8^{ab}	127.4^{abcd}	19.9^{a}
17	1055.8^{abcd}	54.0^{abc}	44.3^{a}	123.4^{abcd}	13.6^{a}
18	1414.5^{a}	84.0^{a}	46.0^{a}	171.3^{a}	24.6^{a}

T: treatment, NS: nutritive solution, Ab0: non-inoculated, Ab1: inoculated, Mix1: 75% BM + 25% S, Mix2: 25% BM + 75% S, Mix3: 50% BM + 50% S, 1: NS-0+Mix1+Ab0, 2: NS-0+Mix2+Ab0, 3: NS-0+Mix3+Ab0, 4: NS-0+Mix1+Ab1, 5: NS-0+Mix2+Ab1, 6: NS-0+Mix3+Ab1, 7: NS-50+Mix1+Ab0, 8: NS-50+Mix2+Ab0, 9: NS-50+Mix3+Ab0, 10: NS-50+Mix1+Ab1, 11: NS-50+Mix2+Ab1, 12: NS-50+Mix3+Ab1, 13: NS-100+Mix1+Ab0, 14: NS-100+Mix2+Ab0, 15: NS-100+Mix3+Ab0, 16: NS-100+Mix1+Ab1, 17: NS-100+Mix2+Ab1, 18: NS-100+Mix3+Ab1. In each column, at levels of each factor, values with the same letter are not significantly different (Tukey, 0.05).

they were fertigated for 12 months with 100% Steiner solution and inoculated with *Azospirillum brasilense* monthly, had a higher relative growth rate, as well as twice the leaf area, root volume, leaf fresh weight, and root, leaf and piña dry weight than plants established in a substrate mixture of 25% bovine manure and 75% sand, irrigated with water and without inoculation.

ACKNOWLEDGEMENTS

To the Consejo Nacional de Ciencia y Tecnología (CONACYT-México) for the support provided, through a research grant. The research was funded by the Tecnológico Nacional de México (TecNM), with project 6617.18-P, entitled “Asexual propagation of two endemic species of Oaxaca”.

DECLARATION OF CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors.

AUTHORS’ CONTRIBUTIONS

The authors contributed equally to the manuscript.

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