Article

Acclimatization and transplantation of *Agave angustifolia* Haw. vitroplants in wild conditions

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Abstract

Agave angustifolia Haw. it has been used in a traditional way in Sonora, for the elaboration of the Bacanora spirituous drink. As a consequence of the legalization of this distillate, wild populations are threatened by overuse. To contribute to this problem, the objective of this study was to evaluate the behavior of *A. angustifolia* vitroplants during their acclimatization to different substrates and transplantation under wild conditions. Four clonal lines (LC15s, LC26s, LC23b and LC24b) were evaluated in four substrates (control: sand, 'clay soil' and gravel (3:1:1); treatment I: peat: control mixture (1:1); treatment II: potting soil: control mixture (1:1) and treatment III: peat: potting soil: control mixture (1:1:1). The transplant site was characterized by analysis of vegetation and physical and chemical soil *in situ*, while the climate with cartography. These biotic and abiotic characteristics defined the transplantation of 1 000 vitroplants, 250 per clone, in three sections of a hillside with subtropical scrub. Height, number of leaves and cover of the agaves were monitored in a period of 66 weeks. After 60 d, the vitroplants were acclimatized with 100% survival in all treatments. The substrate with the highest content of organic matter favored rooting and root growth. The transplant reached a survival of 84 a 98%, under conditions wild with organic management. The response of the clonal lines was dissimilar during acclimatization and transplantation. The stages of the project were developed between 2009 and 2016.

Keywords: *Agave angustifolia*, micropropagation, subtropical scrub, survival.

Reception date: March 2020
Acceptance date: July 2020

Introduction

In Sonora, *Agave angustifolia* has been used for more than 300 years in the elaboration of the bacanora spirituous drink (Salazar *et al*., 2015), which was produced clandestinely for 77 years until the repeal of the bacanora dry law by the H. Congress of the state of Sonora in June 1992 (Salazar, 2007).

As a result, its production resumed economic impact in the mountain region of the state, but caused the wild populations of *A. angustifolia* to decrease significantly due to overuse in the Bacanora Denomination of Origin (DOB) area (Diario Oficial de la Federación, 06-I-2000), which could generate a negative environmental impact even more due to the aridity prevailing in the region (Nobel, 1994).

This situation is aggravated because it is a semelparous species with a life cycle of around seven years, a low establishment of seedlings derived from seed and asexual reproduction through a limited number of rhizomes, bulbils and bulbils insufficient to compensate the extraction. For a sustainable and economically viable production of bacanora, the availability of raw material is imperative.

The micropropagation of agaves through the sprouting of lateral meristems in the stem of the seedlings (Robert *et al*., 1992; Esqueda and Vargas, 2007; Millan-Soto *et al*., 2016) allows the massive production of vigorous, healthy, rejuvenated plants and it would help offset the erosion of wild populations. In addition, it offers the opportunity to increase the yield in foliar biomass through the selection and propagation of highly productive individuals as has been achieved with henequen (Eastmond *et al*., 2000), making fiber production more efficient, reducing costs and generating a system economically attractive for producers.

In vitro culture has been considered a viable alternative for the cloning of *A. angustifolia*, in which somatic embryogenesis stands out (Arzate-Fernández and Mejía-Franco, 2011) and encapsulated somatic embryos (synthetic seeds) (Arzate-Fernández *et al*., 2016). Enriquez-del Valle *et al*. (2005) evaluated different concentrations of inorganic salts and indole butyric acid for the budding of lateral meristems and rooting. The protocol of the present study differs in growth regulators (2,4-dichlorophenoxyacetic and 6-benzyladenine) and the concentration of salts.

A critical stage is the acclimatization of the vitroplants (Santacruz-Rubalcaba *et al*., 2008). Because it is challenging to transfer them from a medium rich in nutrients, growth regulators and saturated humidity, to a non-sterile substrate with uncontrolled relative humidity and temperature. Likewise, they still do not photosynthesize and their nutrition initially depends on the accumulated reserves (Monja-Mio *et al*., 2015). Therefore, organic substrates and fertigation favor the ex vitro adaptation of *A. angustifolia* so that they continue their growth and development (Enriquez-del Valle *et al*., 2009).

To obtain a high survival rate during acclimatization, a soft substrate with good drainage and moisture retention is necessary, which favors rapid root growth without drying out the plant. In this stage the roots acquire functionality to absorb water and nutrients, and the stomata to

control perspiration and therefore, the loss of water. During the *in vitro* to *ex vitro* phase in vitroplants of *A. angustifolia* the stomatal complex develops, increases the deposition of epicuticular waxes and the formation of calcium oxalate crystals on the epidermis of the leaf (Monja-Mio *et al*., 2015).

In predicting climate change for Mexico, Sonora will be the second state with the highest temperature increase (3.57 °C), as well as the decrease in precipitation with 21.3%, the latter shared with Baja California (Martinez-Austria and Patiño-Gomez, 2012). Therefore, the holistic agave transplant under grassland conditions developed by Esqueda *et al*. (2013), in the recovery of wild populations and sustainable management of *A. angustifolia* is important for the producers of the DOB area, where profitable productive projects are required. Some of the clones obtained from specimens from the Sonoran mountains have been characterized in terms of their metabolic efficiency (Millan-Soto *et al*., 2016), development of the stomatal complex (Monja-Mio *et al*., 2015) and epigenetics (Duarte-Ake *et al*., 2016). The objective of the present study was to evaluate the behavior of micropropagated plants of *Agave angustifolia* during their acclimatization in different substrates and their transplantation to wild conditions.

Materials and methods

Vitroplants

They were produced by sprouting lateral meristems in the stem of the seedlings in magenta boxes (Sigma Aldrich V8505), with modified culture medium from Murashige and Skoog (1962) (MS) and Robert *et al*. (1992). The medium of the multiplication and growth phase contained 0.05 and 0.025 mg L^{-1} of 2,4-dichlorophenoxyacetic, respectively; both with 1 mg L^{-1} of 6-benzyladenine (BA). In the rooting medium, 50% MS salts, 1% agar-agar, 0.025 mg L^{-1} of 2,4-D, without 6-BA were used. The culture room had a 12 h photoperiod with a light intensity of 50 µmol m^{-2} s^{-1} supplied by white fluorescent tubes and a temperature of 27 ±2 °C. The clonal lines (LC) evaluated were LC15s and LC26s from mother plants originating from seeds collected in Bacanora, Sonora (28° 58’ 44” north latitude, 109° 23’ 54” west longitude), while LC23b and LC24b came from of bulbilos of Moctezuma, Sonora (29° 48’ 11” north latitude, 109° 40’ 44” west longitude). These LCs were established in 2003, based on apparently healthy, vigorous and representative specimens of the species. The multiplication factor was calculated with the number of vitroplants produced at the end of the culture between the initial number. Nine subcultures with replacements were performed every six weeks.

Substrates

Four substrates were evaluated: control (T): sand, clay and gravel (3:1:1); treatment I (TI): peat: control mixture (1:1); Treatment II (TII): potting soil (black soil, litter soil and sawdust): control mixture (1: 1) and treatment III (TIII): peat: potting soil: control mixture (1:1:1). For each substrate, the physical and chemical factors were analyzed according to the methodologies indicated by Fernandez *et al*. (2006). The percentage of moisture retention and irrigation efficiency were determined with the protocols described in Davidson *et al*. (1999).

Acclimatization

The selected vitroplants were between 5 and 7 cm tall, vigorous and without any symptoms of disease. After removing the seedlings from the magenta boxes, they were washed with running water to remove excess agar, the roots were pruned to 0.5 cm in length and immersed for 3 min in a solution with 5 g L^{-1} of Captan 50%. The acclimatization was carried out in Hermosillo, Sonora located at 29° 07' 31" north latitude and 110° 53' 33" west longitude. During the 60 d of evaluation the temperature and relative humidity were of 18-32 average of 27 °C and 18-48 average of 28%, respectively (HOBO U12-012).

Polystyrene trays with 60 cavities of 5 × 5 × 7.5 cm with a capacity of 9 L were used on a shaded surface with 80% mesh and were irrigated at field capacity three times a week. The survival percentage was determined by multiplying the number of final seedlings by 100 by the initial value. Height was measured from the basal part of the stem to the tip of the longest leaf with a graduated ruler. The number of leaves was quantified and the root length with a ruler graduated in mm. The fresh and dry weight of the plants was recorded with a digital scale; they were dried at 45 °C for 48 h until constant weight. Each seedling was considered an experimental unit and the determination was made with five repetitions.

Transplant

It was established in a subtropical scrub in Ures, Sonora (29° 27' 51.45'' west longitude, 110° 15' 42.31'' west longitude) within the DOB area. The abiotic factors were characterized based on thematic charts and digital maps of climate, physiography, hydrology, geology, including taking in situ samples for the physical and chemical analysis of soils. Vegetation analysis was done using the method of random quadrants and Canfield lines, determining density, dominance, frequency and importance value of the different plant species (Bonham, 1989).

1000 vitroplants were transplanted with a size of 18 to 24 cm, previously hardened for *ca*. 8 months under shade with 50% mesh; 250 per clone in three sections (SI, SII and SIII) of a 439 (base, SI) hill at 446 masl (top, SIII) with a slope of 6%. The transplant was carried out at the beginning of the summer rains based on the abiotic and biotic characteristics of the site, prioritizing the nurse plants. The average distance was 1 m between the agave and the nurse, with one to three individuals per nurse. Height, number of leaves and cover of the agaves were measured, monitoring 24% of the individuals selected at random by clonal line.

Statistical analysis

For acclimatization, a completely randomized design was used with a factorial arrangement (substrates and clonal lines with four levels per factor), monitoring morphological aspects (number of leaves, plant height, dry weight, root length and percentage of rooting) for 60 d. The transplantation data were subjected to an analysis of variance (Andeva) with a completely randomized design, considering the number of leaves, height and cover of vitroplants of the four clonal lines in three sections of a hill for 66 weeks. When there was a significant difference between the treatments, a mean comparison test was performed according to Tukey's multiple range test ($p< 0.05$) (SAS v6.08). The plant species were compared between the site sections according to indices such as Pielou uniformity (J') and Jaccard similarity (J).

Results and discussion

Vitroplants

An average of 12 sprouts were obtained in> 50% of the explants, in a period of six weeks. After nine subcultures, each of the originally induced sprouts produced 729 ±308 new individuals. The sprouts that reached 6-8 cm in height were separated and transferred to a rooting medium. After three weeks, all the seedlings developed 2-3 cm long roots (Figure 1). The dry base biomass was 8.19, 9.82, 13.35 and 14.6% for LC15s, LC26s, 23b and LC24b respectively, being higher in the last two clones ($p< 0.05$). The multiplication factor of LC24b was statistically higher than the rest of the clones with a value of 2.6, while the other clonal lines presented similar values of 1.4 for LC26s and LC23b, and 1.3 for LC15s ($p< 0.05$).

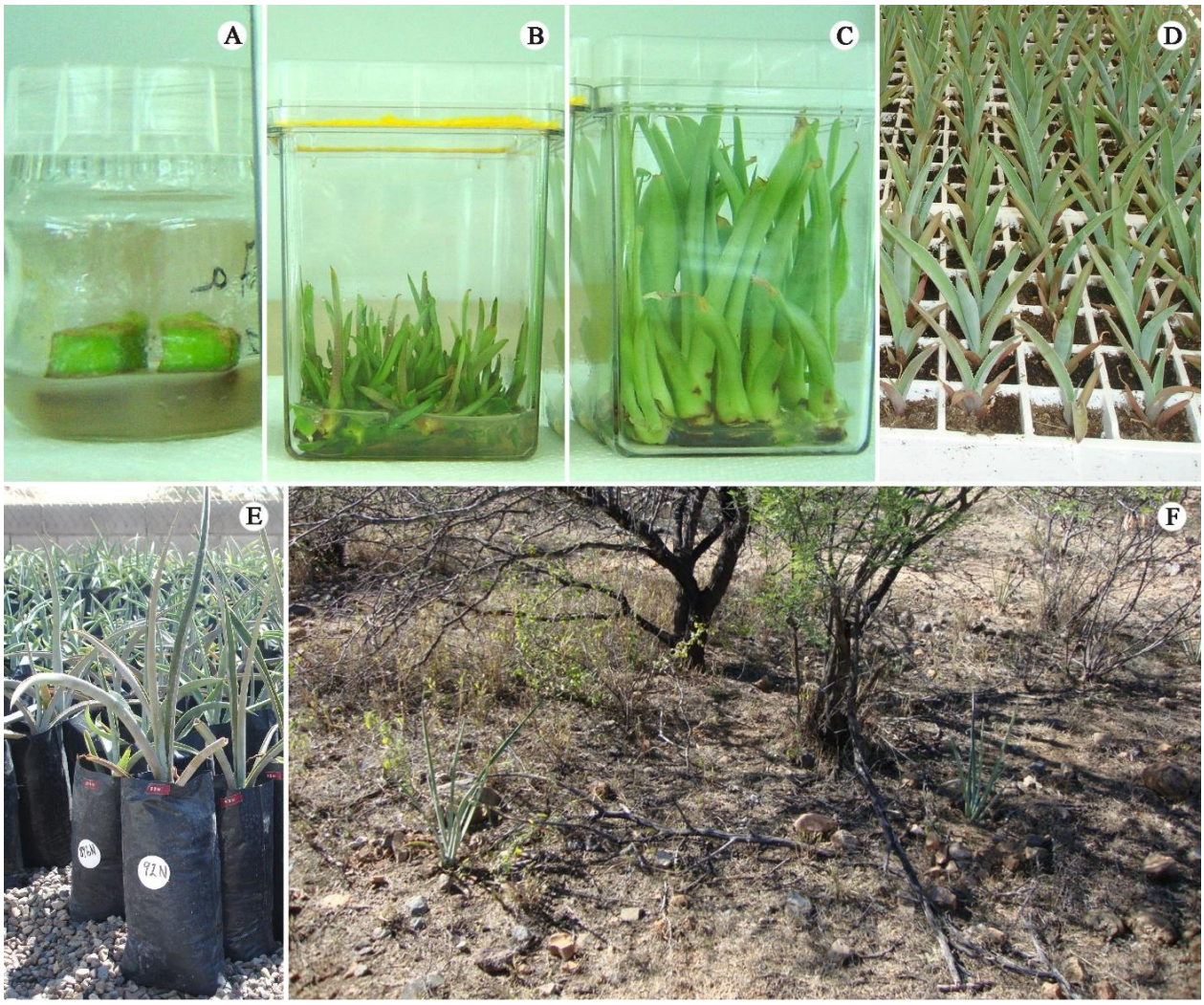

Figure 1. Phases of the micropropagation process of *Agave angustifolia*: Initiation (A) multiplication; (B) and rooting; (C) as well as *ex vitro* acclimatization phase; (D) hardening; and (E) transplantation in wild conditions (F).

Enríquez-del Valle *et al*. (2005) observed that the addition of indolebutyric acid (IBA) up to 1 mg L^{-1} stimulates sprout growth and root formation in *A. angustifolia* with an average per sprout of 8.6 roots and 6.4 leaves in 7 d (75% of MS salts and 0.75 mg L^{-1} IBA) vs 4.2 roots and 5.8 leaves in 14 d (100% MS salts without IBA). In the present study, the culture medium of the multiplication and growth phase contained 0.05 and 0.025 mg L^{-1} of 2,4-D respectively and both with 1 mg L^{-1} of 6-BA. In the rooting medium 0.025 mg L^{-1} of 2,4-D was used. The average number of leaves at the end of the in vitro culture fluctuated from 4.8 ±0.7 (LC15s) to 6.1 ±1.2 (LC24b), being lower than the previous work. However, a rooting medium with 50% MS salts, without 6-BA and 1% agar-agar was selected, considering that the transplantation would be carried out under wild conditions.

Micropropagation could be a cornerstone for sustainable bacanora production. It would make it possible to contribute to the domestication of the species, to produce highly productive agaves on a massive scale, to have enough material for planting, afforestation and recovery of wild populations, in accordance with an economic development program of the bacanora industry.

Acclimatization

Survival was 100% in all treatments. The four substrates showed adequate texture and nutrient content for the four clonal lines. LC15s, LC26s, LC23b and LC24b vitroplants have a low respiration rate (0.42-0.54 nmol CO_2 mg^{-1} seg^{-1}), low metabolic activity (14.3-20.5 µW mg^{-1} dry weight) and high metabolic efficiency (300-447 $kJmol^{-1}$) (Millan-Soto *et al*., 2016), which indicates an efficient use of the nutritional reserves accumulated *in vitro* until the roots are functional for the absorption of water and nutrients, and the stomata in the control of the perspiration.

The number of leaves varied according to the clonal line and the substrate ($p< 0.05$). The initial general average was 5.4 and the final average of 5.2. The greatest loss occurred at 20 d with an incipient recovery at 40 d, which coincided with the beginning of rooting. After 60 d, LC15s and LC26s had 10.2 and 11.1% more than the initial number of leaves; on the contrary, LC24b and LC23b had 5 and 20.1% fewer leaves, respectively. In relation to the substrate, the initial and final general average of leaves was 5.4 and 5.27 respectively; 6% lower in T and TII, the same in TIII and 1.8% more in TI. The observed in TI could be due to its greater irrigation efficiency. The moisture retention capacity of the substrate ranged from 7.5 (T) to 22.6 (TI). A high moisture retention capacity makes irrigation more efficient, which was lowest in T with 36% and the highest in TI with 80%.

The loss, recovery and gain of leaves was also observed during the acclimatization of *A. fourcroydes* (Abreu *et al*., 2007). The substrate and the fertilization dose with a greater contribution of organic matter favors the relationship between loss and formation of leaves, making it gradual. *A. americana* var. *oaxacensis* micropropagated replaces all its leaves formed *in vitro* generally at 240 d, acquiring the phenotypic characteristics that characterize the species (Cruz-García *et al*., 2017). During the *ex vitro* adaptation of *A. angustifolia*, the stomatal complex with guard and subsidiary cells develops, as well as the substomatic cavity, increasing in size and number adaxially and abaxially; increases the deposition of epicuticular waxes from 2.7 to 12.5 mg cm^{-2}, development of papillary structures and formation of calcium oxalate crystals on the epidermis including the substomatic cavity (Monja-Mio *et al*., 2015). All of the above favors a better control of evapotranspiration in the face of hydric and thermal stress under shaded areas.

The height of the vitroplants varied according to the clonal line and the substrate ($p< 0.05$). The initial and final average height was 6.2 vs 5.6 cm respectively. In relation to the substrate, the initial general average was 6.2 cm and at 60 d, 5.4, 6.0, 5.6 and 5.3 cm for T, TI, TII and TIII respectively. At the end of the evaluation period, the height was lower by 3 to 15% of the initial value. The decrease in size is associated with the loss of humidity; however, Pospisilova *et al*. (1999) observed only a slight reduction in stomatal functionality and water loss in *A. tequilana* vitroplants. Enriquez-del Valle *et al*. (2013), with two substrates and five levels of fertilization in *A. americana* var. *oaxacensis* observed a 0.98% correlation between fertilization and leaf length, which increased from 25 to 100% vs. control during acclimatization.

The dry matter content was similar between clones and substrates ($p> 0.05$). The initial and final average dry weight was 0.14 *vs* 0.12 respectively. At 60 d, 11 to 35% less than the initial dry weight was observed in all clonal lines except LC23b with an increase of 66%. In relation to the substrate, the initial general average was 0.14 g and at 60 d, 0.09 (T) and 0.13 g (TI, TII, TIII). This decrease in dry matter was associated with foliar loss. Abreu *et al*. (2007) observed this trend in *A. fourcroydes* and therefore concluded that vitroplants require 60 d in conditioning before transferring them to nurseries.

Rooting is crucial in acclimatization because it ensures the autotrophy of the vitroplant through the absorption of water and nutrients, which was null at 20 d in all treatments, while at 40 and 60 d an average of 17.8 and 94.7 % respectively. At 60 d, 94.7% of the total vitroplants were found rooted. LC15s showed the highest percentage of rooting with 100% and the lowest LC23b with 87.5% (Table 1). In relation to substrates, TIII and TI were more favorable with 98.8 and 97.5% rooting respectively and the lowest of 91.3% in T and TII at 60 d. This could be due to the higher content of phosphates and organic matter in TIII and TI, which leads to greater irrigation efficiency.

Table 1. Root length and rooting percentage in *Agave angustifolia* vitroplants during acclimatization.

LC	Substratum	Acclimatization days					
		Root length (cm)			% rooting		
		40	60	LR/LC	40	60	%/sust
15s	T	0.95 b	3.05 a	4.65 a	60	100	100
	TI	0.46 b	5.07 a		10	100	
	TII	0.21 b	4.52 a		20	100	
	TIII	0.22 b	5.96 a		20	100	
24b	T	0.26 b	1.71 a	3.33 b	20	85	93.8
	TI	0.2 b	3.05 a		20	100	
	TII	0.29 b	3.02 a		30	95	
	TIII	0.12 b	5.54 a		15	95	
26s	T	0.1 b	3.03 a	3.5 b	15	100	97.5
	TI	0.28 b	2.74 a		10	95	
	TII	0.32 b	3.16 a		45	95	

LC	Substratum	Acclimatization days					
		Root length (cm)			% rooting		
		40	60	LR/LC	40	60	%/sust
23b	TIII	0 b	5.08 a	2.51 c	0	100	87.5
	T	0.22 b	1.32 a		20	80	
	TI	0 b	2.63 a		0	95	
	TII	0 b	1.34 a		0	75	
	TIII	0 b	4.74 a		0	100	

Means with different letters in the same row and in LR/LC (root length/clonal line) in the same column are statistically different ($p< 0.05$).

Phosphorous stimulates the formation of roots with a greater availability of micronutrients at a pH of 6.2 (Ortiz and Ortiz, 1990). The pH of the substrates ranged from 6.9 (TI) to 8.7 (T) from neutral to slightly alkaline (Table 2). TI presented the highest nitrate content and TII the lowest with 30.2 and 15 mg kg^{-1} respectively. Although the requirement for this nutrient for *A. angustifolia* has not been determined, the values are below the reference of 35 mg kg^{-1} (Castellanos *et al*., 2000). Likewise, the highest and lowest phosphate content corresponded to TI and T with 92.4 and 59.7 mg kg^{-1} respectively (Table 2). The amount of phosphates is well above the reference value of 30 mg kg^{-1} (Castellanos *et al*., 2000).

Table 2. Physical and chemical analysis of the substrates.

Edaphic factor	Acclimatization substrates				Transplant site			Reference levels
	Control	TI	TII	TIII	SI	SII	SIII	
pH	8.7	6.9	8	8.1	6.4	6.4	6.8	7
$N\text{-}NO^{-}_{3}$ (mg kg^{-1})	16.5	30.2	15	17.1	13.9	14.1	14	35
$P\text{-}PO_4^{\equiv}$ (mg kg^{-1})	59.7	92.4	63.6	68.8	17.3	8.6	12.8	30
Ca (cmol kg^{-1})	5600	5090	5370	5070	940	900	1320	1600
Mg (cmol kg^{-1})	260	380	290	290	140	120	140	250
Na (mg L^{-1})	425	370	461	489	68	57	62	< 100
K (cmol kg^{-1})	146	123	98	87	170	190	191	150
OM (%)	0.6	4	3.4	4	0.94	1	0.67	2
Sand (%)	69	77	81	85	71	71	69	-
Silt (%)	22	18	14	12	20	20	20	-
Clay (%)	9	5	3	3	9	9	11	-
Texture	FA	AF	FA	AF	FAA	FAA	FAA	-
EC (dS m^{-1})	1.74	1.91	1.94	2.14	0.38	0.42	0.42	1
Saturation (%)	17.6	68.2	30.3	36.4	-	-	-	-
CRH	7.5	22.6	10.7	15	-	-	-	-
IE	36	80	66.7	70.4	-	-	-	-

EC= electrical conductivity; CRH= moisture retention capacity; IE= irrigation efficiency; OM= organic matter; FA= sandy loam; AF= loam sandy; FAA= Loam-sand-clay.

Root length varied according to clone and substrate ($p< 0.01$). The highest average after 60 d was recorded in LC15s with 4.7 cm and the lowest in LC23b with 2.5 cm. Regarding substrates, the longest root length (5.3 cm) was observed in TIII and in T, the smallest with 2.3 cm. In TIII, peat and potting soil facilitated root development, which could be due to the electrical conductivity of 2.1 dS m^{-1}, in addition to its high content of organic matter. The electrical conductivity is recommended less than 1.5 dS m^{-1} (Castellanos *et al*., 2000); however, in all treatments it was higher from 1.7 in T to 2.1 dS m^{-1} in TIII (Table 2). Organic matter ranged from very poor in T with 0.6% to rich in TI and TIII with 4%. Abreu *et al*. (2007) observed a survival of 83-93% in *A. fourcroydes* with 8-14% of organic matter in the substrate; however, a content greater than 10% with a high level of humidity favored a higher incidence of pathogens.

Enriquez-del Valle *et al*. (2009) observed that *A. angustifolia* vitroplants with 100% compost in the substrate and with fertigation in the nursery for six months accumulated 37.8 g of total biomass, height of 40.4 cm and 2.1% foliar N *vs*. 18.1 g, 23.5 cm and 1.7 % respectively, with 25% compost and without fertigation. Although this behavior is outstanding, since the transplant would be carried out under wild conditions without irrigation or fertilization, a hardening in the nursery was chosen with a substrate similar to the harsh conditions, limited irrigation and clonal lines of mother plants from an environmental region. similar to the site to be forested.

Transplant

The wild area has a BSo(h')hw(x') climate, which corresponds to hot dry with summer rains, average annual temperature and precipitation of 22-26 ºC and 250-500 mm respectively, and subtropical scrub vegetation. In SI, IBS and IBS from the transplant site, 10, 9 and 8 plant species were observed, with a Jaccard similarity index of 0.5, 0.54 and 0.58, and Pielou uniformity of 0.7, 0.84 and 0.8 respectively. *Mimosa laxiflora* presented the highest importance value (VI= 80.1) followed by *Fouquieria splendens* (VI= 33.2), *Bursera laxiflora* (VI= 32.3) and *Randia thurberi* (VI= 31.3). Thus, these species were prioritized as nurses of the vitroplants.

In the edaphic analysis, the percentage of organic matter fluctuated between 0.9 (SI) and 1% (SII) (poor level), the pH around neutrality from 6.4 (SII and SIII) to 6.8 (SI) and the electrical conductivity between 0.38 (SIII) and 0.42 (SII and SI) dS m^{-1} (Table 2). The phosphate concentration ranged from 8.6 (SII) to 17.3 mg kg^{-1} (SIII) and nitrates between 13.9 (SIII) and 14.1 mg kg^{-1} (SII). The texture was loam-sand-clay throughout the area. Statistical analyzes indicated that the physical and chemical characteristics of the soil are similar in the three sections. Nitrogen limits the growth of both cultivated and wild agave more frequently, followed by phosphorus and potassium (Nobel, 1994). In the Sonoran sierra, *A. angustifolia* is observed mainly in soils poor in fertility and organic matter, high percentage of stony, good drainage, shallow and with slight slopes (Esqueda *et al*., 2013).

The average initial height of the vitroplants was 18.2, 22.2, 22.8 and 24.1 cm in the clonal line 23b, 24b, 15s and 26s respectively (Figure 2). The overall average height increase was 30.5 cm at the end of the evaluation cycle, the highest being 34.6 cm in LC26s and the lowest of 24.6 cm in LC23b. Likewise, it was higher in the lower section with 25.8 cm ($p< 0.05$). Due to the higher multiplication factor, biomass accumulation and metabolic efficiency of clones originating from bulbils (Millan-Soto *et al*., 2016), a higher growth speed was expected in LC24b and LC23b.

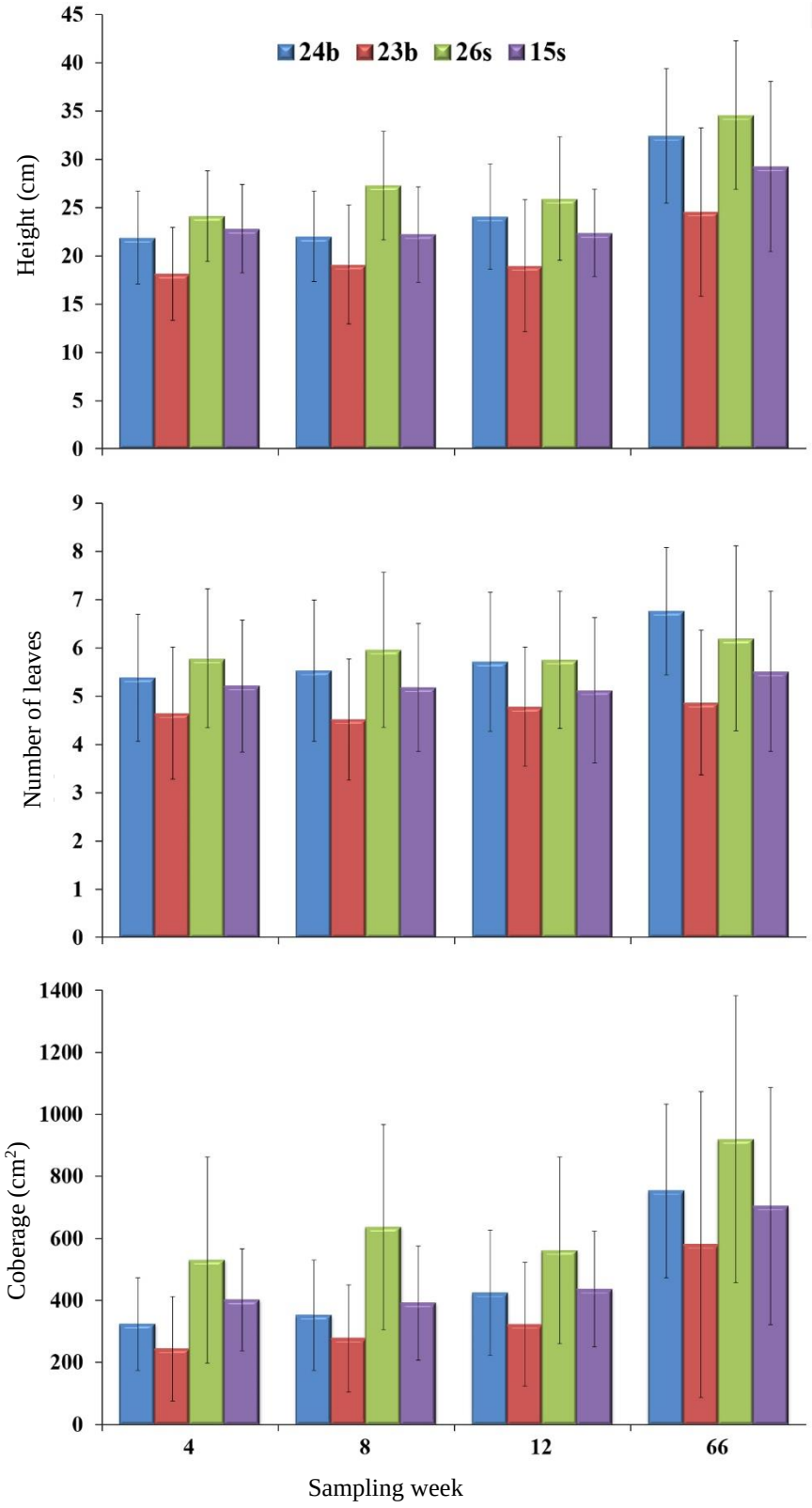

Figure 2. Height, number of leaves and coverage of four clonal lines of *Agave angustifolia* with respect to the sampling time. Bars= standard deviation.

Producers in the DOB zone consider that to ensure the survival of *A. angustifolia* after transplanting, it must have a minimum height of 40 cm to tolerate the low temperatures in winter and the prolonged drought throughout the year (Cervantes-Mendivil *et al*., 2007). In this study with an average initial height of 21.9 cm and an average survival rate of 89%, the dogma established for more than two decades of a minimum of 40 cm was broken. Due to the correlation between growth and initial height (r= 0.89), transplantation is suggested with individuals around 25 cm in height and not less than 20 cm.

The initial average number of leaves ranged from 4.7 (LC23b) to 5.8 (LC26s), with no increase at 60 d ($p> 0.05$). The results are considered consistent with the phenology of the species. The initial average coverage range was from 244 (LC23b) to 530 cm^2 (LC26s) ($p< 0.05$). At the end of the evaluation period, the coverage was statistically equal between LC26s (919 cm^2) and LC24b (752 cm^2), as well as between LC15s (703 cm^2) and LC23b (580 cm^2). The total average coverage increased from 374 to 739 cm^2 ($p< 0.05$) in the evaluation period with differences between sites, which could reflect the existence of microenvironments. Although *A. angustifolia* has a limited ability to switch between C3 and MAC metabolism, its high and persistent nocturnal CO_2 fixation (75-83%) and acidification (339-393 µmol H^+ g^{-1} fresh weight), provides potential for production of biomass in dry environments whose accumulation cannot be explained only by the assimilation of CO_2, but varies in a complex way due to environmental conditions such as photoperiod and temperature (Holtum and Winter, 2014).

Survival was 84, 86, 88, and 98% for LC23b, LC15s, LC26s, and LC24b, respectively. The vitroplants were developed under wild conditions without application of irrigation, fertilization, fungicides or insecticides, successfully coping with drought and winter. Designing the transplant considering critical factors such as nurse plants, orientation, radiation, slope, stoniness, as well as adequate acclimatization and hardening of the vitroplants are crucial for a high establishment.

Due to the unlimited production of seedlings and their possible scheduling in the micropropagation laboratory, the introduction of agaves could be planned as well as their extraction, leading to a sustainable use in overexploited areas. It would be easy to integrate with the management of the ecosystem in the Sonoran highlands, conserving its biodiversity and taking advantage of the nurse plants, likewise the cost of the micropropagated agave would be compensated by not requiring inputs or extra expenses for its cultivation in wild conditions. Currently, producers pay between 20 and 25 pesos per suckling ~ 30 cm high, a hardened vitroplant of ~ 15 cm costs around 60% of a rhizome, which is a viable alternative for the production of raw material destined to the elaboration of bacanora.

Conclusions

The micropropagation protocol by lateral bud sprouting was efficient. The most suitable substrates for the acclimatization of *Agave angustifolia* vitroplants under shade conditions had a loamy sandy texture with a rich content of organic matter, achieving 100% survival and at 60 d the hardening phase can be passed. The response of the clonal lines is dissimilar in the percentage of rooting, root length and biomass. Transplantation based on the biotic and abiotic characteristics of the area was suitable for afforestation with *A. angustifolia* vitroplants, using the canopy of nurse plants with a high survival rate.

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