

Growth of *Agave angustifolia* Haw. in relation to its nutritional condition

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Abstract

Agave angustifolia Haw. plants, which are obtained by asexual propagation (inflorescence bulbils and rhizome suckers), are established in a nursery where they are provided with agronomic management that is considered to include the supply of nutrients, to achieve quality plants in health and vigor that are successfully established in the field. The research was carried out from January 2017 to August 2018, in the nursery of the Technological Institute of the Valley of Oaxaca, located in the Exhacienda de Nazareno, Xoxocotlán, Oaxaca, Mexico, the objective was to evaluate the nutritional contents in foliar tissues and the size of *Agave angustifolia* plants, in nursery conditions, fertigated at different concentrations of nutrients. For seven months, four groups of plants that were in soil of loamy-sandy texture were applied different types of irrigation: water or nutrient solution (SN) at 50, 75 and 100% of the Steiner formulation, after that time, their growth was evaluated. In leaf samples, the nutritional contents were analyzed, and the plants to which water was applied, SN-50%, SN-75% and SN-100%, had in mg kg⁻¹ of foliar dry matter: 7 225, 10 641, 12 575 and 14 957 of N; 3 017, 5 018, 5 297 and 6 719 of P; 7 827 19 604, 16 220.1 and 18 847 of Mg, accumulated, 282.74, 340.11, 431.98 and 453.05 g of foliar dry matter, as well as 75.01, 77.21, 95.92 and 103.14 g of dry matter in the root, respectively.

Keywords: *agave*, fertigation, nutrients.

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Introduction

In the state of Oaxaca, Mexico, *Agave angustifolia* is used as a raw material to produce mezcal. It is a plant with morphological characteristics of succulence, which forms a large rosette 1.5 to 2 m in height and 1.5 to 2 m in diameter, with rigid leaves, with marginal and apical spines; it develops a flower scape that reaches up to 6 m in height. The stem of the rosette, called pineapple, which has an average fresh weight of 80 kg, contains 21.16% of total reducing sugars of the fresh weight (González-González *et al.*, 2007; Cruz *et al.*, 2013), its root is shallow, it grows in land with moderate to steep slopes, stony soils.

The species supports environmental conditions, such as water deficit, high temperature, moderately saline soils and with low fertility, that limit the growth and productivity of other crop plants (Andrade *et al.*, 2007; García, 2007; Enríquez-del-Valle, 2008). This species presents photosynthesis of the Crassulacean acid metabolism type, MAC (Matiz *et al.*, 2013) and it allows them to obtain net carbon gains with minimal water losses (Winter and Smith, 1996).

Being an alcohol-producing plant of economic interest to produce mezcal, plant material is needed to establish in the field. Although this species produces viable seeds, the plantations are established with plants that are obtained by asexual propagation, mainly rhizome suckers and inflorescence bulbils, and in a small proportion, plants obtained by plant tissue cultures have been established (Arizaga and Ezcurra, 2002; Enríquez-del Valle, 2008).

The plants, which are obtained in an asexual way, are kept for six to 18 months in nursery, to obtain 30-50 cm in height, convenient to establish them in the field, and during this period it is necessary to give agronomic management, stage in which the health and the supply of nutrients are considered convenient to achieve plants of quality and vigor that are successfully established in the field. When the plants are extracted from the soil of the nursery to transfer them to the field, the roots are pruned and sometimes immersed for a few minutes in fungicidal solution, to later be placed in a ventilated place in the shade for two days to a week so that their tissues lose slightly moisture, which according to the nurserymen promotes the plant to establish successfully in the field.

Plantations are established during the first months of the rainy season to ensure that plants develop new roots to establish themselves in the soil and restart growth (Díaz *et al.*, 2011). Plants generally depend on the energy of solar radiation captured through the photosynthetic process for carbon fixation and synthesis of structural and metabolic compounds. But the efficiency of the plant to capture solar radiation, fix carbon and accumulate dry matter depends on its physiological condition, which is positively related to the nutrimental condition.

In *A. tequilana*, soil fertility studies have been carried out, which conclude the need to apply fertilizers to increase crop yields (Álvarez-Sánchez *et al.*, 2010). *A. angustifolia* in field conditions (Martínez-Ramírez and Bautista-Sánchez, 2013), as well as in micropropagated plants of *A. americana* var. *oaxacensis* (Enríquez-del-Valle *et al.*, 2013) in greenhouse and *A. potatorum* (Enríquez-del Valle *et al.*, 2016) in nursery conditions, show the favorable effect of nutrient supply on plant growth. There are 17 essential nutrients that are classified into macronutrients (nitrogen,

N, phosphorus, P, potassium, K, calcium, Ca, magnesium, Mg and sulfur, S) and micronutrients (iron, Fe, copper, Cu, manganese, Mn, zinc, Zn, boron, B and molybdenum, Mo) (Vitosh *et al.*, 1994; Grusak, 2001; Zúñiga, 2013).

Of all the essential elements for the plant, carbon (C), hydrogen (H) and oxygen (O) constitute about 90% of the dry weight of a plant (Medina, 1999). Inside the plant, these elements are used for the synthesis of carbohydrates, through photosynthesis, which occurs in the chloroplasts of the cells, using light as an energy source. The remaining 14 elements only represent about 10% of the dry weight of a plant (Rodríguez and Flores, 2004).

In previous works on the development of *A. angustifolia* in greenhouse-nursery, it has been demonstrated the convenience of supplying fertigation, which has positive effects on the development of plants, in characteristics such as number of leaves, plant height, stem diameter, fresh and dry weight (Enríquez-del-Valle *et al.*, 2009). But more information is required for agave to show the relationship between nutrient supply, nutrient content in plant tissues and plant growth. That is why the objective of this work was: To evaluate plants of *A. angustifolia*, which in nursery conditions were fertigated at different concentrations of nutrients, the size they reached in relation to the nutritional contents of their foliar tissues.

Materials and methods

Experimental material

This work is a continuation of the experimental procedure, in which plant growth data were reported, by Ríos-Ramírez *et al.* (2018). The plant material consisted of 27 18-month-old plants of *A. angustifolia* obtained from inflorescence bulbils. Which were in nursery conditions, individually established in pots of 4 dm³ with soil of loamy-sandy texture. They were separated into four groups, which received twice a week for seven months various types of irrigation (treatments): 1) only water; or irrigation with nutrient solution (SN) in different dilutions of Steiner's formulation (1984), 2) SN-50%; 3) SN-75%; 4) SN-100%.

SN-100% contains in 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. Also, all groups were sprayed twice a week with agricultural fungicide N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide of the brand Bayer[®] and systemic antibiotic, agricultural terramycin 5% Oxytetracycline, of the brand Zoetis[®]. After seven months in the aforementioned conditions, the plants were harvested and the following was quantified, their height, number of leaves, leaf area (cm²) determined with a Laser scanner Area Meter (CID Bio-Science, Copyright[®] 2016 CID Bio-Science, Inc. Camas, WA USA), the leaf and root volume (cm³) by dipping them in a known volume of water in a graduated cylinder.

The fresh weight (g) of the leaves and roots was measured on a triple beam scale (IROSA[®] 700 PPW, Mexico) with precision of 0.1 g. Subsequently, the leaves and roots were placed separately in paper bags properly labeled, to be placed for two weeks at a temperature of 65 °C in a convection oven (Felisa[®] Fabricantes Feligneo, SA de CV, Mexico). After this time, the dry root and foliar material were weighed again in the triple beam scale.

Determination of nutrients in foliar tissues

To analyze the nutrient contents, the dry leaves of *Agave* were pulverized in a mill (Apex Construction Ltd V110), of which 0.5 g of sample was weighed in duplicate on an analytical balance (Sartorius CP2245), these samples were subjected to wet digestion with a mixture of $\text{HNO}_3:\text{HClO}_4$ (ratio 2:1) in a Kjeldahl digester of the SEV brand DIK-20 model.

The determination of nitrogen (N) and carbon (C) was performed by the combustion method, in an organic elemental analyzer (Mod: Perkin Elmer Series II CHNS/O analyzer 2400), phosphorus (P) was evaluated by the Vanadate-Molybdate-Yellow method in an ultraviolet visible light spectrophotometer (UV-Vis GBC Cintra 10) at a wavelength of 470 nm, the elements potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were determined by Atomic Absorption Spectrophotometry, in an atomic absorption spectrophotometer (Thermo Scientific, model ICE 3000 series AA) with air-acetylene flame by direct aspiration; and sulfates (SO_4) by the turbidimetric method, in a ultraviolet visible light spectrophotometer (UV-Vis GBC Cintra 10) at a wavelength of 340 nm.

Experimental design and data analysis

The experiment was established according to a completely random design. The experimental unit was a plant and with different number of repetitions per treatment (from four to 11). In the data for each variable, the homogeneity of variance was analyzed using the Bartlett test and the Shapiro-Wilk normality test. The variables N, Na, K, Zn, Fe, Cu were transformed to $\log_{10}(x)$ to satisfy the assumptions of homogeneity and normality. Data were subjected to an analysis of variance, comparisons of means (Duncan, 0.05), correlations (0.05). Correlation analyses between the leaf dry weight and the contents of nutrients in the leaves were performed, as well as between the amounts of the various nutrients. For statistical analysis routines, the SAS® Statistical Analysis System, version 9.0, was used.

Results and discussion

The nutrient contents found in foliar tissues of *A. angustifolia* plants were influenced by the increasing concentrations of fertigation they received for seven months in the nursery. Analyses of variance (Table 1) show that nutrient supply levels had significant different effects ($p \leq 0.05$) in the contents of N, Mg, Zn and not significant effects for K, SO_4 , P, Na, Fe, Cu, as well as in the accumulated leaf dry matter.

The amount of leaf dry matter and root dry matter that the plants accumulated were also positively related to the amount of nutrients supplied to them. Thus, plants irrigated with only water and fertigated plants with SN-100% accumulated 282.7 and 453.05 g of leaf dry matter, as well as 75 and 103.1 g of dry matter in the root, which in each case were significantly different magnitudes (Duncan, 0.05) (Table 2).

Table 1. Summary of the analysis of variance of dry matter and nutrient contents in foliar tissues of *Agave angustifolia* plants, which, in nursery, were fertigated at different concentrations of nutrients.

Fv	Gl	Mean squares and significance					
		P	C	N	Na	Mg	K
Trat	3	14847174.6 ^{ns}	489539571 ^{ns}	68167267.3 [*]	1.60107727 ^{ns}	198638940 [*]	0.33048079 ^{ns}
Err	24	12454715.2	668481315	10267603.5	0.57726192	61911784	0.12158501
Tot	27						
Fv	Gl	Zn	Fe	SO ₄	Cu	MSF	MSR
Trat	3	0.27210426 [*]	0.20388993 ^{ns}	36743275.7 ^{ns}	0.1886378 ^{ns}	2.67 ^{ns}	1508.2 ^{ns}
Err	24	0.08453928	0.4057517	21039730.1	0.35015818	0.96	1869.2
Tot	27						

Fv= sources of variation; Gl= degrees of freedom; Trat= treatment; Err= error; Tot= total; P= phosphorus; C= carbon; N= nitrogen; Na= sodium; Mg= magnesium; K= potassium; Zn= zinc; Fe= iron, SO₄= sulfates; Cu= copper; MSF= leaf dry matter; MSR= root dry matter; ns= non-significant values ($p > 0.05$); ^{*}=values with significant effects ($p \leq 0.05$); ^{**}=values with highly significant effects ($p \leq 0.01$).

Table 2. Dry matter of leaves (g) and root (g), and nutrient contents (mg kg⁻¹) in foliar tissues of *Agave angustifolia* plants, which, in nursery, were fertigated at different concentrations of nutrients.

Variables	Irrigation type treatment			
	Water	SN50%	SN75%	SN100%
MSF	282.74 ±0.6 b	340.11 ±1 ab	431.98 ±0.7 ab	453.05 ±121.5 a
MSR	75.01 ±32.6 a	77.21 ±48 a	95.92 ±25.7 a	103.14 ±53.4 a
P	3017 ±3024.4 a	5018 ±4009.5 a	5297 ±2783 a	6719 ±537.9 a
C	413508 ±250382.1 a	403786 ±423534.2 a	395363 ±217209.9 a	416057 ±55592.8 a
N	7225 ±5773.8 c	10641 ±16475.7 bc	12575 ±9162.3 ab	14957 ±2381.6 a
Mg	7827 ±4950.5 b	19604 ±9389.9 a	16220.1 ±4670.3 ab	18847 ±992.4 a
K	18057 ±16352.4 a	45048 ±16475.7 a	42522 ±9956.9 a	45943 ±3094.2 a
Na	153.2 ±265 a	892.7 ±16475.7 a	543.2 ±463.9 a	779 ±124.9 a
Zn	12.88 ±11.5 a	26.89 ±15.4 a	29.23 ±26.8 a	33.85 ±0.0004 a
Fe	23.55 ±23.3 a	65.32 ±56.8 a	67.37 ±53.6 a	55.02 ±0.0009 a
Cu	14.83 ±12.1 a	36.63 ±30.5 a	46.14 ±42.7 a	30.21 ±0.0004 a
SO ₄	1371 ±1957.8 a	6087 ±4607 a	5843 ±3934.5 a	6510 ±0.07 a

P= phosphorus; C= carbon; N= nitrogen; Na= sodium; Mg= magnesium; K= potassium; Zn= zinc; Fe= iron, SO₄= sulfates; Cu= copper; MSF= leaf dry matter; MSR= root dry matter. In each variable horizontally, means with the same letter are not significantly different (Duncan, 0.05).

According to the concentration for each nutrient determined in the foliar tissues of *A. angustifolia*, the macroelements with the highest concentration were C, N, Mg, K and the microelement Fe, compared to the rest of the nutrients determined (Table 2). The plants accumulated in their foliar tissues more N, P and Zn in response to the increasing amounts of nutrients in fertigation, up to SN-100%, in such a way, the plants irrigated with water and the plants fertigated with SN-100% had in mg kg⁻¹ of dry matter: 7 225 and 14 957 of N, 3 017 and 6 719 of P, 12.88 and 33.85 of Zn, respectively, amounts that in each case were significantly different (Duncan, 0.05).

A similar trend was observed in the case of Fe and Cu, but the maximum accumulation of these nutrients occurred when up to SN-75% was applied. Plants irrigated only with water had, in their foliar tissues, 1 371 mg kg⁻¹ of SO₄ and 18 057 mg kg⁻¹ of K of dry matter, while in fertigated plants, a non-significant tendency to have more SO₄ and K was observed (Table 2). So, it can be said that the increasing supply of nutrient solution to plants of *A. angustifolia* for seven months accumulated greater amount of nutrients in the foliar tissues of these plants, which showed higher growth.

The data of the nutrition analysis *A. angustifolia* obtained in the present study are consistent with those reported by Cruz *et al.* (2013), who, in adult plants of this species from three localities of Oaxaca, Mexico, determined a higher concentration of the macroelements Mg and Ca; and the microelement Fe. Silos-Espino *et al.* (2011) studied plants from eight to 12 years (before flowering) of *A. salmiana* Gentry, in which they analyzed components of nutritional value in forage quality and main minerals (Ca, P, Mg, Fe, Zn, Cu, Bo and Se) of the foliar tissues. Ca was mainly found with 5 870 mg 100 g⁻¹, P with 220 mg 100 g⁻¹, Zn 16.9 mg 100 g⁻¹ and 33.2 mg 100 g⁻¹ of Fe.

Plants fix atmospheric CO₂ through the process of photosynthesis and synthesize organic compounds (Glime, 2017). Subsequently, the products of photosynthesis and mineral substances from the soil are used in the processes of synthesis of proteins, lipids and other organic compounds that are structural and metabolic constituents of plants. And the magnitude of accumulated biomass and economic yield result from the efficiency of those processes (Gutiérrez-Rodríguez *et al.*, 2005). Since it is important that the plants in the nursery show vigorous growth, attention should be paid so that they have high net photosynthesis, as well as plant health, the elimination of other competing plant species and that the plants are in appropriate water and nutritional condition.

The nutritional analyses of the plants of *A. angustifolia* show differences between the different groups of plants according to the amount of fertigation they received (Water, SN-50%, SN-75%, SN-100%) and that they are related to the data reported by Ríos-Ramírez *et al.* (2018), which show that, after seven months in nursery, the plants of *A. angustifolia* increased their number of leaves, the size of the root and the leaves, evaluated in leaf area, leaf volume, leaf dry weight, in positive relationship to the amount of nutrients they received through irrigation.

Since the plants that received higher doses of fertilization had a higher concentration of nutrients in their leaf dry matter and were also the largest (Table 2). Therefore, the convenience of fertigation is shown, since it maintains the availability of water and nutrients in the root zone at optimal levels to obtain a higher growth of the plants and subsequently the potential productivity of the crop (Guzmán, 2004).

In young trees of forest species that are produced in nursery, it has also been shown that the nutritional condition of the plant influences the total height, the diameter of the stem, the aerial and root biomass, characteristics that are related to the ability of the plant to overcome the stress condition when it is established in the field and restarts growth (McDowell *et al.*, 2008). In general, all plants need nutrients for the synthesis of structural and metabolic substances, which determines their photosynthetic efficiency for the assimilation of C, which constitutes between 40 to 50% of the plant dry matter, as well as through photosynthesis, the energy of solar radiation is conserved in compounds that provide this energy for the processes of synthesis (Grusak, 2001).

The higher the concentration of nutrient solution supplied to the plants, the more they grew, and their foliar tissues had a higher concentration of N, and this element N is part of metabolically important compounds such as amino acids, proteins, photosynthetic pigments, so their supply has a direct impact on the increase of dry matter (McDonald *et al.*, 1996; Uchida, 2000). Correlation analyses (Table 3) show that leaf dry weights had a positive and significant correlation with N content ($r= 0.678$, $p\leq 0.01$), with phosphorus ($r= 0.743$, $p\leq 0.01$), with Mg ($r= 0.899$, $p\leq 0.01$), with K ($r= 0.830$, $p\leq 0.01$), with Fe ($r= 0.786$, $p\leq 0.01$), Cu ($r= 0.699$, $p\leq 0.01$), SO₄ ($r= 0.977$, $p\leq 0.01$).

Table 3. Correlations between leaf dry matter (g) and nutrient contents (mg kg⁻¹) in foliar tissues of *Agave angustifolia* plants, which for seven months in nursery were fertigated at different concentrations of nutrients.

	T	MSF	P	C	N	Mg	K	Mn	Zn	Fe	SO ₄
T	1	0.43*	0.36 ^{ns}	-0.002 ^{ns}	0.67**	0.404*	0.34 ^{ns}	0.53 ^{ns}	0.39*	0.22 ^{ns}	0.37*
MSF		1	0.74**	0.036 ^{ns}	0.68**	0.899**	0.83**	-0.11 ^{ns}	0.5*	0.79**	0.98**
P			1	-0.192 ^{ns}	0.03 ^{ns}	0.63**	0.55*	0.05 ^{ns}	0.56**	0.61**	0.74**
C				1	0.11 ^{ns}	0.002 ^{ns}	-0.05 ^{ns}	0.94 ^{ns}	0.09 ^{ns}	-0.06 ^{ns}	0.08 ^{ns}
N					1	0.845**	0.72**	-0.57 ^{ns}	0.45*	0.55*	0.87**
Mg						1	0.89**	-0.24 ^{ns}	0.62*	0.66*	0.87**
K							1	0.22 ^{ns}	0.53*	0.52*	0.82**
Mn								1	0.87 ^{ns}	-0.25 ^{ns}	-0.08 ^{ns}
Zn									1	0.37 ^{ns}	0.45*
Fe										1	0.8**
SO ₄											1

T= fertilization dose; P= phosphorus; C= carbon; N= nitrogen; Mg= magnesium; K= potassium; Ca= calcium; Zn= zinc; Fe= iron, SO₄= sulfates; Cu= copper; MSF= leaf dry matter. In each correlation value, ns= not significant ($p> 0.05$); * = significant ($p\leq 0.05$). ** = highly significant ($p\leq 0.01$).

Conclusions

Plants of *Agave angustifolia* Haw. accumulated more leaf and root dry matter, and had more N, P, K, Zn, Fe, Cu and SO₄ than non-fertigated plants, and the amounts increased in direct relationship to the nutrients they received. Plants irrigated only with water and fertigated plants at 75 or 100% of nutrients of Steiner's formulation had 282.74, 431.98 and 453.05 g of leaf dry matter, 75.01, 95.92 and 103.14 g of dry matter in the root, their leaves had in mg kg⁻¹ of dry matter: 3 017, 5 297 and 6 719 of P; 7 225, 12 575 and 14 957 of N; 12.88, 29.23 and 33.85 of Zn; 23.55, 67.37 and 55.02 of Fe; 14.83, 46.14 and 30.21 of Cu, respectively. In general, leaf dry matter had high positive significant correlations with the contents of N, P, K, Mg, Zn, Fe, Cu, SO₄.

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