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# PHYSICAL AND PHYSIOLOGICAL VARIATIONS OF *Agave salmiana* Otto ex Salm-Dyck SEEDS REGARD THEIR DEVELOPMENTAL ENVIRONMENT

**Minerva Huerta[1], V. Baruch Arroyo Peña[1], José Luis Salinas[1], Daniel Padilla[2] and Cecilia B. Peña-Valdivia[1]***

[1] Programa de Posgrado en Botánica, Colegio de Postgraduados, Montecillo, Estado de México, 56230. Carretera México-Texcoco km 35.5. México.

[2] CONACYT- Programa de Posgrado en Botánica, Colegio de Postgraduados, Montecillo, Estado de México, 56230. Carretera México-Texcoco km 35.5.

*(Corresponding author cecilia@colpos.mx)

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## Abstract

Seed quality attributes relate to their physical, physiological, and genetic health. *Agave salmiana* Otto ex Salm-Dyck mostly propagates asexually, although each plant produces hundreds of viable seeds. This research aims to assess the effects of native environments on the partial chemical composition, and physical and physiological characteristics of *A. salmiana* seeds on their initial plant development. Seeds were collected at the Estado de México (EM; at 19º 48’ 18” N, 99º 49’ 46” W, 2822 m a.s.l., and 9.1-15.5º C annual mean temperature) and San Luis Potosí (SLP; 22º 40’ 10” N, 100 º 44’ 38” W, 1870 m a.s.l., and 13.4-21.7º C annual mean temperature) States, Mexico. Seeds that developed in a temperate environment and greater humidity (EM) accumulated a higher glucose and fructose concentration but less protein seed food reserves than those developed in a warmer and drier environment (SLP). Plants growing in drier and hotter environments also produce heavier, thicker, shorter, and less wide seeds than those growing in a wet and cool environment. Seed germination

(25 °C and complete darkness) in both samples (without pre-germinative treatments, and no scarification) was synchronous, following a logistic trend and maximum germination over 96 %, in under 170 h. According to their Dickson and slenderness indices, seedlings of *A. salmiana* have low quality but resist wind, cold, and drought. Initial plants growth, for 120 days, was remarkably similar between locations. Chemical composition and physical quality contrast to the physiological quality in *A. salmiana* seeds depending on the plant growing region.

**Keywords:** *Agave*, seed germination, maguey, seedling, plant growth.

## 1. Introduction

The basic parameters and attributes to evaluate seed quality are: (a) physical qualities (size, shape, color), (b) physiological qualities (*i.e.*, germination capacity or viability and seed vigor), (c) specific genetic quality of the variety, and (d) health (diseases and pests' absence) of the seed lot (ISTA 2016). From those, the seed germination, and seed vigor are seedling's indicators of their ability to emerge from the soil and produce a plant in the field in natural conditions and survive the potentially stressful field conditions, or rapidly grow in favorable ones (Baskin & Baskin 1988). Maternal effects on the seed's characteristics have been documented in many plant species (Donohue 2009). Emphasis has been focused on the environmental effects on mother plants and how it alters seed germinability. However, other assessed maternal effects include other seed characteristics, such as size, color, chemical composition, and quality of the offspring (Nguyen *et al*. 2021).

*Agave salmiana* Otto ex Salm-Dyck is a species of the *Agave* genus, Agavaceae family (*sensu stricto*) (García-Mendoza & Chávez-Rendón 2013) or Asparagaceae (APG III 2009, Chase *et al*. 2009). It distributes from the northern Coahuila state to the south of the Mexican territory (Chiapas state). It is endemic to Mexico, considered semi-domesticated, and found both cultivated and growing in the wild, with different degrees of anthropocentric interaction (Mora-López *et al*. 2011; Reyes-Agüero *et al*. 2019). *Agave salmiana* plants reduce erosion in their ecosystems, and as such, are elements that shape the agricultural landscape, and provide food and habitat for birds, mammals, and insects (Aguilar *et al*. 2014; Eguiarte *et al*. 2000; Gómez-Aiza & Zuiria 2010). Their anthropogenic uses include ornamental, as construction materials, textile fiber extraction, as a food source (flower buds, flowers, and young leaves), sugar source for fermented drinks and medicines, firewood, and fodder; also, these plants act as windbreaks, among other applications (Aguilar *et al*. 2014; García-Mendoza 2011; Mora-López *et al*. 2011; Reyes-Agüero *et al*. 2019).

*Agave salmiana* are long-living (up to 25 years) plants, have a single flowering event during their life cycle (semelparous species), and produce several hundred viable seeds (up to 200,000 and 600,000) per plant (Huerta-Lovera *et al.* 2018). Although, this species reproduces both, sexually and asexually, the latter, through aerial bulbils and basal suckers and rhizomes is the most common (Ramírez-Tobías *et al*. 2012). *Agave salmiana* mostly propagates asexually, even with its high number of viable seeds production (Huerta-Lovera *et al*. 2018). According to Nobel (1988) and Arizaga & Ezcurra (2002), asexual propagation ensures the plants´ establishment and gives them certain uniformity that facilitates agronomic management but can also make them susceptible to diseases (Piven *et al*. 2001). The asexual propagation of most commercial *Agave* species is mostly due to the misbelief that maguey seeds are unviable and, therefore, dispersion happens *via* suckers and bulbils (Gentry 1982; Peña-Valdivia *et al*. 2006). This asexual propagation tendency probably relates to unsuitable *in situ* conditions for germination and decreasing quantity and quality of seeds as a result of herbivory, or diminished seed production due to plants' usage before flowering, *i.e.*, for fermented and distilled beverages like tequila, and during flowering (to consume inflorescences) or low seedlings quality (Peña-Valdivia *et al.* 2006).

In *Agave* spp. the relation between the physical and physiological characteristics of the seeds to their ability to germinate and produce healthy seedlings has been incipiently assessed. Probably due to the belief that reproduction by seeds requires more care and lengthens the growth period (Anonymous 1988). Also, it is documented that depending on the species, a variable proportion of seeds in the wild can remain dormant (Peña-Valdivia *et al*. 2013). Seed germination, seedling emergence patterns, and initial plant growth depend on the morphological, physiological, and biochemical characteristics of the seeds, which also geographically variate their response to the environment (*e.g.*, to precipitation, and temperature) (Jimenez-Torres *et al*. 2021; Peña-Valdivia *et al*. 2006); although, they likely depend on a combination of two or more of these factors (Baskin & Baskin 1988). Also, it has been postulated that the physiological and biochemical characteristics of seeds partially depend on their developmental environment and their interaction with the initial growth environment (Elizalde *et al*. 2017; Jimenez-Torres *et al*. 2021). Seed germination and viability of *H. perotensis* significantly varied between the year's seeds were collected, due to the contrasting climate between the seed's development cycle (Elizalde *et al*. 2017). Also, moderate-high night temperatures drastically affected the initial plant growth and the metabolism of *A. mapisaga* and *A. salmiana*, suggesting the responses to stressing temperatures is partly specific, but less on the seed´s origin (Jimenez-Torres *et al*. 2021).

It is reported that *A. americana*, *A. asperrima*, *A. cupreata*, *A. duranguensis*, *A. lechuguilla*, *A. salmiana*, and *A. striata* lack seed dormancy and can reach greater than 90 % maximum germination in laboratory conditions (Campos *et al*. 2020; Peña-Valdivia et al. 2006; Pritchard & Miller 1995; Ramírez-Tobías *et al.* 2012). Similar results are reported for *A. parryi* (Freeman 1975), *A. lechuguilla* (Freeman *et al*. 1977; Jiménez-Aguilar & Flores 2010), *A. americana* (Pritchard & Miller 1995), *A. macroacantha* (Arizaga & Ezcurra 2002), *A. attenuate*, *A. jarcia* and *A. victoria-reginae* (Maiti *et al*. 2005), and in *A. striata* (Jiménez-Aguilar & Flores 2010). This lack of dormancy contrasts with the widespread vegetative reproduction of these species, the huge reproductive effort implied by semelparity, and the great number of small seeds produced (Arizaga & Ezcurra 2002). Still, the lack of dormancy and massive production of viable small seeds provide hints at a strategy to maximize the repopulation of sexually originated plants.

This research evaluates the partial chemical composition and physical, and physiological characteristics of *A. salmiana* seeds collected in two distinct Mexican regions, contrasting in altitude and climate. The tested hypothesis was that the chemical composition, and physical and physiological characteristics of the *A. salmiana* seeds and the initial growth of their plants are significant, regarding their area of collection and their environments.

## 2. Materials and methods

### *2.1 Seed samples*

For this research, *A. salmiana* Otto ex Salm-Dyck seeds were collected from plants growing at two different Mexican locations (Figure 1 A). One, the “Cerro Tepari”, San José del Tunal, Atlacomulco, Estado de México, with a (Cb (w2)(w)(i')g climate; this is the most humid of the sub-humid climates, with predominant rainfall during summer, summer rains, and less than 5 % of the total annual rainfall during winter. The mean annual temperature of 12.7 ºC, lower than 21 ºC mean temperature during the warmest month and greater than −2 ºC in the coldest. In this environment, along with the rain, dew accumulates during morning hours, fog from the mountains and frosts during winter, which are a significant source of humidity (García 1987; INEGI, National Institute of Statistics and Geography 2009a) (Figure 1 B). The other location, San Luis Potosí, SLP, Mexico with a (BSokw(e)gw" climate. This climate corresponds to a dry steppe, arid temperate, with summer rains, extreme annual mean monthly oscillation temperatures between 7 and 14 oC (e), with maximums and minimums during cold and warm seasons between 5-13 and 22-30 oC, type Ganges (g) temperature during march and canicula (w"). During its rainy season (6.6 months) total precipitation reaches 95 mm and during the dry season (5.4 months) total precipitation amounts to 5 mm (García 1987; INEGI 2009b) (Figure 1

C).

The capsules from plants with mature fruiting stems (Figure 2 A) were harvested after collecting the 5-6 m tall stems as Huerta-Lovera *et al*. (2018) described. The evaluated seeds were obtained from 250 capsules per site and kept in paper bags in cardboard boxes at 25 ± 3 ºC one-two months until evaluation. All the evaluated seeds were mature and separated from sterile ones in the capsules. Mature seeds seemed normal, well-formed, and without apparent damage. These, differ from sterile ones in their black color and thickness, greater than that of the sterile ones, which tend to be clear and thin (Figure 2 B).

### *2.2 Evaluated variables*

Whole seeds (25 g) were grounded in a mortar. The flour powder was used to quantify their chemical composition; the assessment included glucose, fructose, sucrose, total protein, lipids, ashes, and soluble phenolics concentration. Sugars were quantified in 100 mg of flour, following the enzymatic method described by Viola & Davies (1992) and Bernal *et al*. (2005). The flour powder was also used to quantify moisture, total lipids, crude proteins, and ash, following the AOAC methods (2005). The lipids were assessed from 0.5 g flour samples *via* the Soxhlet method; crude protein was quantified in 0.4 g flour samples using the Kjendahl method; and 1 g flour samples were used to determine the ash content (AOAC 2005). Phenolics were quantified in ethanolic 100 mg flour extracts following the spectrophotometric method described by Magalhães *et al*. (2010) in a microplate reader (Thermo Multiskan FC), using gallic acid as standard. The seeds´ chemical analysis included ten repetitions. On 250 seeds from each site, the width, thickness, and length were individually evaluated with a Vernier digital calibrator, standard millimeter (Truper, CALDI-6MP, 14388), and their biomass determined in an analytical balance (precision of ± 0.0001 g, Scientech® SA 120).

Seed germination was evaluated in 15 replicates, with 10 seeds per experimental unit at 25 oC in constant darkness, following the methodology by the ISTA (2016). Seed germination was recorded every 12 h and their final percentage was determined after 200 h. The seedlings’ quality was assessed with the slenderness or robustness index (height/diameter), following Sáenz *et al*. (2010), and Dickson (Rueda *et al*. 2012), following equation 1:

$$\text{Dickson index} = \frac{\text{total dray biomass (g)}}{\frac{\text{height (cm)}}{\text{diameter (mm)}} + \frac{\text{seedling dray biomass (g)}}{\text{root dray biomass (g)}}} \qquad \text{(Equation 1)}$$

A hundred and twenty seeds from each sample were sown in pots (12 cm length ×12 cm width × 7 cm depth) with 70 g of peat moss and kept at field capacity. Plants were placed in a glasshouse (day/night temperature of 33/12 ºC and natural photoperiod). At noon, the average greenhouse PPFD at plant height was 332 µmol $m^{-2}$ $s^{-1}$ under a white anti-aphid mesh, placed approximately 50 cm above plant height. This is because young *Agave* plants are sensitive to high light intensity (Jimenez-Torres *et al*. 2021). The seedlings' emergence was recorded every 12 h for 70 days. The emergence rate was calculated using the Maguire equation (1962), M = n1 / t1 + n2 / t2 + n120 / t70; where n1, n2, n120 are the number of seedlings emerged at times t1, t2, t14 in days.

Other one hundred and fifty seeds of each sample were sown in similar conditions to those described to evaluate the seedlings´ emergence. After 40 days in the greenhouse, five plants were sampled every eight days and their number of roots, length of their main root, stem diameter, plant eight, leaves per plant, leaves length, leaves base width, and fresh and dry biomass of the roots and shoot were evaluated up to 120-day.

### *2.3 Experimental design and statistical analysis*

The experimental design was completely random, and different repetitions were carried out depending on the evaluation. The results were then analyzed with the ANOVA and Tukey multiple means comparison tests ($P \leq 0.05$). When the statistical assumptions of heterogeneity were not met, the Kruskall Wallis test (H) was used, followed by a pairwise comparisons test performed in the InfoStat software, version 2008. When the data did not meet the H test assumptions, the Mann-Whitney U test was used ($P \leq 0.05$). In addition, the biomass and seed dimensions were evaluated with the Pearson correlation test; the germination parameters, the seedling emergence, and plant growth variables were analyzed in the STATGRAPHICS Centurion XVI statistical software.

## 3. Results

### *3.1 Partial seed chemical composition*

Among the evaluated soluble sugars, the EM seed sample contained twice ($P \leq 0.05$) as much glucose compared to the SLP sample (Figure 3 A). In contrast, the differences in the concentration of fructose and sucrose were not significant ($P > 0.05$) among samples (Figure 3 B-C). Both seed samples contained similar concentrations of lipids, minerals, and phenolics. In contrast, the seeds of the SLP samples were 13.65 % higher ($P \leq 0.05$) in protein content (Fig 4).

### *3.2 Seeds biomass*

The seed´s moisture content varied, from 5.28 to 6.06 %, between sampling sites and was not significant ($P > 0.05$); the 100 seeds' mean biomass, ranged between 1180 mg and 1200 mg ($P \leq 0.05$). The seeds biomass from the EM sample ranged between 2.2 and 14.8 mg and in the SLP sample between 3.3 and 16.7 mg. The seeds biomass distribution was asymmetric (left-skewed) in both samples. The maximum frequency occurred in the same interval (12.00 to 13.00 mg per seed) in both samples (Figure 5 A-B).

### *3.3 Seed width*

The width of the assessed seeds heterogeneously varied among samples; values fluctuated between 2.76 and 7.87 mm in the EM sample and from 3.81 to 6.79 mm in the SLP sample. The seed width frequency distribution in the EM sample was asymmetric with two maximums. The highest frequency in the EM sample occurred in the 5.50-7.49 mm interval and included 89 % of the sample. In the SLP sample, the seed width frequency distribution was left asymmetric, occurring between 3.81 and 6.79 mm. This sample included 46.6 % of the seeds in the 5.5 to 5.99 mm interval (Figure 5 C-D).

### *3.4 Seed thickness*

The seeds´ thickness in the EM sample ranged from 0.28 to 2.36 mm. The seed thickness frequency of this sample showed a closely symmetric distribution. The distribution of this seed character in the EM sample indicates the prevalence of seeds between 0.5- and 0.7-mm thicknesses. The seed thickness in the SLP sample ranged from 0.32 to 1.26 mm. The distribution of the seed thickness of this sample showed some flatness (platykurtic distribution) and was multimodal (Figure 5 E-F).

### *3.5 Seed length*

Seed length among the EM sample varied between 5.26 and 10.22 mm. The seed length frequency distribution was asymmetric (left-skewed) with three maximums. Most seeds (71.6 %) were in the length range between 7.52 and 9.45 mm. In contrast, the distribution frequency of the seed length in the SLP sample ranged between 5.79 and 9.28 mm and was asymmetric (left-skewed). Most seeds (51.27 %) of this sample were at the higher end of the range between 8.39 and 8.99 mm (Figure 5 G-H).

### *3.6 Seed germination*

The seeds from both sites started germination around 60 h, and both samples reached their maximum accumulated germination of 95-97 % within 160-168 h, with no significant difference among samples ($P > 0.05$). As with the above, both seed samples reached a similar maximum seed rate germination of 33 seeds d-1, in around 90 h (Figure 6 A-B).

### *3.7 Seedling emergence*

The maximum cumulative emergence among samples was not significantly different ($P > 0.05$). On average, amounted to 90- 91 % and reached around day 30. Seedling emergence rate was no different among samples, their maximum reached after 19 d (Figure 6 C-D).

### *3.8 Seedling quality*

The Dickson and the slenderness indexes were evaluated on 20- and 23-day-old seedlings with lengths between 10 and 120 mm. The difference between samples was that the 20-day-old seedlings had not exposed their first nomophile. Regardless of the seedling length, the Dickson index ranged between 3.14 x 10-4 in shorter seedlings and 5.93 x 10-4 in seedlings around 50-60 mm in length. There were no significant differences ($P > 0.05$) among samples and seedlings with and without exposed nomophile. The slenderness index increased, from 1 to 8, linearly along the seedling length (10 to 125 mm) (Figure 6 E-F). This index positively correlated with the size of the seedlings ($r = 0.9979$ and 0.9989, $P \leq 0.05$).

### *3.9 Initial plant growth and development*

Plant growth was evaluated during the first 125 days of development. During this time, the root number and leaves per plant grew in an exponential manner ($y = a(1-e^{-bx})$) in plants of both seed samples. Similarly, the root and some of the younger leaf’s growth in their length, stem diameter, and base width also showed an exponential fit ($y = a(1-e^{-bx})$) increase in the plants of both samples. The

EM sample plants showed similar height in some dates compared to those of the SLP sample; as in 64-72 and 96-120 days of age. However, when comparing the average height of both groups of plants, the *t*-test showed that the EM sample plants were 26 % taller ($P \leq 0.0001$) than those from the SLP seed sample (Figure 7 A-D; Figure 8 A-C).

The total accumulated biomass and dry biomass in the roots and shoots in the evaluated period linearly increased ($y = mx + b$) in the plants of the seeds from both provenances. Also, the average biomass accumulation, total and dry, of the plants from each region showed no significant differences ($P > 0.05$), according to the *t*-test comparison between the plants of the seeds from both samples (Figure 9 A-D).

## 4. Discussion

The evaluated seeds were collected in localities at different longitudes, latitudes, and altitudes; the difference in the latter was more than 700 m (Figure 1 A). Localities also contrasted in their climate (Figure 1 B-C). The highest monthly precipitation at the EM locality, from May to November, on average almost double that of the SLP locality.   The minimum temperatures (Tmin) in EM fluctuated between -1.9 ºC in January, the coldest month, and 7.4 ºC in June and July; the mean maximum temperatures (Tmax) fluctuated between 20.1 and 25 ºC ºC in January and April. In contrast, in the SLP locality, Tmin fluctuated between 6.6 and 15.2 ºC, in January and June, and Tmax ranged from 20.2 ºC to 28.6 ºC (Figure 1 B-C).

Seeds from both localities showed low fructose, intermediate glucose, and high sucrose concentration. These differences amounted to three and about four times more glucose than the fructose in the EM and SLP samples, respectively; Also, the sucrose concentration was 20 and 36 times higher than that of fructose in each sample. In contrast to the monosaccharides, the difference between sucrose concentrations was not significant between samples. According to the above, soluble sugars in the EM sample were 27 % more abundant than in SLP seeds. Lipids and proteins were the most abundant seed reserves in both samples, amounting on average around 20 % and up to 23 % of the dry mass, respectively. However, the SLP sample was about 14 % richer in protein compared to that from EM. In contrast, differences in minerals and phenolics concentrations were not significant between samples. The chemical composition of the seeds of the *Agave* genus is poorly documented. In this regard, Gutiérrez-Hernández *et al.* (2020) quantified the partial seed chemical composition of *A. potatorum* (“Tobalá”) collected for three years from Oaxaca, Mexico. On average, Tobalá seeds contain 15.7 % lipids, 37.7 % protein, and 4.3 % minerals; only protein varied, from 36.4 to 38.3 %, between years. In this research, *A. salmiana* seeds were on average 30 % richer in lipids but had 73 % less protein than those of *A potatorum*. Regardless of this, due to its seed composition, *A. salmiana* corresponds to the oleoproteaginous seed type. Regarding their mineral content, these depend on genotypic and phenotypic influences, including maternal and environmental effects (Fenner 1992). However, the seeds of EM and SLP, such as those three collected samples of *A. potatorum*, from Oaxaca (Gutiérrez-Hernández *et al*. 2020), independently of the species or site of collection, all had similar mineral content (4 %). Mineral content affects seeds germination and seedlings' development, and at the same time relates to the seed size and plant species; this is due to the role of Ca, Cu, Fe, Mg, Mn, Mo, Ni, S, and Zn, as enzymatic cofactors, and others, such as B and Ca, in stabilizing cell walls and cell elongation (Eggert & von Wirén 2013). The results indicated that the environment of development influenced the seed´s sugars and protein content; those in cooler areas and greater humidity availability (EM) accumulated a higher concentration of glucose and fructose, but less protein seed food reserves compared to those developed in a warmer-dryer environment (SLP).

The intervals of the seed biomass, their dimensions, *i.e*., width, thickness, length, and the distribution and maximum frequency of each of these seminal variables were different among seed samples. The SLP sample was 5.3 % heavier than the EM one. Its lower *C.V.* indicated a greater homogeneity (Table 1). The EM sample included a wider seed width interval and two maximums, compared to the SLP sample; thus, the EM sample, had greater variability, almost 9 % wider compared to the SLP one (Table 1). In contrast to the width, the SLP sample showed more than one maximum seed thickness and, exhibiting less variability, was 8.5 % thicker than the EM sample. Still, seeds in the last were 2.26 % larger than those of SLP; but, equally homogeneous (*C.V*. 10.9 and 8.3 %, respectively) (Table 1). Also, the distribution of the seed length of the EM sample showed two maxima and the highest proportion (72 %), between 7.5 and 9.5 mm in length. In contrast, the negative bias of the frequency distribution of this seed character, in the SLP sample, indicated that seeds ranging between 8 and 9 mm prevailed (62 %). These results indicate that *A. salmiana* plants growing in a drier and hotter environment, such as San Luis Potosí, produce less wide, heavier, thicker, and shorter seeds than those growing in a wet and cooler environment, as that in Atlacomulco, Estado de México.

The average seed biomass of both samples in this study is within the interval (9.31 to 12.41 mg) of other *A. salmiana* seeds collected in SLP by Huerta-Lovera *et al*. (2018) and (9.5-14.0 mg) by Peña-Valdivia *et al*. (2006); but, heavier (70 %) than those collected by Ramírez-Tobías *et al*. (2012) also in SLP (7.17 mg). The average seed length of both assessed samples here coincides with the intervals documented by Gentry (1982) in *A. salmiana* seeds, which was between 8 and 9 mm; also, with that documented in seeds from the Mexico Valley (Rzedowski & Rzedowski 2005) and from the

Tehuacán-Cuicatlán Valley (7.0-9.0 mm) (García-Mendoza 2011). The main seed´s dimensions concur with those of the seeds of the Xamini and Salmiana variant, of *A. salmian*a from Hidalgo, México, ranging between 6.56 and 8.33 mm, 4.75 and 6.21 mm, and 0.69 and 0.75 mm in length, width, and thickness (González-Cruz *et al*. 2014). The seed dimensions also matched those of three variants of *A. salmiana* collected in SLP by Vázquez *et al*. (2011) (seed width, length, and biomass between 7.1 and 8.4 mm, 5.4 and 6.5 mm, and 10.8 to 14.5 mg). Overall, all these results indicate that *A. salmiana* seed shows low variability in the physical characteristics, regardless of the area of origin and therefore growth environment.

Seed germination in both samples (without pre-germinative treatments, with no scarification in 25 °C and darkness) was synchronous, following a logistic trend, with r2 of 0.9634 and 0.9355 (EM and SLP samples, respectively). This model includes parameters to describe the germination onset and maximum percentage of seed germination. Both samples started germination after 60 h, and reached an average maximum germination of over 96 % in less than 170 h. Also, the seed germination rate throughout the 170 h described a symmetric bell-shaped trend with a mean maximum of 35 seeds $h^{-1}$ after 90 h in both samples. Regarding the seed’s germination, Peña-Valdivia *et al.* (2006) and Ramírez-Tobías *et al.* (2012) determined, in a 7 to 40 °C interval, that the optimum temperature for *A. salmiana* is around 25-26 °C. The maximum accumulated seed germination of the EM and SLP samples is similar to that quantified by Ramírez-Tobias *et al*. (2012) and Pérez-Sánchez *et al*. (2011) (96 and 98 %), from seeds collected in SLP and in the Southern Chihuahuan Desert, Mexico, respectively. In contrast, the germination of the seeds of eight samples of *A. salmiana*, from four locations, at altitudes between 1370 and 2030 m a.s.l., and diverse climates, from SLP varied between 44 and 95 % (Peña-Valdivia *et al.* 2006). These authors demonstrated between 30 and 55 % of dormancy in six of these samples, which increased their synchronous germination up to 98 ± 2 % at 84 h with mechanical scarification; furthermore, they observed the inverse relation between dormancy and seed storage time. The seed dormancy percentage in samples from EM and SLP in the present research was lower than 1-3 %, including seeds not germinating because of a lack of embryonic axis; this result is like that reported by Ramírez-Tobías *et al*. (2012) and Pérez-Sánchez *et al*. (2011).

The differences in the maximum cumulative emergence (90 and 91 %), and maximum seedling emergence rates (0.4 seedling h-1 around 500 h) were not significant between EM and SLP samples. Both took 3 days to start seedling emergence. Furthermore, the maximum accumulative germination in the laboratory was not significantly different from that of seedlings emerging in a greenhouse. In this regard, seed germination is frequently higher than seedling emergence, both in greenhouse and field conditions. This difference depends on the seed vigor, or the embryo´s inability to generate vigorous seedlings that traverse the soil and emerge to the surface (Bewley 1997). Soluble sugars as seed reserves, can support seed germination and good seedling development, as previously reported in other Cactaceae seeds (Alencar *et al.* 2012). However, the sugar content differences in the seeds among both samples did not affect any of these processes. Maximum seedling emergence in the present study was higher than in other *A. salmiana* samples from San Luis Potosí by Sánchez-Urdaneta *et al.* (2011) and Vázquez *et al.* (2011), which reached a maximum of 71 % seedling emergence. These differences likely relate to seed reserves and the efficiency to translocate them to the growing seedling (Werker 1997). In the present research, Dickson´s and slenderness index, or robustness coefficient, showed no differences between both samples. The slenderness index linearly increased with the seedling's height; At the same time, regardless of the size of the seedlings, the Dickson and slenderness indexes show that *Agave* seedlings of up to 100 mm length, with and without nomophile, had the potential to withstand certain adverse environmental conditions. However, according to the Dickson index, its quality was relatively low. The slenderness index relates to plant resistance and its potential photosynthetic capacity. Values between 5 and 10 in this index indicate high plant quality and a good balance between morphological and physiological characteristics (Rueda *et al*. 2012). The Dickson index reported here indicates that the *Agave* seedlings have low quality because they are far from the maximum value (one). Still, according to the slenderness index, they are resistant to wind, cold, and drought, given they had greater than six values (Sáenz *et al*. 2010). This information is useful to partially explain the low sexual *in situ A. salmiana* reproduction and its common asexual propagation *via* bulbils, shoots, and rhizomes (Pérez-Sánchez *et al*. 2015).

Plants from the seeds of both locations showed more similarities than differences during their first 120 days of development. The number of roots per plant and their length, as well as the diameter of their stems and number of leaves per plant, exponentially increased regardless of their locality of origin. In contrast, the root and shoot biomass linearly increase and did not differ between samples. On average, the shoot and root biomass per plant was 3.57 and 1.32 g, that is a shoot: root index of 1:3. All these increases contrasted with the relative stability of plant height, leaf length, and basal leaf width in plants of seeds of both samples.

Seeds from both maternal environments had high germinability and similar initial plant growth in the absence of stressful conditions. However, it is documented that the maternal environment interacts with the growing temperature during initial plant growth in *A. salmiana*. As a result of the interaction, young plants accumulate different amounts of biomass,–soluble sugars, total free amino acids, total phenols, and in maximum quantum efficiency of PSII photochemistry (Jimenez-Torres *et al*., 2021). Similarly, *Petunia* x *hybrida* seeds from contrasting maternal environments had high and similar germinability and seedling vigor with no stress treatment but exhibited significant differences in their response to stressful conditions (Nguyen *et al*. 2021); the authors, concluded that the epigenetic mechanisms are involved in this response.

In conclusion, chemical composition and physical quality, in contrast to the physiological quality of *A. salmiana* seeds depends on the maternal environment.

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Table 1 Biomass, width, length, and average thickness of *Agave salmiana* Otto ex Salm-Dyck seed plants from Cerro Tepari, Atlacomulco, Estado de México and San Luis Potosí, México.

| Characteristic | Estado de México | | | |
|---|---|---|---|---|
| | Biomass (mg) | Width (mm) | Thickness (mm) | Length (mm) |
| Mean | 0.0113 a | 6.1419 b | 0.6313 a | 8.4281 b |
| Minimum | 0.0022 | 2.7633 | 0.2800 | 5.2567 |
| Maximum | 0.0148 | 7.8667 | 2.3567 | 10.2233 |
| Standard error | 0.00014 | 0.0547 | 0.0122 | 0.0593 |
| *C.V.* (%) | 19.1071 | 13.7938 | 29.8907 | 10.8981 |
| | San Luis Potosí | | | |
| | Biomass (mg) | Width (mm) | Thickness (mm) | Length (mm) |
| Mean | 0.0119 b | 5.6434 a | 0.6849 b | 8.2422 a |
| Minimum | 0.0033 | 3.8067 | 0.3173 | 5.7933 |
| Maximum | 0.0167 | 6.7867 | 1.2567 | 9.2833 |
| Standard error | 0.0001 | 0.0368 | 0.0109 | 0.0446 |
| *C.V.* (%) | 15.5000 | 9.9621 | 24.2371 | 8.2575 |

*n* = 250

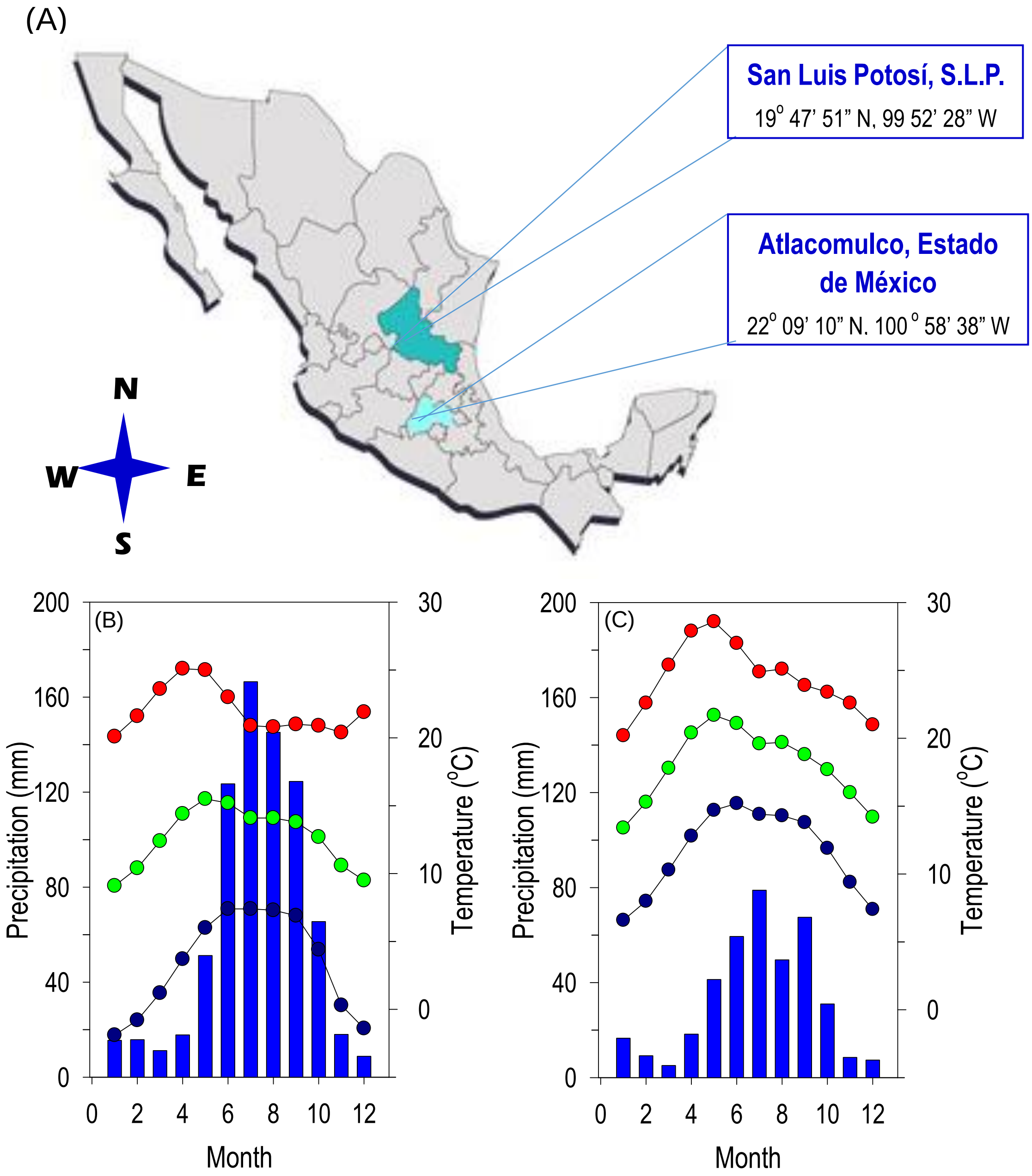

Figure 1. Localities sampled for *Agave salmiana* Otto ex Salm-Dyck of the seeds in Mexico and climographs based on average precipitation (bars), and average minimum (black circles), medium (green circles), and maximum (red circles) temperature at Cerro Tepari, Atlacomulco, Estado de México(B), and San Luis Potosí, Mexico (C); each bar and circle represent the mean monthly (1: January … 12: December) data over a three-decade period from 1981 to 2010 (CONAGUA, 2020).

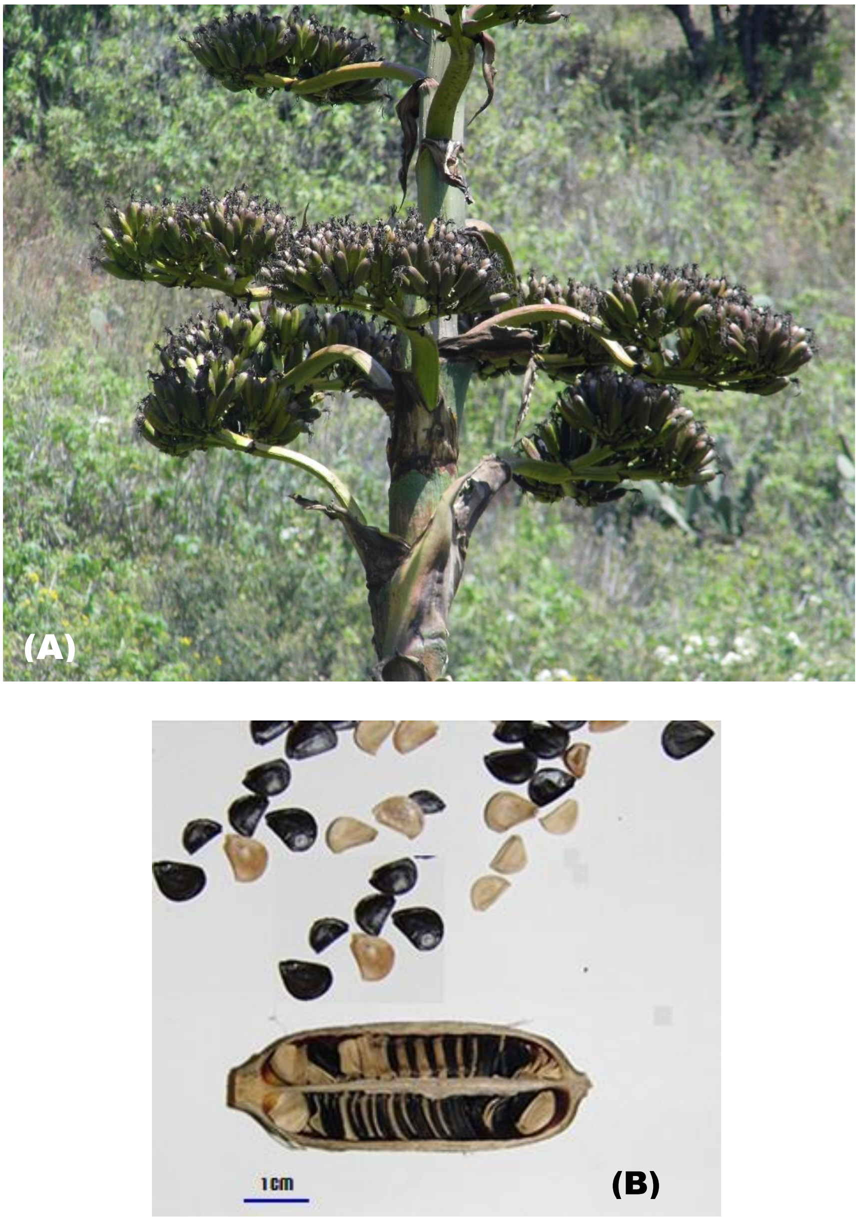

Figure 2. Mature capsules on the lateral branches or umbels in the upper third of the 5-6 m long reproductive stalk of *Agave salmiana* Otto ex Salm-Dyck (A). Normal (black color) and sterile (light color) seeds and their arrangement inside a capsule of *A. salmiana* (B).

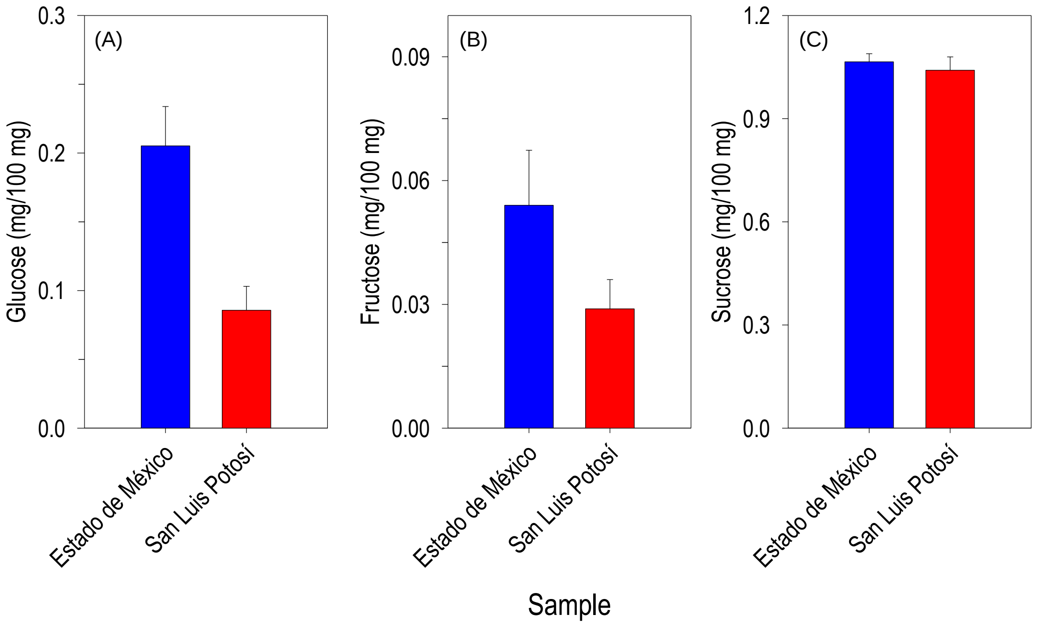

Figure 3. Glucose (A), fructose (B), and sucrose (C) concentration in seed of *Agave salmiana* Otto ex Salm-Dyck from Estado de México (blue bars) and San Luis Potosí (red bars), Mexico ($n = 10$).

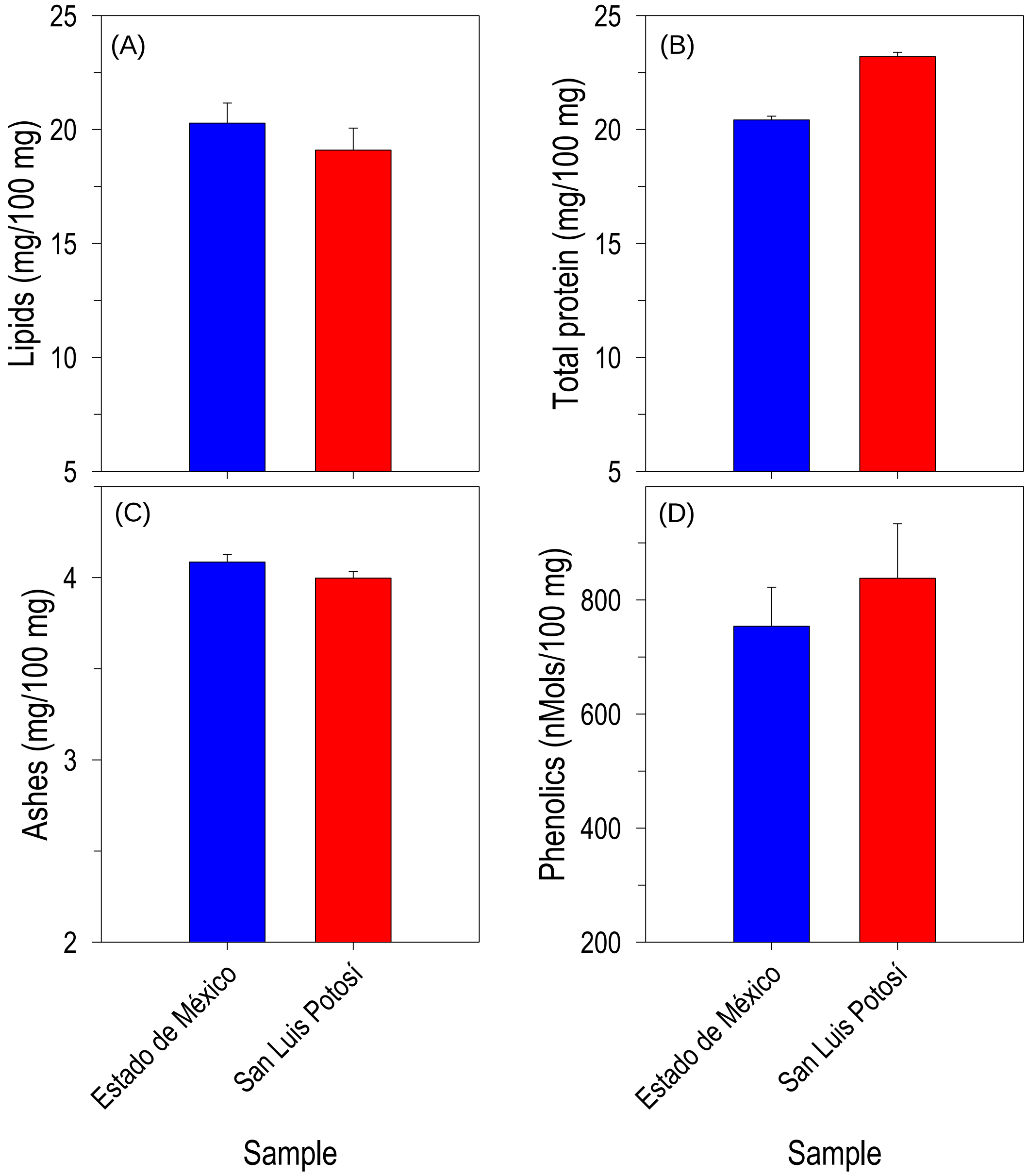

Figure 4. Lipids (A), total protein (B), ashes (C), and total phenolics concentration in seed of *Agave salmiana* Otto ex Salm-Dyck from Estado de México (blue bars) and San Luis Potosí (red bars), Mexico (*n* = 10).

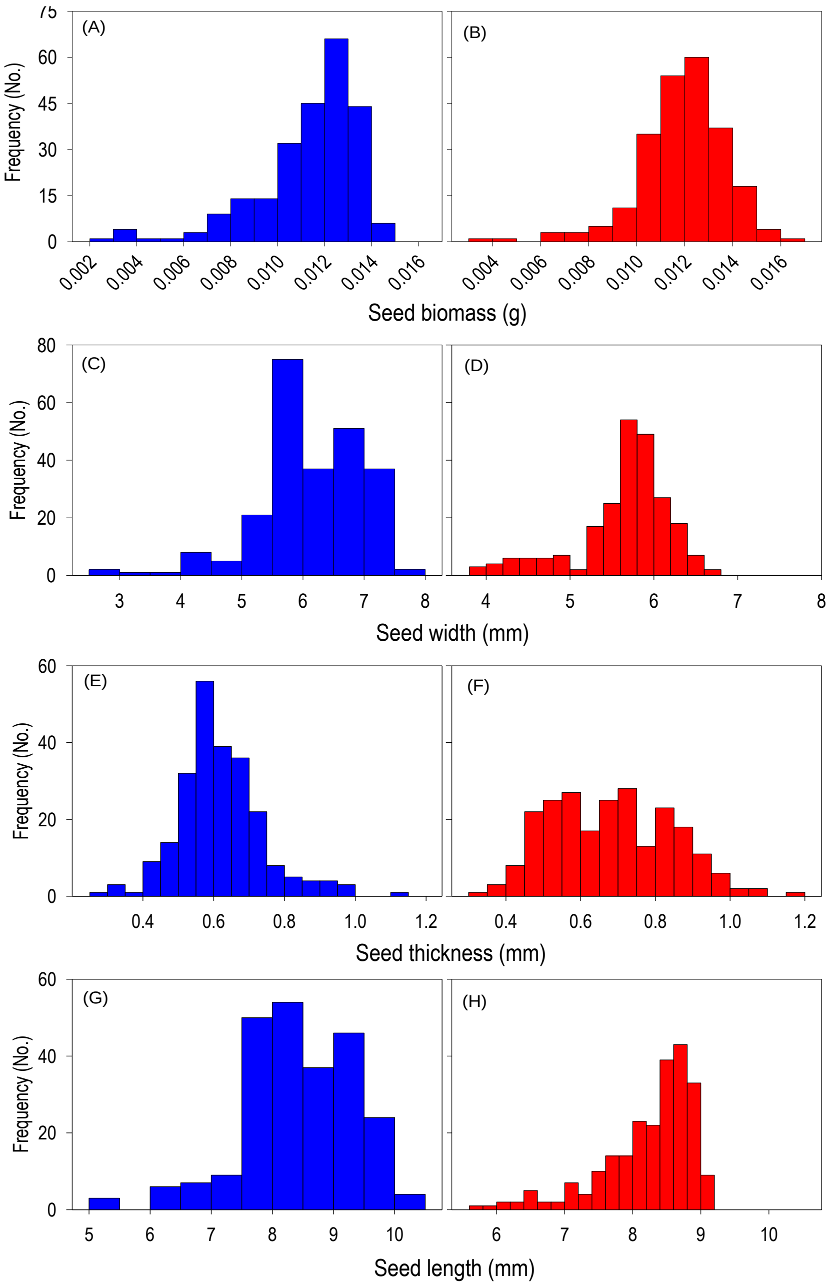

Figure 5. Frequency distribution of seed biomass (A, B), seed width (C, D), seed thickness (E, F), and seed length (G, H) of *Agave salmiana* Otto ex Salm-Dyck from Estado de México (blue bars) and San Luis Potosí (red bars), Mexico ($n = 250$).

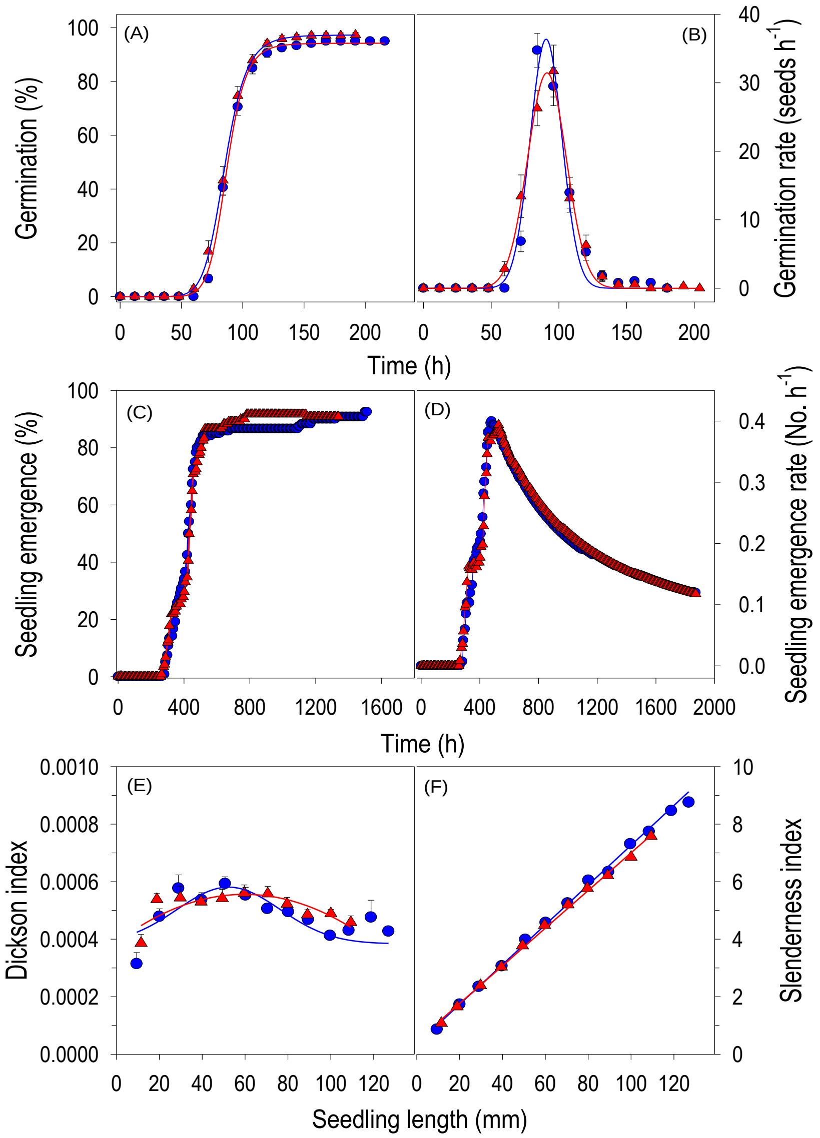

Figure 6. Cumulative seed germination ± *s.e.* (A), seed germination rate + *s.e.* (B) (at 25 ºC in darkness), cumulative seedling emergence + *s.e.* (C), cumulative seedling emergence rate + *s.e.* (D) in greenhouse conditions, Dickson's index + *s.e.* (E) and slenderness index + *s.e.* (F) of seedlings of *Agave salmiana* Otto ex Salm-Dyck seeds (*n* = 30). Seeds were collected from plants at the Estado de México (blue circles), and from San Luis Potosí (red triangles), Mexico.

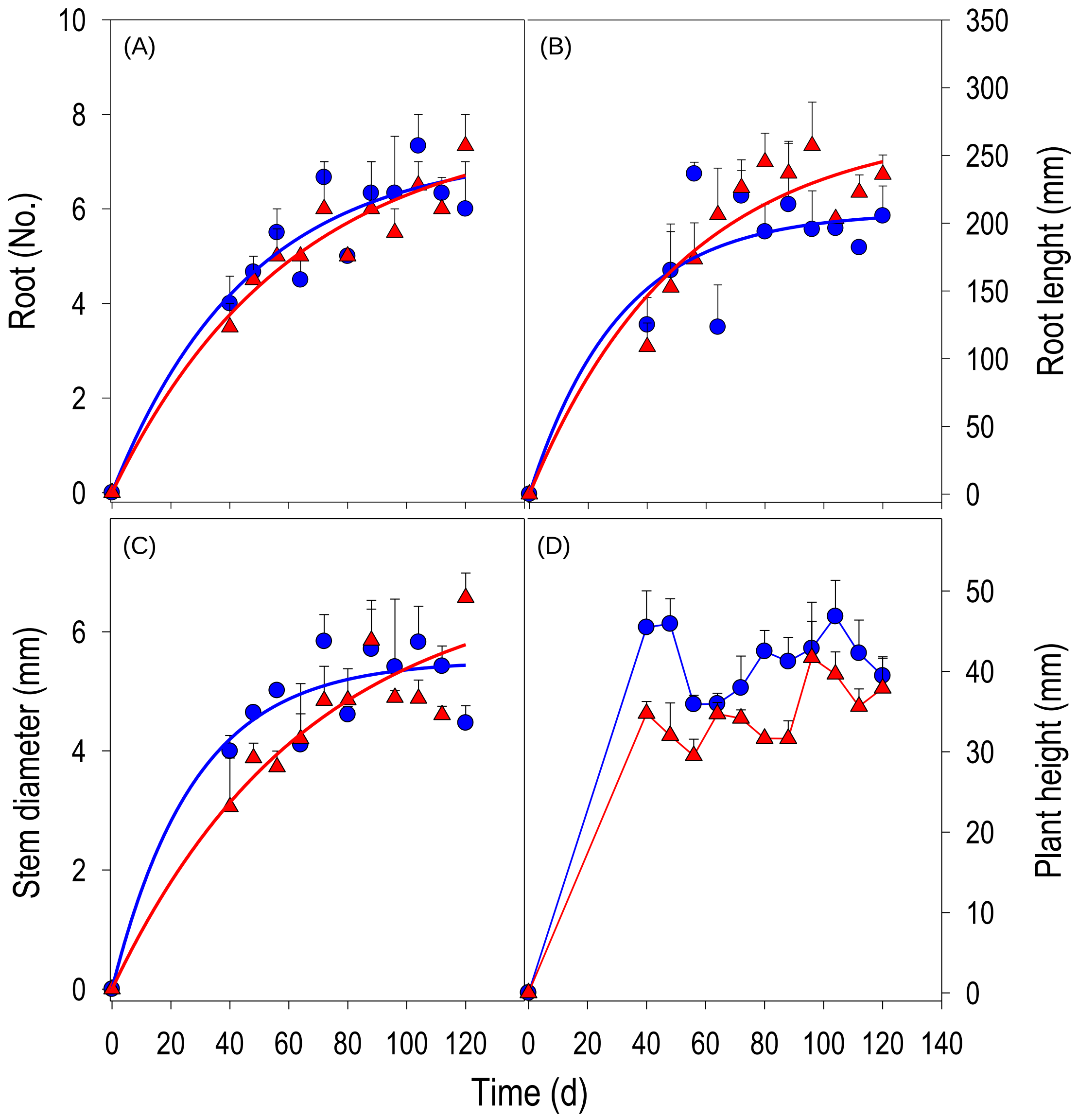

Figure 7. Increase of root number per plant ± *s.e.* (A), maximum root length ± *s.e.* (B), stem diameter ± *s.e.* (C), and plant height ± *s.e.* (D) of plants from seeds of *Agave salmiana* Otto ex Salm-Dyck (*n* = 5) collected at Estado de Mexico (blue circles), and San Luis Potosí (red triangles), Mexico.

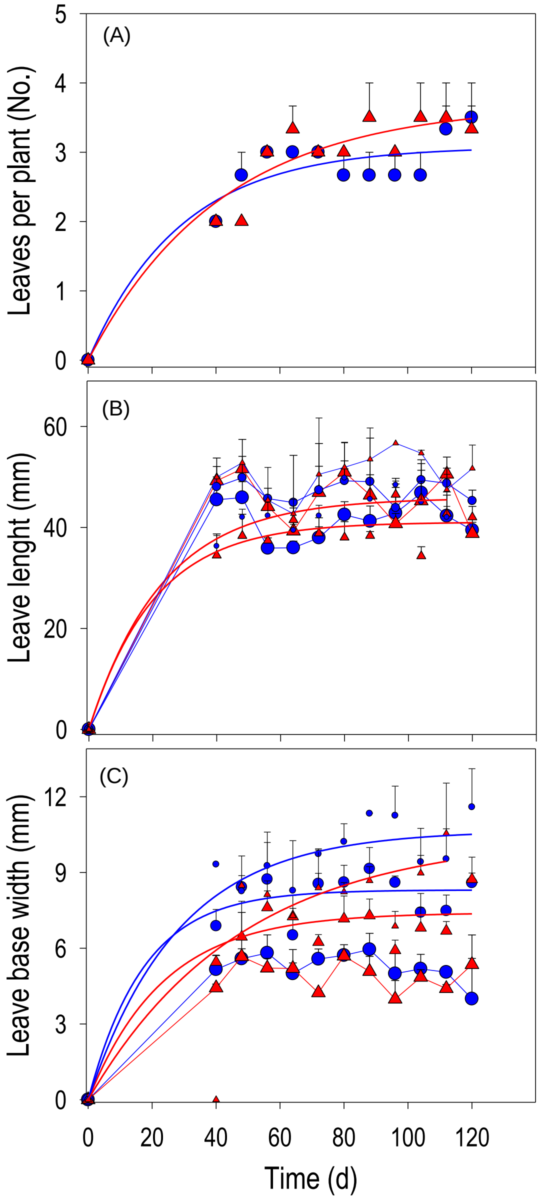

Figure 8. Increase of leaves per plant ± *s.e.* (A), leaves length ± *s.e.* (B), and leaves base width ± *s.e.* (C) of plants of seeds of *Agave salmiana* Otto ex Salm-Dyck (*n* = 5) collected at Estado de Mexico (blue circles), and San Luis Potosí (red triangles), Mexico. Large, medium and small symbols size in (B) and (C) correspond to the basal, central and upper leaves, respectively, on the rosette.

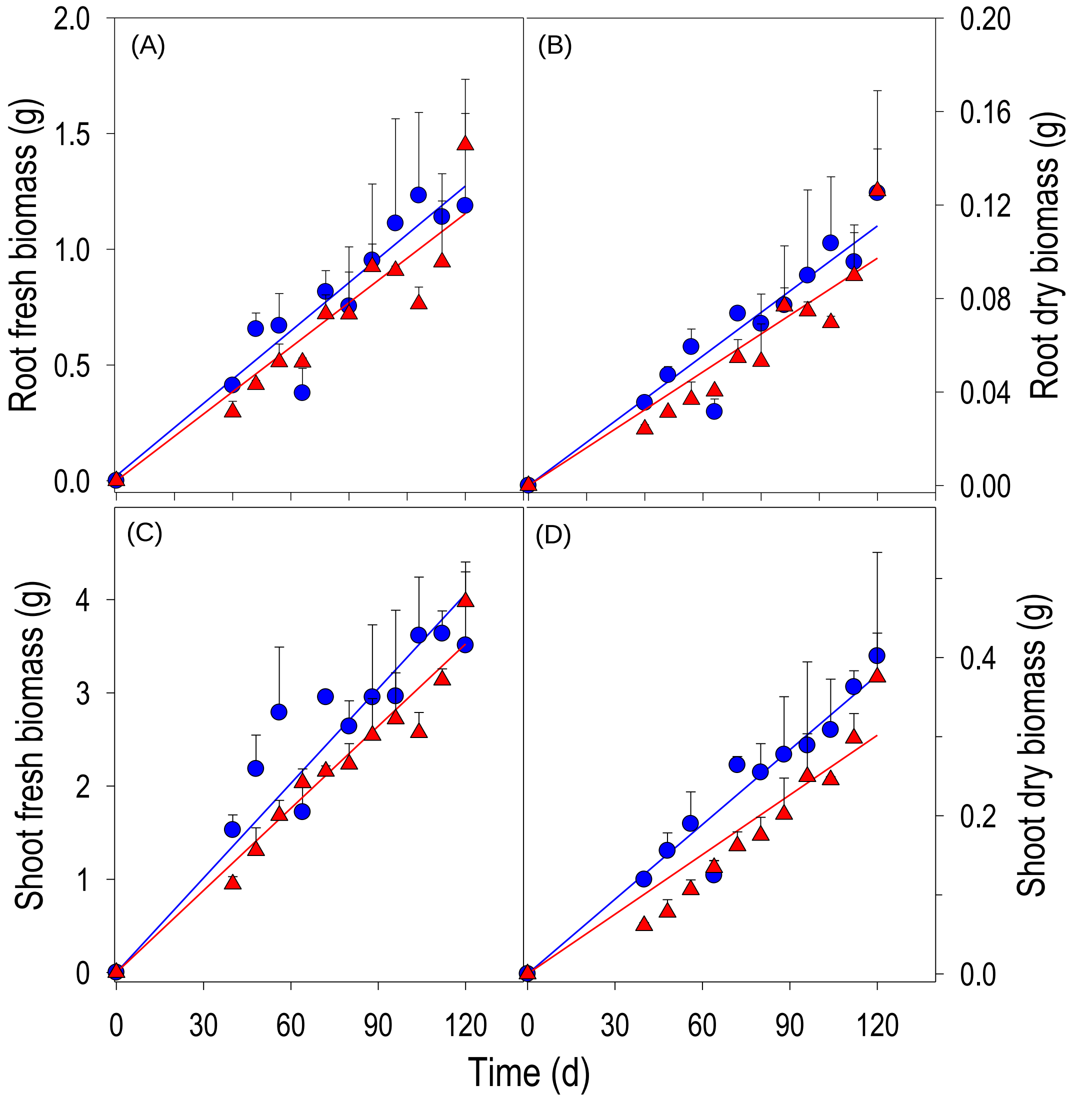

Figure 9. Increase of root fresh (A) and dry biomass ± *s.e.* (B), and shoot fresh (C) and dry biomass ± *s.e.* (D) per plant of seeds of *Agave salmiana* Otto ex Salm-Dyck (*n* = 5) collected at Estado de Mexico (blue circles), and San Luis Potosí (red triangles), Mexico.