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Research article

Ash *maguey*: incidence of the growth medium on the quality of the plant in nursery

Maguey cenizo: incidencia del medio de crecimiento sobre la calidad de planta en vivero

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Abstract

Agave durangensis is widely used in the production of mescal in the state of *Durango*. Despite its great economic and ecological importance, over time its natural populations have decreased, requiring its propagation in nursery for plantation programs, either for restoration or commercial purposes. The objective of this study was to determine the most appropriate mixture of substrates for the morphological growth of *A. durangensis* in the nursery stage. During nine months, the growth of plants produced in seven mixtures of substrates were evaluated, with different proportions of peat, composted pine bark, vermiculite and perlite. Regarding the morphological variables, the most favorable response was obtained by treatment with mixture of peat, composted pine bark and vermiculite in equal parts (33.3 %), while the plant with lower quality was produced in treatment with mixture of peat, composted pine bark and perlite in equal parts (33.3 %). The differentiated response of the development of the species to the mixtures of evaluated substrates, create the need to adjust the types of substrates and their proportions to the requirements of the species to be produced.

Keywords: *Agave durangensis* Gentry, composted pine bark, morphological development, mescal, substrate, peat.

Resumen

Agave durangensis es ampliamente usado en la producción de mezcal en el estado de Durango. A pesar de la gran importancia económica y ecológica que tiene, con el paso del tiempo sus poblaciones naturales han disminuido, requiriéndose su propagación en vivero para programas de plantación, ya sea con fines de restauración o comerciales. El objetivo de este estudio fue determinar la mezcla de sustratos más apropiada para el crecimiento morfológico de *A. durangensis* en etapa de vivero. Durante nueve meses se evaluó el crecimiento de la planta producida en charola tipo bloque de 54 cavidades con 200 mL de volumen; se utilizaron siete mezclas de sustratos con diferentes proporciones de turba, corteza de pino compostada, vermiculita y perlita. Respecto a

las variables morfológicas, la respuesta más favorable la obtuvo el tratamiento con la mezcla de turba, corteza y vermiculita en partes iguales (33.3 %), mientras que la planta con calidad menor, con la mezcla de turba, corteza y perlita en partes iguales (33.3 %). La respuesta diferenciada del desarrollo de la especie a las mezclas de sustratos evaluadas crea la necesidad de ajustar los tipos de sustrato y sus proporciones a los requerimientos de la especie que se desea producir.

Palabras clave: *Agave durangensis* Gentry, corteza de pino compostada, desarrollo morfológico, mezcal, sustrato, turba.

Introduction

In Mexico, the production of agave plants in nurseries for restoration or commercial plantations is regulated by the NMX-AA-SCFI-170-2016 standard (Certification of Forest Nursery Operations), which establishes minimum quality criteria for 139 species that the plant must meet prior to being planted in the field (Secretaría de Economía [SE], 2016). Although the goal of producing quality plants is a priority for the more than 150 nurseries in the country (Comisión Nacional Forestal [Conafor], 2020), the path taken by each nursery grower varies, leading to different methods of plant production (Aldrete et al., 2023).

Agave durangensis Gentry (ash *maguey*) is a non-timber natural resource notable for its economic importance, as it is used in the production of agave distillate. In 1997, the state of *Durango* obtained the "Mescal Designation of Origin," marking the beginning of a boom in its use, which resulted in the decline of wild populations (Loera-Gallegos et al., 2018). The species reproduces asexually through offshoots and bulbils; however, this leads to the loss of genetic diversity (Figueredo-Urbina et al., 2021). Another propagation alternative is sexual propagation. García-Rodríguez et al. (2023) mention that when the seed is properly managed, the germination rate exceeds 90 %. However, in the natural environment, predation and the limitation of

optimal temperature and humidity conditions affect its establishment, which puts its preservation at risk (Ramírez-Tobías *et al.*, 2012).

As an alternative to counteract the adverse effects of harvesting wild populations of *A. durangensis*, cultivating them from seed in a nursery is a feasible option. This involves managing various factors that influence plant development, from germination to planting in the field: inputs, environmental conditions, and pest and disease monitoring, among other aspects (Rosales-Serna *et al.*, 2020). Among the inputs, the substrate used to create the correct growth medium for the plant stands out (Aldrete *et al.*, 2023). The aforementioned standard recognizes that the function of a substrate is to support plant growth and is generally composed of a mixture of organic and inorganic materials (SE, 2016).

Peat was one of the first materials used as a substrate in container production, beginning in 1930 in England (Landis *et al.*, 1990); its use has now spread worldwide, leading to problems of overexploitation of the material (Gayosso-Rodríguez *et al.*, 2016; United Nations Environment Programme [UNEP], 2022). In the 1960s, the combined use of peat (50-60 %), vermiculite (20-30 %), and perlite (20-30 %) became popular in the United States; in Mexico, the use of these substrates in forest nurseries did not begin until 1990 (Aldrete & Aguilera-Rodríguez, 2019). In fact, most forest nurseries currently consider these components as part of the mix, which may be the response to what is established in the Mexican standard, which promotes three mix options, formed with different proportions of peat, vermiculite and perlite; while three other options include pine bark to produce plants in the container system (SE, 2016), without considering the species of interest.

Over time, changes in substrate components have varied. Thus, Prieto-Ruíz *et al.* (2009) indicate that only two of 11 nurseries in the state of *Durango* used pine bark as the substrate; the rest used mixtures containing 50 to 60 % peat, 20 to 25 % vermiculite, and 15 to 25 % perlite. This is despite the fact that, according to Aguilera-Rodríguez *et al.* (2021) and Madrid-Aispuro *et al.* (2020), it had already been

scientifically proven that the use of composted pine bark, as well as fresh pine sawdust, represent viable alternatives.

Furthermore, the use of a mixture composed of peat, vermiculite, and perlite (3:1:1; vol.) has become widespread. It is characterized by a low bulk density and high porosity for aeration and moisture retention, both for pine (Aldrete et al., 2023) and agave (García-Rodríguez et al., 2023; Rosales-Mata et al., 2013) production. However, the intrinsic requirements of each type of plant are different. Agave, having a crassulacean acid metabolism, has the ability to regulate the opening of its stomata to reduce water loss (Cruz-Vasconcelos et al., 2020). Therefore, in nursery production, substrate components that provide good drainage should be chosen for the mixture, since these types of plants are intolerant to waterlogging or excess moisture in the substrate.

In addition, peat provides moisture retention (Aldrete et al., 2023) and in the proportion used in a base mix for pine plants is usually beneficial, but its response to agave should be evaluated. Therefore, the objective of this study was to determine the most appropriate substrate mix for the development of *A. durangensis* in the nursery stage, based on the substrates most nursery growers use (peat, vermiculite, perlite, and composted pine bark). The hypothesis is that the substrate used should be formulated based on the needs of the species to be produced, rather than the production system, so at least one substrate mix should support its growth. The aim of the project was to contribute knowledge to the technical management of *A. durangensis* in the nursery production system.

Materials and Methods

Study area

The study was conducted at the *Praxedis Guerrero* forest nursery, operated by the Ministry of Natural Resources and Environment of the state government of *Durango*, Mexico, located at 12.5 km of the *Durango-Mezquital* highway, at 23°56'58.3" N and 104°34'07.4" W, at 1 890 masl.

Production conditions

The seed was collected from a natural stand where the predominant vegetation is composed of *A. durangensis*, *Dasyllirion* spp. and *Neltuma* spp., located in the town of *Nombre de Dios*, *Durango*, Mexico (23°58'59" N and 104°19'29" W, at 1 846 masl).

Prior to sowing, as a pre-germination treatment, the seed was soaked in water at room temperature for 8 h. At the end of this process, the seedhead was coated with Tecto®60 fungicide (Syngenta®, Mexico). Sowing took place in a greenhouse with a metal frame protected with 720-gauge white polyethylene film, covered in 50 % shade netting and side curtains; the average maximum and minimum temperatures were 38 and 5 °C, respectively.

Sixty days after sowing, the plant was transplanted into a rigid, black polyethylene block-type tray with 54 cavities and internal root guides (200 mL volume, 4.8 cm upper diameter, 2.83 cm lower diameter, and 15 cm long). Five months after sowing, the plant material was moved to an area with 50 % shade netting, with an average maximum temperature of 37 °C and a minimum of 13 °C for four months. The plant was grown in a nursery for nine months, from December 17th, 2019, to September 18th, 2020.

Treatments and experimental design

Seven substrate mixtures based on peat, composted pine bark, vermiculite and perlite were evaluated (Table 1). During the preparation of each mixture, 4 g L⁻¹ of slow-release granular fertilizer Multicote 8[®] (Haifa Chemicals Ltd., Israel) with a 12N-25P-12K+ME composition was added. In addition, 50 ppm of Peters[®] soluble fertilizer (ICL, USA) with a 9N-45P-15K composition was applied once a week during the establishment stage (two to five months after planting). From the fifth to ninth month after planting, during the rapid growth stage, irrigation was carried out with the addition of 100 ppm of Peters[®] soluble fertilizer (ICL, USA) composed of 20N-10P-20K.

Table 1. Substrate evaluated mixtures.

Treatment	Composition
1	Peat, composted bark, vermiculite and perlite (50:20:15:15)
2	Peat, composted bark, vermiculite and perlite (30:40:15:15)
3	Composted bark, vermiculite and perlite (50:25:25)
4	Peat, vermiculite and perlite (50:25:25)
5	Peat, composted bark and perlite (33.3:33.3:33.3)
6	Peat, composted bark and vermiculite (33.3:33.3:33.3)
7	Peat and composted bark (50:50)

The treatments were distributed in a randomized complete block experimental design. Each treatment consisted of four replications with 54 plants each. The total porosity, aeration porosity, and water retention capacity of the substrates evaluated were

determined using the methodology described in Mexican standard NMX-AA-170-SCFI-2016 (SE, 2016) (Table 2).

Table 2. Porosity of the substrate evaluated mixtures.

Treatment	Total porosity (%)	Aerating porosity (%)	Water retention capacity (%)
1	48	27	21
2	58	35	23
3	63	37	26
4	55	37	18
5	58	35	23
6	58	35	23
7	61	38	23
Reference value	60-80*	20-35**	25-55*

1 = Peat, composted bark, vermiculite and perlite (50:20:15:15); 2 = Peat, composted bark, vermiculite and perlite (30:40:15:15); 3 = Composted bark, vermiculite and perlite (50:25:25); 4 = Peat, vermiculite and perlite (50:25:25); 5 = Peat, composted bark and perlite (33.3:33.3:33.3); 6 = Peat, composted bark and vermiculite (33.3:33.3:33.3); 7 = Peat and composted bark (50:50).

*Landis et al. (1990); **SE (2016).

Morphological variables evaluated

The plant was evaluated nine months after planting. Ten central plants were removed from each replicate (a 54-well tray) to avoid edge effects (40 plants in total per treatment). The root substrate was removed with water to avoid damaging

the roots. The height (cm), root length (cm), and rosette diameter (cm) were then determined using a model RAS-30 PILOT® graduated ruler; the root base diameter (mm) and stem diameter (mm) were determined using a model CAL-6MP Truper® digital caliper; and the dry biomass of the shoot and root portion (g) were determined (Figure 1).

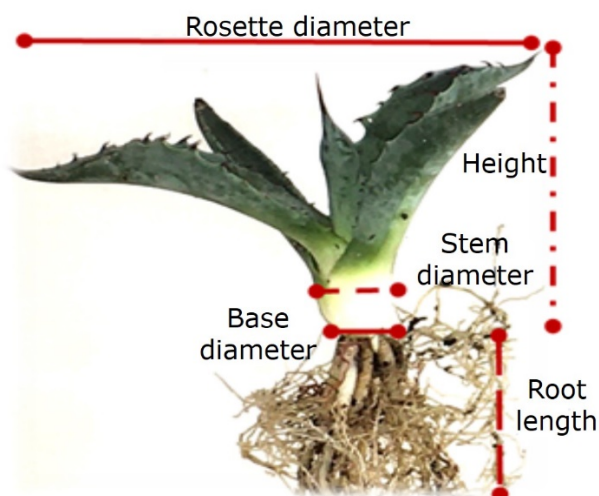


Figure 1. Schematic of the variables evaluated.

To estimate dry biomass, each plant was cross-sectioned to separate the root from the aboveground portion. The aboveground portion was then segmented to remove the leaves from the stem, and each leaf was scored on both sides to facilitate drying. The fresh root and aboveground segmented biomass was placed inside a Kraft paper bag and dehydrated in a forced-air oven (model 9024A Ecoshel®) at 65 °C for 72 h (Ramírez-Tobías et al., 2014). Once the plants reached a constant weight, the dry weight was determined using an analytical balance (model Pioneer PA512 Ohaus®). The data were analyzed using the PROC GLM procedure in SAS 9.2® (SAS Institute Inc., 2009); when significant differences were found, Tukey's mean separation tests were performed ($p \leq 0.05$). In all cases, the assumption of normality and homogeneity

of variance were tested using the Shapiro-Wilk and Levene tests, respectively (Contreras-Cruz, 2023).

Results and Discussion

For the height variable, treatments 1, 2, 4, and 6 stood out statistically ($p \leq 0.05$). The difference between the extreme values was 1.8 cm. For root length, the results showed significant differences ($p \leq 0.05$) between treatments, with the highest value in T2 and the lowest in T5 and T7; the difference between the extreme values was 1.2 cm (Table 3 and Figure 2).

Table 3. Root height and length of *Agave durangensis* Gentry with various substrate mixtures, nine months after planting.

Treatment	Height (cm)	Root length (cm)
1	6.1±0.1 a	12.0±0.3 ab
2	6.1±0.2 a	12.4±0.2 a
3	5.6±0.1 ab	11.8±0.1 ab
4	5.8±0.1 a	11.9±0.2 ab
5	4.3±0.1 c	11.2±0.3 b
6	6.1±0.2 a	11.8±0.1 ab
7	5.1±0.1 b	11.4±0.3 b
<i>p</i> value	<0.0001	<0.0028

1 = Peat, composted bark, vermiculite and perlite (50:20:15:15); 2 = Peat, composted bark, vermiculite and perlite (30:40:15:15); 3 = Composted bark, vermiculite and perlite (50:25:25); 4 = Peat, vermiculite and perlite (50:25:25); 5 = Peat, composted bark and perlite (33.3:33.3:33.3); 6 = Peat, composted bark and

vermiculite (33.3:33.3:33.3); 7 = Peat and composted bark (50:50). Different letters in the same column indicate differences ($p \leq 0.05$).



Figure 2. Morphological appearance of the plant, by treatment, nine months after planting.

Hernández-Vargas et al. (2006) evaluated the response of *A. durangensis* seedling transplants to unmulched soil, mulched soil, and pots. Seven months after transplanting, they recorded an average height of 7.4 cm, with the highest growth being obtained by transplanting to mulched soil, a value higher than that obtained in the present investigation, in which the growth medium was limited by the volume of the container. Furthermore, this trial began during the winter season, which could have influenced the final height reached, since temperatures below zero and frost have been reported to directly affect agave development (Arreola-Tostado et al., 2020), and they recover once temperature becomes higher.

Sandoval-Ramírez (2019) reported an average root length of 10.9 cm in *A. angustifolia* Haw. seedlings when evaluating the effect of two types of substrate (coconut fiber and perlite) and the mixture of both with a concentration of nutrient solution on growth and N and P concentrations, a value lower than that of the present investigation; however, this can be explained by the different phenotypic characteristics in the growth of both species.

It should be noted that the treatments with shorter root height and length (T5 and T7) share the characteristic of omitting the vermiculite substrate from the mixture. This could have reduced Cation Exchange Capacity (*CEC*) and, therefore, affected nutrient assimilation, impacting the morphological development of the plant; as mentioned by Monsalve-Camacho *et al.* (2021), vermiculite is a material of geological origin with a high *CEC*, which helps retain nutrients in the growth medium, so that plants can take advantage of them.

The average values for the variables base diameter, stem diameter, and rosette diameter showed significant differences ($p \leq 0.05$) between treatments. For base diameter, the difference between extreme values was 2.9 mm; the best treatments were T4 and T6, while T5 and T7 recorded the lowest values. Regarding stem diameter, the difference between extreme values was 4.2 mm, with T4 being the most prominent, while T5 had the lowest value. Regarding rosette diameter, the dissimilarity between extreme values was 2.3 cm, with T6 in the highest statistical group and T5 at the lowest (Table 4).

Table 4. Base, stem and rosette diameters of *Agave durangensis* Gentry in various substrate mixtures nine months after planting.

Treatment	Diameter		
	Base (mm)	Stem (mm)	Rossete(cm)
1	8.8±0.3 ab	13.6±0.3 bc	7.3±0.3 abc
2	8.6±0.3 ab	14.5±0.3 ab	7.2±0.3 abc
3	7.8±0.2 bc	13.1±0.3 cd	6.8±0.2 bcd
4	8.9±0.3 a	14.9±0.3 a	7.5±0.2 ab
5	6.8±0.2 c	10.7±0.2 e	5.8±0.2 d
6	9.7±0.3 a	14.0±0.4 abc	8.1±0.3 a
7	7.2±0.2 c	12.0±0.3 d	6.3±0.3 cd
<i>p</i> value	<0.0001	<0.0001	<0.0001

1 = Peat, composted bark, vermiculite and perlite (50:20:15:15); 2 = Peat, composted bark, vermiculite and perlite (30:40:15:15); 3 = Composted bark, vermiculite and perlite (50:25:25); 4 = Peat, vermiculite and perlite (50:25:25); 5 = Peat, composted bark and perlite (33.3:33.3:33.3); 6 = Peat, composted bark and vermiculite (33.3:33.3:33.3); 7 = Peat and composted bark (50:50). Different letters in the same column indicate differences ($p \leq 0.05$).

Base diameter is not a variable that has been previously used in agave studies, but it was considered an appropriate indicator of the robustness of the root system with respect to stem diameter. Enríquez-del Valle et al. (2013) reached a lower result; they evaluated the effect of substrates and fertigation doses on the acclimatization and growth of *A. americana* L. var. *oaxacensis* Gentry seedlings obtained *in vitro*, and recorded an average value of 13 mm. Sandoval-Ramírez (2019) studied the effect of substrates and nutrient solution concentration on the growth and concentration of N and P in *A. angustifolia* seedlings and obtained an average value of 9.71 mm, lower than the one calculated in the present investigation. The difference between the values may be due to the composition of the mixture used, which was coconut fiber and perlite in equal proportions; in this case for this variable, the best results were achieved when the mixtures were integrated from 0 to 25 % perlite.

Ramírez-Tobías et al. (2014) described that this type of plant has succulent leaves and thick cuticles that allow for water storage; in the present study, it was found that the use of peat, composted pine bark, and vermiculite allowed for improved rosette development, that is, a better response in environments with humidity restrictions. Hernández-Vargas et al. (2006) established an average rosette diameter of 10 cm seven months after transplanting *A. durangensis* into a bag; this value is higher than that recorded in this study, a difference that can be explained by the age of the plant at the time of evaluation.

The average dry aboveground biomass value showed a difference ($p \leq 0.05$) between treatments; the difference between extreme values was 0.8 g, with T6 being the most notable; the opposite was observed in T5 and T7. In the case of dry root biomass, there were also significant differences between treatments, with a difference of 0.23 g between extreme values; T6 showed the best results, while the opposite was true for T5 (Table 5).

Table 5. Dry aboveground and root biomass of *Agave durangensis* Gentry in treatments with various substrate mixtures nine months after planting.

Treatment	Dry biomass (g)	
	Aerial	Root
1	1.1±0.07 b	0.28±0.02 cd
2	1.2±0.06 ab	0.33±0.02 abc
3	1.1±0.06 b	0.32±0.02 bc
4	1.2±0.05 ab	0.36±0.02 ab
5	0.6±0.04 c	0.17±0.01 e
6	1.4±0.10 a	0.41±0.03 a
7	0.8±0.06 c	0.23±0.02 de
<i>p</i> value	<0.0001	<0.0001

1 = Peat, composted bark, vermiculite and perlite (50:20:15:15); 2 = Peat, composted bark, vermiculite and perlite (30:40:15:15); 3 = Composted bark, vermiculite and perlite (50:25:25); 4 = Peat, vermiculite and perlite (50:25:25); 5 = Peat, composted bark and perlite (33.3:33.3:33.3); 6 = Peat, composted bark and vermiculite (33.3:33.3:33.3); 7 = Peat and composted bark (50:50). Different letters in the same column indicate differences ($p \leq 0.05$).

When assessing the effect of substrates and fertigation rates on the acclimatization and growth of *A. americana* seedlings obtained *in vitro*, Enríquez-del Valle et al. (2013) determined an average value of 0.72 g for aboveground dry biomass, while for root

biomass it was 0.30 g; lower values than those obtained in this study. Rosales-Mata et al. (2013) analyzed agave production in a nursery and concluded that the best plant growth was achieved with the combination of peat, perlite and vermiculite, in a proportion of 55:21:24, respectively; in this case the results showed that the combination of peat, composted pine bark and vermiculite in equal proportions made possible a better development of the plant; in addition, they pointed out that the intrinsic characteristics of the substrates had more effect than the porosity characteristics of the mixtures; therefore, despite the fact that all treatments received the same fertilization, nutrient absorption was favored in the mixtures with vermiculite, which were the ones that recorded the highest dry biomass production.

Conclusions

Plant growth was best in the mixture composed of peat, composted pine bark, and vermiculite in equal parts (33.3 %). The lowest-quality plant was obtained when the mixture of peat, composted pine bark, and perlite was used in equal parts (33.3 %). The differentiated developmental response of the species to the mixtures alludes to the need to adjust the types of substrates and their proportions to the requirements of the species to be produced.

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Conflicts of interest

The authors declare no conflicts of interest.

Contribution by Author

Alejandra Medina García: establishment, monitoring and evaluation of the experimental trial; Silvia Salcido Ruiz: design and supervision of the trial, as well as writing of the manuscript; José Ángel Prieto Ruíz: design, supervision of the experiment and review of the manuscript; Enrique Santana Aispuro: establishment and monitoring of the experimental trial; Rosa Elvira Madrid Aispuro: statistical analysis and review of the manuscript.

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