Nutrient Solutions and Drought in Plant Growth and Fructans Content of *Agave potatorum* Zucc

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Abstract. The *Agave potatorum* Zucc. is a wild species endemic to Oaxaca and Puebla, Mexico. The stem or “head” of the plants of this species contains a large amount of fructans, which, in conjunction with their crassulacean acid metabolism (CAM), helps the agave to survive droughts. The soluble carbohydrates are used to produce mezcal. The objective was to evaluate growth and content of fructans of *A. potatorum* young plants grown in soil and perlite substrate, fertigated with three nutrient solutions, and subjected to drought. Eight-month-old plants were used and, for 15 months, were fertigated with nutrient solutions: 1) Steiner, 2) Hoagland and Arnon, and 3) Urrestarazu. Irrigation was later suspended to simulate a 5-month drought and induce stress. During fertigation, the vegetative growth was greater in plants irrigated with Hoagland and Arnon and Urrestarazu solutions in perlite and in soil. After the period of water deficit stress, plants in perlite substrate fertigated with the Hoagland and Arnon solution accumulated more fructans in the heads, reaching a maximum of 75%, than plants in soil substrate (42%).

Agave potatorum Zucc. is a wild species that grows in pine-oak forests in mountainous areas of the states of Oaxaca and Puebla, Mexico. In Oaxaca, since the last decade, this species has been collected and used as a raw material to produce the distilled beverage known as “tobalá” mezcal (Martínez-Ramírez et al., 2013), which has excellent flavor and aroma, making it one of the most demanded alcoholic beverages. For this reason, in the state of Oaxaca, *A. potatorum* is being domesticated; its cultivation is incipient and in small areas of indigenous communities. However, the main techniques for its management are unknown. Like most agaves, *A. potatorum* adapts to diverse environments and survives long periods of drought because of its succulent leaves with

thick cuticles that allow it to store water in its tissues (Martínez-Ramírez et al., 2013). Also, as the result of CAM, it can perform some physiological functions, such as the assimilation of carbon dioxide by using the water stored in its tissues when moisture is restricted for an extended period of time (Pimienta-Barrios et al., 2006; Seki et al., 2007).

Supplying nutrients to plants through nutrient solutions is a basic aspect in hydroponic crops, for production or research purposes (Trejo-Téllez and Gómez-Merino, 2012). Because the nutritional demand of each species differs, different formulations are used (Cadahia, 2005). However, the nutritional needs of *Agave* species are unknown.

Although *A. potatorum* grows and reproduces naturally in conditions of stress caused by high temperatures, long drought periods, and rocky unfertile soils (Torres et al., 2016), it has been proven that this species responds positively in growth and quantity of stored sugars to the application of organic or inorganic nitrogen (Enríquez del Valle et al., 2016; Martínez-Ramírez et al., 2013) and water during the drought period (Nobel et al., 1989). For an *A. potatorum* plant to be used for mezcal production, it must reach physiological maturity and emit its floral

scape; this occurs when the plant is 8 to 10 years old. To reduce time to harvest, one alternative is to determine the content of fructans in young plants under different culture management techniques, partially simulating natural growing conditions. Therefore, the objective of this work was to evaluate the growth and content of fructans of young *A. potatorum* plants grown in soil and perlite, fertigated with three nutrient solutions and subjected to drought.

Material and Methods

Experiment location and conditions. The research was carried out from January 2015 to Aug. 2016 at the Interdisciplinary Research Center for Integral Regional Development of the Oaxaca Unit of the National Polytechnic Institute, located at 17°1'31"N and 96°43' 11" W and an elevation of 1530 m, in a plastic (200- μ m) tunnel-type greenhouse with permanently open lateral and zenithal vents.

We used 8-month-old *Agave potatorum* Zucc bare-rooted plants acquired from a commercial nursery in the Sierra Sur of Oaxaca. Two independent experiments were established in the greenhouse and conducted in parallel: 1) 30-L white/black polyethylene bags with perlite substrate (Agrolita, Tlalpan, Mexico) and 2) agricultural soil with the following characteristics: sandy-loam texture, with a moisture retention capacity of 7.01%, slightly basic pH 7.6, low organic matter content (1.6%), and 23, 92, 142, and 4318 mg·kg⁻¹ of nitrogen, phosphorus, potassium, and calcium, respectively [NOM-021-REC-2000 (SEMARNAT, 2016)]. Planting density was 8333 plants per ha (1.5 m between plants in rows 0.8 m apart). Chemical analysis of the water used found pH 7.82 and CE 0.85 dS·m⁻¹, macroelements (meq·L⁻¹) calcium (4.51), magnesium (1.56), potassium (0.05), sulfate (1.18), nitrate (1.60), and microelements (mg·L⁻¹) boron (0.31), iron (0.11), manganese (0.034), copper (0.002), and zinc (0.02).

Treatments and experimental design. In both experiments three nutritive solutions were used (Table 1) based on 1) Steiner, 2) Hoagland and Arnon, and 3) Urrestarazu nutritive solutions, which were adjusted according to the water and soil analysis. The pH was maintained at 6.5 and the CE at about 2.8 dS·m⁻¹ in all the used solutions.

Irrigation frequency was every 7 d for 15 months and the volume applied was 600 mL of nutrient solution per plant. At the end of this period, fertigation was suspended during the subsequent 5 months to simulate drought. Each experiment was established according to a completely randomized design with a factorial arrangement, where the factors were the period without irrigation (“Before” and “After”) and the nutritive solutions (Steiner, Hoagland and Arnon, and Urrestarazu). There were six treatments with four replications in each experiment. The experimental unit consisted of ten plants.

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Table 1. Nutrient solutions applied to *A. potatorum* plants grown in soil and perlite substrate.

Nutrient solution ^z	NO ₃ ⁻	H ₃ PO ₄ ⁻	SO ₄ ²⁻	K ⁺	Ca ²⁺	Mg ²⁺	NH ₄ ⁺
	meq·L ⁻¹						
Steiner	12	1	7	7	9	4	—
Hoagland and Arnon	14	1	4	6	6	4	1
Urrestarazu	15	1	5	8	8	5	1

^zSource: Urrestarazu (2004).NO₃⁻ = nitrate; H₃PO₄⁻ = phosphoric acid; SO₄²⁻ = sulfate; K⁺ = potassium; Ca²⁺ = calcium; Mg²⁺ = magnesium; NH₄⁺ = ammonium.Table 2. Growth variables of *A. potatorum* plants established in perlite, before and after a 5-month drought period.

Nutrient solution	Leaves			Head	
	Number	Length (cm)	Thickness (mm)	Fresh wt (g)	
Before drought period					
Steiner	26 ± 1.0 a ^z	14.9 ± 0.5 b	9.5 ± 0.1 b	34.8 ± 2.1 b	223.0 ± 18.7 a
Hoagland and Arnon	25 ± 0.5 a	17.4 ± 0.4 a	10.7 ± 0.3 a	51.1 ± 1.4 a	241.2 ± 12.2 a
Urrestarazu	29 ± 1.5 a	15.5 ± 0.5 b	9.8 ± 0.3 ab	42.5 ± 3.4 b	203.4 ± 16.9 a
After drought period					
Steiner	19 ± 0.3 c	17.1 ± 0.2 b	3.3 ± 0.1 b	29.9 ± 0.9 b	356.1 ± 22.1 ab
Hoagland and Arnon	24 ± 0.4 b	16.7 ± 0.3 b	4.8 ± 0.1 a	31.3 ± 0.8 b	413.2 ± 11.7 a
Urrestarazu	28 ± 0.9 a	20.1 ± 0.3 a	4.9 ± 0.3 a	51.4 ± 1.9 a	352.0 ± 14.8 b

^zMeans in each column and each factor with the same letter do not differ statistically (Tukey's, 0.05); ± SE. Fig. 1. Fructans content in heads of *A. potatorum* plants grown in perlite, fertiligated with three nutrient solutions, before and after a drought period. Means in each column and each factor with the same letter do not differ statistically (Tukey's, 0.05).

Evaluated variables. Two evaluations were carried out, the first one was done 15 months after the transplant, ("Before" the drought period), and the second one at 20 months ("After" the drought period). The evaluated variables were as follow: number of unfolded leaves, length, thickness, and weight of three mature fully developed leaves and weight of stem (head), measured with a digital vernier caliper (CD-6" CSX; Mitutoyo America Corporation, Aurora, IL) and an analytical balance (Sartorius AX; Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany).

The content of fructans in the heads of *A. potatorum* was determined using the commercial kit "Fructan Assay Procedure" (K-FRUC) analytical test kit of the Megazyme brand (Megazyme International Ireland, Wicklow, Ireland). The assay protocol was carried out according to the manufacturer's specifications.

Data analysis. The data obtained were subjected to analysis of variance using the statistical package SAS, version 9.0 (SAS, 2002) and comparison of the means as conducted using the Tukey test ($P \leq 0.05$).

Results and Discussion

Perlite experiment. Before the drought period *A. potatorum* plants fertiligated with Hoagland and Arnon and Urrestarazu solutions, which contain the greatest amount of nitrogen (14 and 15 meq·L⁻¹ NO₃⁻ + 1 meq·L⁻¹ ammonium), developed longer (17.4 and 15.5 cm), thicker (10.7 and 9.8 mm), and heavier (51.1 and 42.5 g) leaves than plants fertiligated with Steiner solution (12 meq·L⁻¹ NO₃⁻), which had leaves 14.9 cm long, 9.5 mm thick, and 34.8 g in weight (Table 2). These results are similar to those obtained by Enriquez del Valle et al. (2013) in young *A. americana* plants and by Díaz

et al. (2011) in *A. cocuy*, which had a favorable response in the vegetative growth with increasing amounts of nitrogen fertilization.

After the drought period, regardless of the nutrient solution applied, the plants had fewer leaves, which were up to 50% thinner than before the drought period (Table 2). Although succulent plants such as *Agave* have significant water reserves in the leaves resulting from their specialized morphology and the presence of an impermeable foliar cuticle (Ramírez-Tobías et al., 2014), after 5 months of drought in a low water retention capacity substrate such as perlite (Sánchez et al., 2009), it was found that the leaves lost turgor because a large part of the stored water was used to maintain plant functions. Ramírez-Tobías et al. (2014) found similar results in several species of *Agave* to which restricted was moisture provided; these plants had fewer less turgid leaves than plants with frequent irrigations.

Table 2 shows that the drought period did not affect head growth. Weight increased up to 62% with Hoagland and Arnon solutions, and even under a drought regime, *A. potatorum* plants continued to assimilate and store carbon dioxide in the stem, which is the main reserve organ in *Agave* plants (Pimienta-Barrios et al., 2006).

After the drought period, the plants watered with Urrestarazu solution had low fructan content in the heads (Fig. 1); this contrasted with vegetative growth (Table 2). With this solution, the plants developed the largest number of leaves (28), possibly because it has greater nitrogen concentration (Table 1), and the plants prioritized vegetative growth over fructans accumulation (White et al., 2016). Likewise, plants fertiligated with Hoagland and Arnon solution accumulated up to 75% fructans. This content of carbohydrates is comparable with that described by Mellado-Mojica and López (2012) in 6- to 7-year-old *A. tequilana* plants,

Table 3. Growth variables of *A. potatorum* plants established in soil, before and after a 5-month drought period.

Nutrient solution	Leaves			Head	
	Number	Length (cm)	Thickness (mm)	Fresh wt (g)	
Before drought period					
Steiner	20 ± 1.2 b	19.7 ± 0.4 b	9.6 ± 0.1 a	66.5 ± 3.4 a	238.4 ± 26.7 ab
Hoagland and Arnon	20 ± 0.9 b	19.4 ± 0.5 ab	8.6 ± 0.3 b	47.4 ± 1.1 b	180.0 ± 6.3 b
Urrestarazu	24 ± 1.1 a	21.8 ± 0.9 a	9.9 ± 0.2 a	67.0 ± 3.7 a	294.2 ± 35.6 a
After drought period					
Steiner	27 ± 0.6 b	21.5 ± 0.5 c	7.3 ± 0.4 b	65.5 ± 3.4 b	387.5 ± 18.8 b
Hoagland and Arnon	35 ± 0.3 a	23.6 ± 0.4 b	7.9 ± 0.3 ab	76.3 ± 2.1 b	520.1 ± 18.2 a
Urrestarazu	33 ± 1.6 a	25.2 ± 0.3 a	9.0 ± 0.1 a	109.5 ± 3.9 a	459.8 ± 26.9 a

^aMeans in each column and each factor with the same letter do not differ statistically (Tukey's, 0.05); ± SE.

Fig. 2. Fructans content in heads of *A. potatorum* plants grown in soil, fertigated with three nutrient solutions, before and after a drought period. Means in each column and each factor with the same letter do not differ statistically (Tukey's, 0.05).

possibly due to its agronomic handling in a greenhouse and to fertigation that affected the factors that control fructan accumulation in the plant stem.

Soil experiment. *A. potatorum* plants cultivated in soil and fertigated with Hoagland and Arnon and Urrestarazu solutions had up to 24 leaves per plant that were up to 21.8 cm long, with a maximum thickness and weight of 9.9 mm and 67 g, respectively (Table 3). This trend was similar to that obtained in perlite (Table 2), in which the plants fertigated with Steiner solution had less vegetative growth.

After the drought period, the plants continued to grow in all the fertigation treatments, increasing the length of their leaves by 1.8, 4.2, and 3.4 cm and 7, 15, and 9 leaves per plant in Steiner, Hoagland and Arnon, and Urrestarazu solution treatments, respectively, during this period. This growth may be due to high soil moisture retention in the greenhouse (data not presented) or to the native nitrogen content in the soil.

Before the drought period, the heads weighed between 180 and 294.2 g, and after 5 months without irrigation, head weight increased in all the treatments; the plants fertigated with Hoagland and Arnon solution were the heaviest (520 g).

The only variable that decreased after the period of drought was leaf thickness, coinciding with the results of the experiment in perlite (Table 2), since the plants used the water stored in the leaves to continue their physiological and growth functions (Pimienta-Barrios et al., 2006).

In soil, the greatest percentage of fructans (42%) was found in those plants fertigated with Hoagland and Arnon solution (Fig. 2); these plants also had the heaviest heads (Table 2). In plants with CAM such as agaves, distribution of assimilates to the various structures of the plants is affected by long periods of drought, when they are mainly accumulated in the reserve organs such as the stem and leaves (Barreto et al., 2010), so that the percentage of fructans present in the heads was significantly greater after the dry season.

Finally, it is concluded that *A. potatorum* plants have positive vegetative growth when they are fertigated. The nutrient solutions with high levels of nitrogen increase the biomass of the plants grown both in perlite and in soil. The 5-month drought period led to the accumulation of fructans in *A. potatorum* plant stems; a greater concentration was found in the plants grown in perlite and fertigated with the solution based on the Hoagland and Arnon formulation.

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