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Seed morphometry and NaCl and sucrose effect on germination rates and phytochemicals in sotol (*Dasylirion acrotrichum*) from the Chihuahua state, Mexico

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Abstract. In Mexico, "sotoles" (*Dasylirion* spp.) are used to elaborate the "sotol" liquor, handicraft making, and construction materials. These activities have enhanced the probability that sotol populations could decline because plants are extracted directly from their native ecosystems, given no commercial sotol plant species exist. Furthermore, from an ecological point of view, this genus has also been little studied regarding its seed morphology and germination process. The objective of this study was to characterize the morphometry of the *D. acrotrichum* seeds and to evaluate the effect of different concentrations of NaCl (30, 60, and 90 mM) and sucrose (30, 60, and 90 mM) on seed germination rates, seedling morphology, and the phytochemical content in the seedling leaves under in vitro conditions from plants grown in the Chihuahua State. The exogenous NaCl and sucrose treatments influenced the germination speed index, mean germination time, and mean germination speed. The NaCl treatment impacted the total weight, root weight, and leaf length of seedlings, the content of chlorophyll a, sugar, and phenolics, and the antioxidant activity determined by FRAP. Besides, the sucrose treatment affected the seedling length, the content of chlorophyll a, chlorophyll b, total chlorophyll, and sugars, and the antioxidant activity determined by FRAP and DPPH. These results could provide the guidelines to generate better afforestation programs in the areas affected by the overexploitation of the *Dasylirion* spp. and new information about the capacity of these species for developing under these types of stress in the natural environment.

Key words: Sotol, stress, seeds, Chihuahua State, *Dasylirion* spp.

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Introduction

The genus *Dasylirion* Zucc. belongs to the order Asparagales and Asparagaceae family (Bogler, 1998). This genus includes twenty-two plant species (Bogler, 1998), many of them developed in the xerophytic scrub of the arid and semi-arid climate regions of north-central Mexico and the south of the United States of America. In Mexico, these species are distributed mainly along the Chihuahuan Desert, encompassing the states of Chihuahua, Coahuila, Durango, Nuevo León, and Zacatecas (Bogler, 1998; Villarreal-Quintanilla *et al*., 2016). However, the species of this genus form two groups, one distributed in the south, center, and northeast of Mexico, which is different from the second one that spreads by the south of Texas and the north of Coahuila and Sonora States (Reyes-Valdes *et al*., 2012).

Most species constituting this genus grow on rocky soils, in basins and mountain ranges on high plateaus with extreme geographical and climate conditions (IMPI, 2002), especially in the arid zones of the Chihuahuan desert. Some plant species included in this genus are *Euphorbia antisyphilitica*, *Agave lechuguilla*, *Bouteloua ramose*, *Coldenia canescens*, and *Jatropha dioica*.

The plants of the *Dasylirion* genus are commonly named sotoles. These plants are perennial, and polycarpic, with a height between 0.6 m and 1.5 m, and are found mainly in wild populations, although some have been eventually established under cultivation (Orozco-Sifuentes *et al*., 2019). Furthermore, the sotol plants present long, flexible, rosette leaves stemming from the stalk, with pencil-like tips and thorny edges. Their floral scapes or sticks can reach up to 5 m in height, comprising an inflorescence of little flowers inside panicles and elliptical narrow fruits. The individuals of these plants are allocated into two reproductive forms, males, and females, being a dioecious genus (Reyes-Valdes *et al*., 2012). The sotol plants are important because some vestiges studies conclude that plants were essential for the human diet in American prehistory, especially for those human populations who were growing in the arid and semiarid regions (Short *et al*., 2015), where natives cooked the stalk in pits filled with hot stones. Nowadays in Mexico, the sotol plants have different uses, i) to produce an alcoholic beverage known by the same name, ii) local people use the leaves as a source of handcraft making and construction materials, iii) to feed humans, cattle, and wild animals during droughts stations, iv) to reforest the urban, and v) sotol is an important ecological component of desert zones since it contributes to soil maintenance while being a food source for some of the desert fauna, particularly rodents and birds (IMPI, 2002; Olhagaray *et al.*, 2004; Reyes-Valdes *et al*., 2012; Sierra *et al.*, 2008). Under natural conditions, sotol plants only reproduce sexually by seed. The sotol seeds are also a food source for birds and other wild animals (Villavicencio-Gutiérrez *et al*., 2007). These activities have enhanced the probability that the sotol populations could decline because plants are extracted directly from their native ecosystems, as there are no commercial sotol species crops (Villavicencio-Gutiérrez *et al.*, 2007).

The *D. acrotrichum* (Schiede) Zucc. is an endemic plant species of Mexico located in the arid areas where grows on gravelly soils with suitable soil drainage, on creek edges and mountains hillsides where the submontane shrubs and xerophytic vegetation occur. This species is a polycarpic plant with a slowly developing evergreen and woody stem. This species has a high cultural value because is used as firewood (Arias *et al*., 2000), the floral scapus is used for human consumption and livestock (Torres, 2016). The leaves are used for tying roofs and creating ornaments for religious festivals (López-Gutiérrez, 2010). According to the Mexican Official Norm NOM-059-SEMARNAT 2010, *D. acrotrichum* is categorized as a threatened species due to certain factors that cause the deterioration of its habitat or directly reduce the size of the wild populations (Torres, 2016).

The *Dasylirion* genus has been slightly studied, focusing on the distribution and diversity of the species (Encina-Domínguez *et al*., 2013), the sex distribution (Reyes-Valdés *et al*., 2017), and genetic diversity (Pinales-Quero *et al*., 2017). In a previous study, some *Dasylirion* species showed tolerance to humidity and high temperature for the storage duration (Probert *et al*., 2009). More species of this genus generate many seeds per female plant (95,000 seeds per kg), which ensures the establishment of a high seedlings number in natural conditions (Reyes-Valdés *et al*., 2012). Another study on sotol seed germination established that seedling emerges 14 days after seed imbibition *in vitro* condition, and the root reaches 6 cm 48 days after sowing (Francisco-Francisco *et al*., 2016). However, the variation in germination time and subsequent growth of the seedlings is substantial in the natural

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population, making it difficult to establish an average characteristic for the seedlings; besides, the action of adverse events such as drought and foraging by wildlife or livestock could sharply diminish the population of sotol plants (Vega-Cruz *et al*., 2006).

The studies on the seed phytochemical content in *Dasylirion* species are scarce. In a previous proximal analysis, the seed flour from *D. cedrosanum* showed a high content of fat (18.4%), fiber (16.2%), protein (27.7%), and minerals (Ca, Fe, Zn, and Cu) compared to the wheat flour (Orozco-Sifuentes *et al*., 2019). However, detailed information regarding these compounds in seeds from the *Dasylirion* genus is lacking. Additionally, as the seed is the crucial stage for the propagation and perpetuation of species, is essential to determine its morphology and germination. This knowledge may reveal the strategies that these species use for establishing and maintaining themselves in their environments and to resist the climatic fluctuations, making possible to evaluate the potential success of the species in ecological restoration. Because of climate change, the concern about the effect of abiotic stresses such as salinity and drought has increased, as it affects the development of both natural plants and crops and extends more than 800 million ha of lands worldwide (Acosta-Motos *et al.,* 2017). The salinity stress mainly reduces the plant production and growth in semiarid and arid regions, where soil salt content is naturally higher, and the precipitation in these areas may be insufficient for leaching (Farooq *et al.*, 2022). In the present study, the effect of different concentrations of NaCl and sucrose under *in vitro* conditions on seed germination rates, seedling morphology, and phytochemicals in the *D. acrotrichum* species from the Chihuahua state, Mexico was evaluated. In addition, the study contributed with seed morphometric data of this sotol species. This information could provide the guidelines to generate better afforestation programs in the areas affected by the overexploitation of the species and their development under saline stress and drought conditions in the natural environment.

Materials and Methods

Plant material

The *D. acrotrichum* plants were selected on September 28, 2019, from a population in Nuevo Casas Grandes (30° 23’ 54’’ N, 107° 59’ 51’’ O), Chihuahua, México (Figure 1A). The seed collection was performed by sampling ten healthy plants selected randomly per area. The mature inflorescences were cut manually, their seeds hand-harvested, stored in an airtight polyethylene bag, and transported immediately to the laboratory. Then, the seeds were air-dried for 48 h and stored in a paper bag covered with sealed plastic under refrigeration at 4 ºC until processing (Figure 1B). The seeds were cleaned, and the shells were eliminated by hand (Figure 1C).

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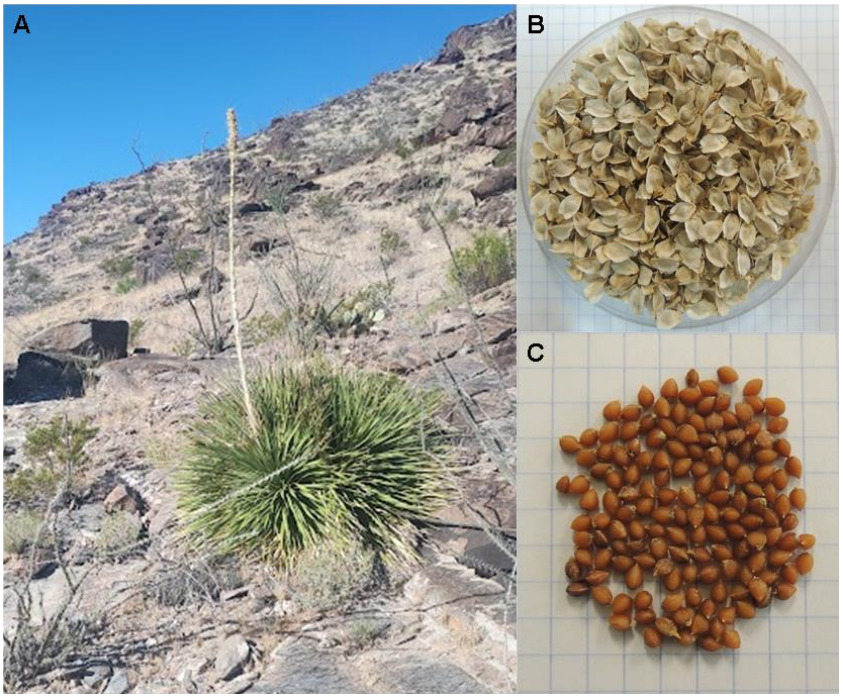

Figure 1. The plant specimen of sotol growing in natural conditions (A). General view of non-scarified (B) and scarified (C) *D. acrotrichum* seeds.

Seed morphometry analysis

The morphometry was determined in 90 seeds randomly selected and individually weighed using an analytical balance (Mettler Toledo AJ150, Ciudad de México, México). Then, the seeds were placed on a millimeter sheet for digital photographing. The images were used to measure the seed length, diameter, area, and perimeter by the free software ImageJ (ImageJ, Bethesda, MD, USA).

Seed germination

The seeds were previously sterilized using 10% (v/v) sodio hypochlorite (from commercial bleach 6%) for 5 min, rinsed for 2 min in distilled water to remove any hypochlorite residues, and then treated with the antifungal Captan 50 WP ADAMA for 2 min (Reyes-Corral *et al*., 2022). Three different concentrations of sucrose (control, 30, 60, and 90 mM) (99.9%, Fagalab®) and NaCl (control, 30, 60, and 90 mM) (99.5%, J.T Baker®) were evaluated for the treatment groups. Fifteen seeds were placed in a Petri dish (11 × 11 × 3.5 cm) between two discs of blotting paper cut according to their diameter to allow seed imbibition. Every group was performed in triplicate. For applying the different treatments and allowing the seed imbibition, the paper discs were moistened with 3 mL per solution for the treatments or distilled water for control group every third day under a laminar flow hood (Envirco® mod. PN1370) to avoid contaminants (Reyes-Corral *et al*., 2022). Then, the Petri dishes were kept in a germination chamber at 25 °C and 12 h photoperiod. The number of germinated seeds was counted daily for 21 days, using the emission of radicle as the germination criterion. For considering one seed germinated, the radicle tip should protrude ≥ 1 mm out of the operculum opening. The percentage of germination, average germination time, average germination rate, and relative germination frequency were estimated using the formulas proposed by Souza *et al*. (2016).

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Seedlings phenotypical characterization

After 21 days, the Petri dishes containing seedlings were removed from the environmental chamber, and ten homogeneous seedlings were selected from each treatment to evaluate the morphometric characteristics. The seedlings were collected separately by hand from the Petri dishes and then photographed on a millimeter sheet. The length of the aerial part and roots of each seedling were measured from images using ImageJ software (Bethesda, MD, USA). Subsequently, the aerial part and roots were manually separated from each seedling per each treatment and individually weighed using an analytical balance (Denver Instrument Apx-200).

Extraction and quantification of chlorophylls

A standard extract was obtained according to the method proposed by Lichtenthaler (1987) with some modifications. Briefly, 0.1 g of leaves were ground in a mortar using a pestle until a fine powder for each sample (one per seedling as biological replicate). Then, 2 mL of 100% (v/v) methanol (JT Baker®, USA) was added to the pulverized and blended with the same pestle. Subsequently, the homogenate was recovered into a 1.5 mL microtube with the help of a micropipette. Later, the sample was stirred and sonicated at 4 °C in darkness for 30 min. Afterward, the extract was centrifuged at 12,000 rpm at 4 °C for 5 min, and the supernatant was collected into a new 1.5 mL microtube. Three independent replicates (three biological replicates per each treatment) of 200 µL of the extract were placed in three separate wells of a 96-well microplate. The absorbances were measured at 632 nm, 652 nm, 665 nm, and 696 nm in a Bio-Rad xMark Plus (Hercules, USA) spectrophotometer. The data were obtained with the Microplate Manager 6.0 (Tokyo, Japan) computer software. The concentration of chlorophyll a, chlorophyll b, chlorophyll c, chlorophyll d, and total chlorophylls was determined using the equations proposed by Ritchie (2008).

Phytochemicals quantification

The leaf extracts were obtained using the protocol proposed by Álvarez-Parrilla *et al*. (2011) with minor changes. In summary, 100 mg of each tissue from each treatment was weighed separately into a 2 mL microtube. Then, 1 mL of 80% (v/v) methanolic solution was added, and the sample was stirred and sonicated for 30 min at 4 ºC in the dark. Next, the extracts were centrifuged at 3000 × *g* for 10 min at 4 ºC using a microcentrifuge (Eppendorf model 5810 R), and the supernatant was transferred into a new 2 mL microtube. The procedure was repeated, obtaining a total extract volume of 2 mL. Every extract was made in triplicate for each treatment. The quantification of phenolics, flavonoids, reducing sugars, and antioxidant activity were determined using these standard extracts.

The reducing sugar content was determined according to the modified methodologies from Ávila-Núñez *et al*. (2012) and Reyes-Corral *et al*. (2022). Briefly, 100 µL of each standard extract was blended individually with 300 µL of 0.43 M of 3,5-dinitrosalicylic acid (DNS) reagent in a 2 mL microtube. Then, samples were incubated at 95 °C in a thermoblock (FELISA®, USA) for 5 min, and immediately later, the mixture was cooled in an ice bath for 5 min. Next, 250 µL of each sample was placed in a 96-well microplate (Microtiter Thermo Scientific®, USA), and absorbance was measured at 540 nm. A calibration curve was performed using glucose as standard, and the results were expressed as mg glucose per g^{-1} of dry weight (DW) (mg glucose·g^{-1} DW).

The phenolics were quantified according to the modified methods determined by Georgé *et al.* (2005). In brief, 150 µL of the extract was transferred on a 96-well microplate (Microtiter Thermo Scientific®, USA) and mixed with 150 µL of 10% (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, México), allowing it

to stand for 2 min. Subsequently, 100 µL of 7.5% Na_2CO_3 (Sigma-Aldrich®, USA) were added. Then, the mixture was agitated and incubated at 50 ºC for 15 min in darkness. The absorbance was measured at 740 nm. The calibration curve was performed using gallic acid (Sigma-Aldrich, México) as standard, and results were expressed as mg gallic acid equivalents by g^{-1} of dry weight (DW) (mg GAE·g^{-1} DW).

The flavonoids were quantified according to the modified methods specified by Georgé *et al*. (2005). Briefly, 41.7 µL of standard extracts were placed in a well of a 96-well microplate (Microtiter Thermo Scientific®, USA). Subsequently, 31 µL of 5% (w/v) $NaNO_2$ solution and 31 µL of a 10% (w/v) Al_2Cl_3 solution were added and allowed to stand first for 5 min and then for 3 min, respectively. Finally, 47.1 µL of a 0.5 M NaOH solution was added, incubating the mixture for 30 min in darkness. The absorbance was measured at 510 nm. The calibration curve was performed using catechin (Sigma-Aldrich, México) as standard, and results were expressed as mg catechin equivalents by g^{-1} of dry weight (mg CE·g^{-1} DW). The determinations were made in triplicate (three biological replicates per each treatment), measured in a Bio-Rad ×Mark Plus spectrophotometer (Hercules, USA), and data obtained with the Microplate Manager 6.0 computer software (Tokyo, Japan).

Antioxidant activity quantification

The antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) was quantified using the methodology proposed by Moreno-Escamilla *et al*. (2017). Briefly, 25 µL of each standard extract was mixed with 200 µL of the DPPH solution (190 mM of DPPH (Sigma-Aldrich, México) in 100% (v/v) methanol) into a well of a 96-well microplate (Microtiter Thermo Scientific®, USA). Then, the mixture was incubated for 30 min at room temperature in darkness, and absorbance was read at 517 nm every minute for one hour. A calibration curve was performed using TROLOX (Sigma-Aldrich, México) as standard, and results were expressed as µM of TROLOX equivalents per g^{-1} of dry weight (mg TE·g^{-1} DW).

The antioxidant activity determined by ferric reducing antioxidant power (FRAP) was quantified using the methodology proposed by Moreno-Escamilla *et al.* (2017). Briefly, 24 µL of the standard extract was mixed with 180 µL FRAP reagent (10 mM of TPTZ (Thermo Fisher, México); 300 mM of $C_2H_3O_2Na$ (Thermo Fisher, México); 20 mM of $FeCl_3$ (Thermo Fisher, México)) into a well of 96-well microplate. Then, the mixture was incubated for 30 min at 37 °C and the absorbance was read at 595 nm every minute for 30 min. The calibration curve was performed using TROLOX (Sigma-Aldrich, México) as standard, and results were expressed as µM of TROLOX equivalents per g^{-1} of dry weight (mg TE·g^{-1} DW). All determinations were made in triplicate (three biological replicates per each treatment), measured in a Bio-Rad ×Mark Plus spectrophotometer (Hercules, USA), and data obtained with the Microplate Manager 6.0 computer software (Tokyo, Japan).

Statistical analysis

The normality of frequency distribution was estimated using the Kolmogorov-Smirnov test. A one-way ANOVA was performed to test for differences in seed germination rates, seedling morphometric, and phytochemical content data. The significance of differences between treatments and variables was assessed using one-way ANOVA at 95% confidence level. The post-hoc Duncan multiple comparison mean test was used to estimate homogeneous groups for comparing more than two samples. A Pearson's correlation was carried out to measure associations between the different treatments (NaCl

and sucrose effect) and the variables evaluated (seed germination rates, seedling morphometric, phytochemical content, and antioxidant activity).

Results and discussion

Seed morphometric traits

The sotol seeds showed a purple-red color and trigon forms with flat and rough surfaces (Figure 1C). These characteristics were like those previously described for *Dasylirion* seeds (Ortiz-López *et al*., 2023; Rodríguez-Trejo *et al*., 2021). The accumulation of pigments in the fruits determines their color (Ortiz-López *et al*., 2023). The anthocyanins and flavonoids are attributed to the predominance of pink, purple or blue. Furthermore, color polymorphism may be associated with environmental diversity, stress tolerance, better tolerance to cold and fungal pathogens (Ortiz-López *et al*., 2023). Such features have been commonly used as criteria to be harvested once the inflorescence reaches maturity and before seeds begin shedding in the *Dasylirion* genus (Sierra-Tristán and Morales-Nieto, 2003).

The morphometric seeds showed 0.013±0.02 g in weight, 3.2±0.4 mm in length, 2.6±0.2 mm in diameter, 1.2±0.2 in the relation between the length and diameter, 0.5±0.1 mm in area, and 8.9±0.6 in perimeter. Although there are few studies related to the morphometric characteristics of the seeds of the genus *Dasylirion*, the morphometric data of length has been reported as 5.1 to 6.8 mm, 3.2 to 4.7 mm of diameter, and the shape coefficient from 0.6 to 0.7 mm in *Dasylirion* seeds collected from Oaxaca, México (Ortiz-López *et al*., 2023). The morphometric data of the seeds of *D. acrotrichum* compared to other species that develop in the same arid zone environments were lower than those determined for the species of cacti such as *Cylindropuntia spinosior* and *C. imbricata* (González-Fernández *et al*., 2023; Reyes-Corral *et al*., 2022), and *Opuntia polyacantha*, *O. phaeacantha*, *O. macrocentra* and *O. engelmannii* (Núñez-Gastélum *et al*., 2018). Furthermore, these were lower than those found for huisache, mesquite, and ahuehuete seeds (Rivas-Medina *et al*., 2005).

Seed germination index

This study explored the effect of exogenous NaCl and sucrose at different concentrations on the sotol seed germination. The results showed significant differences in the germination speed index (GVI), mean germination time (t), and mean germination speed (R) in both treatments; however, no significant differences were observed in the percentage of germination (GP) (Table 1).

Regarding to NaCl treatment, the GVI and R indexes decreased by about 42.4 and 36%, respectively, and the t index increased by roughly 52% at 90 mM NaCl compared with the control, 30 mM, and 60 mM (Table 1). Concerning sucrose treatment, GVI, and R indexes were reduced by about 48 and 57%, respectively, and the t augmented by roughly 46% at 90 mM sucrose compared with the control, 30 and 60 mM. A Pearson correlation was conducted to determine the relationship between treatments and germination indexes. However, this analysis did not show any association between the variables in both treatments.

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Table 1. Effect of the NaCl and sucrose concentration on *D. acrotrichum* seeds germination.

Index	NaCl concentration				ANOVA
	Control	**30 mM**	**60 mM**	**90 mM**	
GVI (days)	2.12±0.04^{b}	2.07±0.12^{b}	2.03±0.05^{b}	1.22±0.15^{a}	0.000
t (days)	7.16±0.27^{a}	6.88±1.58^{a}	7.93±0.47^{a}	10.92±1.88^{b}	0.015
R (days^{-1})	0.14±0.01^{b}	0.15±0.04^{b}	0.13±0.01^{ab}	0.09±0.02^{a}	0.045
GP (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	80±23.09^{a}	0.160
	Sucrose concentration				**ANOVA**
	Control	**30 mM**	**60 mM**	**90 mM**	
GVI (days)	2.07±0.07^{b}	1.57±0.50^{ab}	1.69±0.39^{ab}	1.07±0.00^{a}	0.030
t (days)	6.89±0.19^{a}	7.81±1.11^{a}	6.82±0.76^{a}	10.04±1.22^{b}	0.008
R (days^{-1})	0.14±0.01^{b}	0.13±0.02^{b}	0.15±0.02^{b}	0.06±0.00^{a}	0.000
GP (%)	97.78±3.85^{ar}	80.00±20.00^{a}	82.22±17.37^{a}	64.44±7.70^{a}	0.240

Data were analyzed using a one-way ANOVA. Results are expressed as the mean ± SD (n= 45 per treatment). Different letters (a-c) indicate a significant difference at $p \leq 0.05$ by Duncan's multiple comparison mean test.

Some studies have shown that plants subjected to abiotic stresses during germination exhibit a wide range of responses at whole plant levels, including morphological changes (Atta *et al*., 2023). The results of these study are agreed with those observed in wheat, where seeds under NaCl treatments showed a negative effect on GVI and R and a positive effect on *t* (Fuller *et al*., 2012). Instead, the results observed in the t-index also agree with those shown *Cyamopsis tetragonoloba* (Akram *et al*., 2020), where the t-index augmented with the increase of salt levels. Although in the present study, no affectation of both treatments was observed on the percentage of germination of seeds, these results were like those found in species that develop in arid zone environments such as *Agave durangensis* (Asparagaceae) where seeds under NaCl treatments exhibited the ability to germinate under low, moderate, and high salinity condition (Gallegos-Hernández *et al*., 2024). However, in other studies, a reduction in the percentage of germination has been observed principally in seeds of wheat (Ali *et al.*, 2023), *Suaeda salsa* (Guo *et al*., 2015), and *Brassica insularis* (Santo *et al*., 2017). Some authors have hypothesized that the osmotic stress produced by seeds exposed to abiotic stress may affect the seed water uptake, the starch synthesis reactions, and the seed respiration, causing that the seed does not germinate or reduce the initial germination, the germination rate, the germination speed index, and thus in the delay of the germination time (Atta *et al*., 2023, Steiner *et al*., 2017).

The sucrose is the main carbon form translocated in higher plants and is an important signaling molecule that regulates some genes involved in photosynthesis, metabolism, and developmental processes (Yoon *et al*., 2020; Xu *et al*., 2010). Moreover, the sucrose supply to a culture medium indicates that it serves as a balanced carbon source for cell growth, and the released hexoses directly participate in glycolytic and pentose phosphate pathways (Zha *et al*., 2007). In the present study, the exogenous sucrose also affected the seed germination (Table 1). These results agree with those observed in the seed germination of *Brassica napus* (Xu *et al*., 2010), *Cymbidium aloifolium* (Deb and Pongener, 2011), and *Bletia purpurea* (Johnson *et al*., 2011), where the increasing sucrose concentration resulted in decreased germination.

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Seedling growth

Concerning the morphometric characteristics of seedlings, the study showed significant differences between the NaCl, and sucrose concentrations applied for seed germination. These differences were observed in the total weight of the seedlings, the root weight, the leaf and root length, and the leaf weight (only in the case of NaCl) (Table 2).

The NaCl treatments damaged the *D. acrotrichum* seedling growth. The concentrations at 60 and 90 mM induced a decrease in the total weight of seedlings (26 and 44%), the weight of leaves (28 and 52%), and the length of leaves (46 and 67%), respectively, compared with the control. Furthermore, the weight of roots decreased by 6.3, 25, and 31.3 at 30, 60, and 90 mM NaCl, respectively. By contrast, concerning sucrose treatments, the 30 mM sucrose concentration had a positive effect on *D. acrotrichum* seedlings, causing an increase in the total weight (29%) and in the weight (53%) and length (59%) of roots. However, this treatment decreased the leaf length as the concentration increased, lowering to 46 for 90 mM sucrose.

Table 2. Effect of NaCl and sucrose concentrations on morphometric characteristics of *D. acrotrichum* seedlings.

Characteristics	NaCl concentration				ANOVA
	Control	30 mM	60 mM	90 mM	
Total weight (g)	0.041±0.001^{c}	0.040±0.004^{c}	0.030±0.001^{b}	0.023±0.002^{a}	0.000
Leaf weight (g)	0.025±0.004^{c}	0.025±0.004^{c}	0.018±0.003^{b}	0.012±0.003^{a}	0.000
Root weight (g)	0.016±0.002^{c}	0.015±0.003^{ab}	0.012±0.001^{a}	0.011±0.003^{a}	0.025
Leaf length (cm)	7.0±1.0^{c}	6.1±1.2^{c}	3.8±0.6^{b}	2.3±0.4^{a}	0.000
Root length (cm)	3.3±1.2^{ab}	4.2±0.8^{b}	3.6±0.3^{b}	2.3±0.2^{a}	0.008
	Sucrose concentration				**ANOVA**
	Control	**30 mM**	**60 mM**	**90 mM**	
Total weight (g)	0.027±0.011^{a}	0.035±0.001^{b}	0.029±0.007^{a}	0.026±0.004^{a}	0.003
Leaf weight (g)	0.021±0.007^{a}	0.022±0.005^{a}	0.019±0.004^{a}	0.018±0.003^{a}	0.390
Root weight (g)	0.006±0.003^{a}	0.013±0.001^{c}	0.010±0.003^{bc}	0.008±0.002^{ab}	0.003
Leaf length (cm)	8.1±0.4^{c}	6.7±0.5^{b}	6.2±0.6^{b}	4.4±0.6^{a}	0.000
Root length (cm)	3.2±8.3^{ab}	5.1±1.2^{c}	4.4±0.5^{bc}	2.9±0.8^{a}	0.005

Data were analyzed using a one-way ANOVA. Results are expressed as the mean ± SD (n= 10 per treatment). Different letters (a–c) indicate a significant difference at $p \leq 0.05$ by Duncan's multiple comparison mean test.

The correlation analysis showed negative associations between the treatments and some of the morphometric parameters of *D. acrotrichum* seedlings. The NaCl treatments affected the total weight (Figure 2A), the weight of roots (Figure 2B), and the length of leaves (Figure 2C), and the sucrose treatments showed a negative correlation with the length of leaves (Figure 2D).

These results agree with those effects observed in species that belong to the family Asparagaceae that grow in arid zone environments as *A. durangensis* (Gallegos-Hernández *et al*., 2024), *A. parry* (Bergsten *et al*., 2016), *A. salmiana* (Puente-Garza *et al.*, 2021), and *A. utahensis* (Bergsten *et al*., 2016), where seedling under NaCl treatments, showed a decreased of fresh weight, length, dry weight, water content, shoot, leaves, and radicle length. Besides, the results of this study also agree with those

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effects observed in *A. lechugilla* and *A. salmiana*, where seedlings under drought stress exhibited a decreased seed water uptake, relative water content, and radicle length (Campos *et al*., 2020). The plants under saline stress suffer osmotic stress, and because of that, they exhibit a wide range of responses at cellular and whole plant levels, including changes at morphometric, physiological, and developmental levels (Atta *et al*., 2023). In response to this stress, plants must synthesize compatible metabolites and thus need extra energy, and for this reason, causing a substantial decrease in the growth processes. This effect on the seedling growth rate could be related to the reduction of water uptake by plants, wall extensibility, and lower cell turgor pressure (Acosta-Motos *et al*., 2017). Otherwise, roots play a significant role by excluding Na^+ salt or by controlling the easy pass of Na^+ to the shoot under saline conditions. In previous studies, the accumulation of Na+ ions caused by changes in the ionic balance in roots reduced cell division and plant growth (Atta *et al*., 2023).

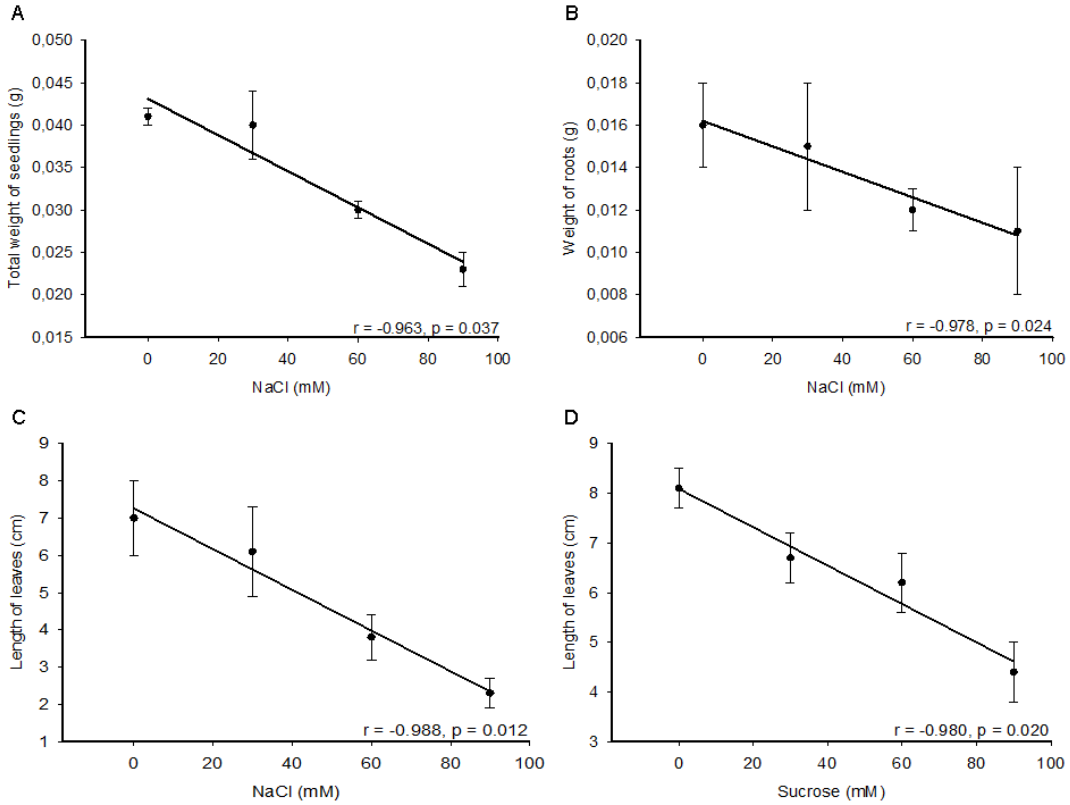

Figure 2. Person's correlation between NaCl and sucrose treatments, and the morphology of *D. acrotrichum* seedlings. Correlation between NaCl treatments and the total weight of seedling (A), the weight of roots (B), the length of leaves (C), and the length of leaves (D). The Pearson's correlation coefficient (r) is specified with a level of significance ($p \leq 0.05$).

Chlorophylls contents

The NaCl and sucrose treatments caused significant changes in the content of chlorophylls (Table 3). The NaCl decreased the content of chlorophyll a by 27, 45, and 49% at 30, 60, and 90 mM NaCl, and the total chlorophyll by 30, 42, and 46%, respectively, compared with the control. The sucrose treatment also decreases chlorophyll a by 13, 42, and 81%, chlorophyll b by 22, 33 and 84%, and the total chlorophyll by 7, 35, and 80% at 30, 60, and 90 mM, respectively, compared with the control.

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Table 3. Effect of NaCl and sucrose concentrations on chlorophyll contents of *D. acrotrichum* seedling leaves.

Chlorophylls	NaCl concentration				ANOVA
	Control	30 mM	60 mM	90 mM	
Chlorophyll a*	13.98±0.49^{c}	10.18±0.58^{b}	7.63±0.30^{a}	7.17±0.07^{a}	0.000
Chlorophyll b*	3.85±0.06^{d}	2.55±0.05^{b}	3.17±0.19^{c}	2.01±0.05^{a}	0.000
Chlorophyll c*	0.67±0.07^{b}	0.27±0.02^{ab}	0.03±0.04^{a}	0.60±0.28^{b}	0.032
Chlorophyll d*	0.22±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.18±0.23^{a}	0.191
Total chlorophyll*	18.73±0.61^{c}	13.08±0.69^{b}	10.78±0.42^{a}	9.96±0.78^{a}	0.001
	Sucrose concentration				**ANOVA**
	Control	**30 mM**	**60 mM**	**90 mM**	
Chlorophyll a*	13.50±0.29^{d}	11.68±0.27^{c}	7.83±0.31^{b}	2.54±0.15^{a}	0.000
Chlorophyll b*	4.22±0.07^{c}	3.31±0.25^{b}	2.84±0.35^{b}	0.69±0.03^{a}	0.000
Chlorophyll c*	0.33±0.02^{a}	0.71±0.23^{a}	0.72±0.71^{a}	0.31±0.06^{a}	0.580
Chlorophyll d*	0.06±0.07^{a}	0.23±0.09^{a}	0.38±0.37^{a}	0.11±0.01^{a}	0.450
Total chlorophyll*	18.12±0.31^{c}	16.77±0.82^{c}	11.76±1.74^{b}	3.65±0.25^{a}	0.000

* $mg \cdot g^{-1}$ of dry weight. Data were analyzed using a one-way ANOVA. Results are expressed as the mean ± SD (n= 3 per treatment). Different letters (a–c) indicate a significant difference at $p \leq 0.05$ by Duncan's multiple comparison mean test.

The correlation analysis showed a negative association between the NaCl treatments and the chlorophyll a content (Figure 3A) and between the sucrose treatments and the content of chlorophyll a (Figure 3B), chlorophyll b (Figure 3C), and total chlorophyll (Figure 3D). In this study, both treatments induced a significant decrease in the content of chlorophyll a and total chlorophyll. These results were like those previously observed in *A. durangensis* (Gallegos-Hernández *et al*., 2024), *Lactuca sativa* (Shin *et al*., 2020), *Triticum aestivum* (Lan *et al*., 2020), *Cucurbita maxima* (Tarchoun *et al*., 2020), *P. vulgaris* (Taïbi e*t al*., 2016), and *Stevia rebaudiana* (Shahverdi e*t al*., 2019) species, in which the photosynthetic apparatus lowered as a response to salinity stress. A reduction in the content of chlorophyll content in salt-stressed plants has been related to degradation by the enzyme chlorophyllase, oxidative stress, and inhibition of chlorophyll synthesis mechanisms (Atta *et al*., 2023). Furthermore, a high exogenous sucrose applied to *Cocos nucifera* plantlets reduced their photosynthetic capacity. This reduction is associated with a low rate of trioses phosphate utilization because of decreased rates of ribulose 1,5-bisphosphate regeneration, with restricted electron transport to PSII, and a reduced carboxylation efficiency and lesser amounts of Rubisco (Fuentes, 2005).

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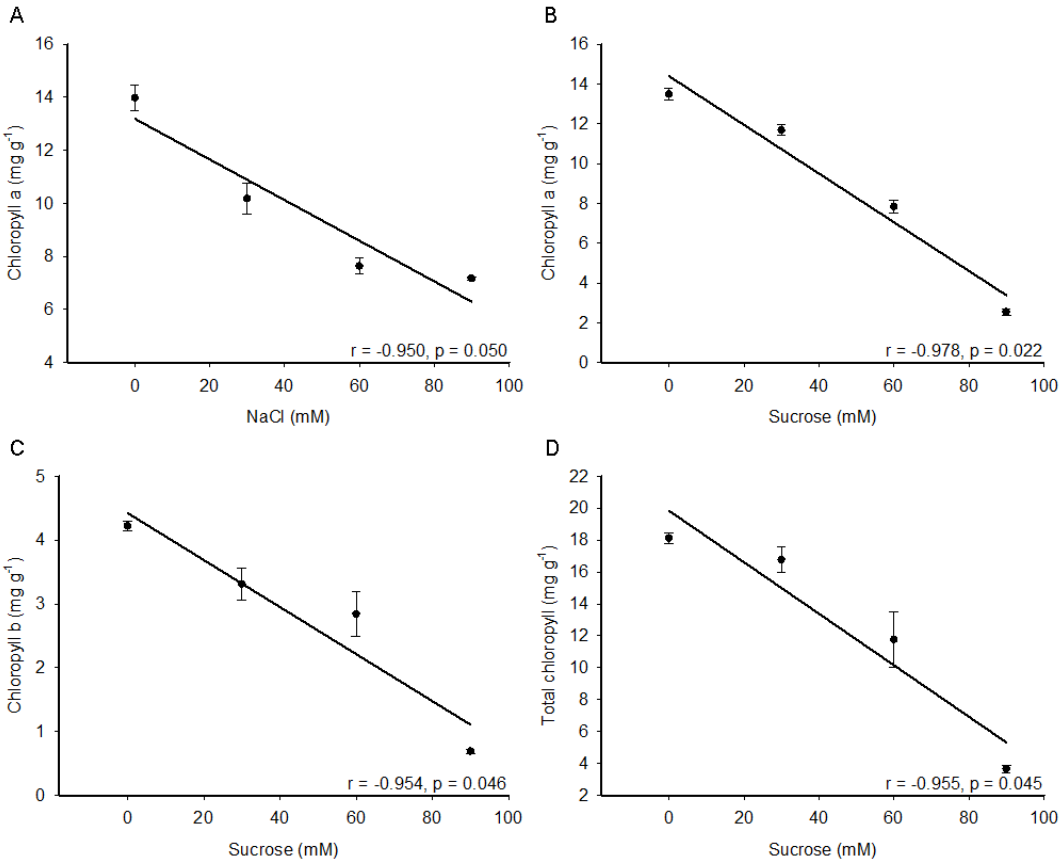

Figure 3. Pearson's correlation between NaCl and sucrose treatments, and chlorophyll contents of leaves *D. acrotrichum* seedlings. Correlation between the NaCl treatment and chlorophyll a (A), sucrose treatment and chlorophyll a (B), chlorophyll b (C) and total chlorophyll (D). The Pearson's correlation coefficient (r) is specified with a level of significance ($p \leq 0.05$).

Phytochemical contents and antioxidant activity

The NaCl and sucrose treatments caused significant changes in levels of reducing sugars, total phenolics, and antioxidant activity (Table 4). Regarding NaCl treatments, the content of reducing sugars increased 2.1 and 1.5 times at 30 and 60 mM NaCl, respectively, compared with the control. However, the highest NaCl concentration caused its decrease at the control level. The total phenolic levels were 1.7 and 4.7 times higher than the control at 60 and 90 mM NaCl, respectively. The antioxidant activity quantified by FRAP assay increased 1.2, 1.2, and 3.4 times at 30, 60, and 90 mM NaCl, respectively.

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Table 4. Effect of NaCl and sucrose concentrations on the phytochemical content and the antioxidant activity in *D. acrotrichum* seedling leaves.

Characteristics	NaCl concentration				ANOVA
	Control	30 mM	60 mM	90 mM	
Reducing sugars*	7.78±0.29[a]	16.31±0.17[c]	11.31±0.35[b]	7.91±0.07[a]	0.000
Total phenolics**	0.09±0.04[a]	0.10±0.03[a]	0.16±0.03[a]	0.43±0.23[b]	0.027
Flavonoids***	4.98±1.97[a]	4.90±0.97[a]	7.02±2.19[a]	7.46±0.34[a]	0.164
DPPH****	1.25±0.34[a]	1.86±0.72[a]	1.38±0.26[a]	1.39±0.27[a]	0.392
FRAP****	0.19±0.07[a]	0.23±0.02[b]	0.23±0.03[b]	0.66±0.19[c]	0.001
	Sucrose concentration				ANOVA
	Control	30 mM	60 mM	90 mM	
Reducing sugars*	9.73±0.11[a]	18.03±0.60[b]	21.18±0.13[c]	21.41±0.04[c]	0.000
Total phenolics**	0.19±0.07[a]	0.14±0.03[a]	0.23±0.08[a]	0.18±0.01[a]	0.385
Flavonoids***	7.27±0.65[a]	6.50±1.31[a]	7.14±0.54[a]	8.35±0.59[a]	0.128
DPPH****	2.70±0.15[c]	1.30±0.04[a]	2.12±0.30[b]	1.42±0.16[a]	0.000
FRAP****	0.33±0.02[b]	0.15±0.03[a]	0.25±0.01[ab]	0.41±0.16[b]	0.023

* mg of glucose equivalents (GE)·g^{-1} of dry weight ** mg gallic acid equivalents (GAE)·g^{-1} of dry weight; *** mg catechin equivalents (CE)·g^{-1} of dry weight; **** mM Trolox equivalents (TE)· g^{-1} dry weight. Data were analyzed using a one-way ANOVA. Results are expressed as the mean ± SD (n = 3 per treatment). Different letters (a–c) indicate a significant difference at $p \leq 0.05$ by Duncan multiple comparison mean test.

Regarding the sucrose treatments, the level of reducing sugars increased by 1.9, 2.2, and 2.2 at 30 mM, 60, and 90 mM sucrose, respectively, compared with the control. The antioxidant activity quantified by the DPPH assay decreased by 2.1, 1.3, and 1.9 times at 30, 60, and 90 mM sucrose, respectively, compared with the control. In the same way, the FRAP assay showed a decrease of 2.2 times at 30 mM sucrose compared with the control.

The correlation analysis of these results only showed a positive association between the sucrose treatment and the reducing sugar content (r=0.902, p=0.05).

Some studies reported that the salt stress excess produces an excessive reactive oxygen species (ROS), detonating the induction of oxidative stress (Acosta-Motos *et al*., 2017). The cells employ defensive mechanisms to prevent the damaging effects of accumulated ROS, which involves the buildup of osmoprotective agents, such as proline, glycine, betaine, and sugars, for helping to maintain cellular function and stability (Panieri *et al*., 2017; Soliman *et al*., 2020). In this study, the reducing sugar content increased at 30 and 60 mM NaCl but reduced at 90 mM NaCl. In the sucrose treatment, this content increased as the sucrose concentration increased. Our results agree with those observed in *S. rebaudiana* (Shahverdi *et al.,* 2019), *Polygonum equisetiforme* (Boughalleb *et al*., 2020), and *T. aestivum* (Alnusairi *et al*., 2021), in which an increase in sugar content has been observed with the increase of salt concentration.

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Other studies have also shown that plants hold a variety of antioxidant molecules, such as phenolics, flavonoids, carotenoids, and ascorbic acid, which participate in counteracting oxidative stress produced by abiotic stresses (Schafer *et al*., 2002). In the present study, the phenolic content increased upon salt stress (Table 4), like what was reported in *P. vulgaris* (Taïbi e*t al*., *2*016). The phenolics and flavonoids have been frequently induced by abiotic stress because these compounds accumulated in plant tissue could help to protect them from damaging effects and may help to inhibit lipid peroxidation in stressed plants (Taïbi e*t al*., 2016).

Conclusions

This study reported the first data on morphometric characteristics of sotol seeds from a population in the Chihuahua state. An increase in the NaCl and sucrose concentration decreased the germination speed index and mean germination speed; however, both treatments increased the mean germination time. In contrast, both treatments did not affect the germination percentage of sotol seeds. At the physiological characteristics, the NaCl and sucrose concentrations reduced the physiological water status and photosynthesis-related parameters in sotol seedlings. An increase in NaCl content decreased the total and root weights and the leaf length of the sotol seedlings; instead, sucrose content only affected the leaf length. Concerning the content of pigments, the increase in NaCl concentration only decreased the total chlorophyll; however, sucrose content affected the content of chlorophyll a, chlorophyll b, and total chlorophyll in the leaves of the seedlings. At the phytochemical characteristics, an increase in NaCl and sucrose concentration increased the phenolics and reducing sugars, respectively, and the antioxidant activity determined by FRAP. However, a more comprehensive analysis, including more seedlings provenances, remains to be done in future studies for understanding the variability in the sotol seedling response to drought and sugar stress.

ETHICS STATEMENT

Not applicable

CONSENT FOR PUBLICATION

Not applicable

COMPETING INTEREST

The authors declare that they have no competing interests.

AVAILABILITY OF SUPPORTING DATA

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Conceptualization, J.V-G. Methodology, J.V-G., G.I.G-V., Validation, R.G-F. Formal analysis, R.G-F. Research, R.G-F., M.Q-M. Resources, J.V-G., R.G-F., M.Q-M. Visualization, J.V-G, G.I.G-V. Literature review, J.V-G. Supervision R.G-F. review of the final version and approval of the manuscript before sending it, J.V-G., R.G-F., G.I.G-V., M.Q-M.

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