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Seed Vigor Variation of *Agave durangensis* Gentry (Agavaceae)

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ABSTRACT

Agave durangensis propagates basically by seeds. This species is economically important because it supports a mescal industry in Durango, Mexico. At present, its exploitation is overall by collecting plants from the wild populations. In order to reduce the negative impact of the collection in the natural populations of *A. durangensis* and to improve the mescal industry, it is necessary to establish plantations with selected seeds for a high vigor. In this paper, the variation in morphological features, physiological behavior of germination, and biochemical indicators of seed vigor among three natural populations of *A. durangensis* was assessed. Variation was found in the seed weight (0.68 to 1.15 mg/seed), seed dimensions (3.51×5.29 to 4.62×5.92 mm), germinability reduction at 15˚C related to 25˚C (4% to 51%), germination rate at 25˚C (44.0 to 48.5 seeds/day) and at 15˚C (5.17 to 6.77 seeds/day), development reduction at 15˚C related to 25˚C (86.66% to 91.99%), levels of seed accumulated phenols (71 to 85 µg/seed), antioxidant potential (42% to 50% reduction of DPPH*), seed alcohol dehydrogenase activity (ADH) (180 to 1100 µmol NAD$^+$/mg protein/min), highest ADH activity after imbibitions at 25˚C (310.24 to 520.2 µmol NAD$^+$/mg protein/min), and highest ADH activity after imbibitions at 15˚C (170.74 to 440.71 µmol NAD$^+$/mg protein/min). The variation in the seed vigor was revealed by a principal component analysis (PCA) based on all the parameters evaluated. PCA clearly discriminated among the three populations.

Keywords: Agave Durangensis; Seed Vigor; Germinability; Natural Variation

1. Introduction

Agave durangensis Gentry is the base of a mescal industry in Durango, Mexico. At present, its exploitation is carried out by collecting plants from the wild populations [1], which is causing the reduction and fragmentation of its natural distribution [2]. *Agave durangensis* propagates basically by seeds, and under natural conditions it is infrequent to observe offshoots; this favors high levels of genetic variability [2], which may represent a source of worthy alleles to select for the establishment of commercial plantations. Thus, it is relevant to carry out studies to assess the variability in the seed vigor among its natural populations. Natural variability in the seed vigor has

been found among varieties of cultivated species [3] as well as among the natural populations of wild species [4].

The definition of seed vigor given by the Association of Official Seed Analyst's Vigor Committee in 1979 was mentioned by McDonald [5] as those seed properties determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions. Seed vigor has genetic and environmental determinants [3]. Most tests to evaluate the vigor of seeds consider the germination behavior [6,7] and the deterioration of that behavior under condition of stress [8]. The correlation between the morphological, physiological and biochemical indicators of seed vigor may assist to broad the understanding of how the vigor is

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expressed and facilitate the selection of high vigor seeds for breeding programs. Among the biochemical characters used as indicators of seed vigor are the ADH activity [3,9,10] and the levels of antioxidant phenols [11,12]. The aim of the present study was to evaluate the variation in the seed vigor among three natural populations of *A. durangensis* as a way to select seeds to establish plantations.

2. Materials and Methods

2.1. Seeds

Seeds of *Agave durangensis* were collected from three natural populations in August 2009. The **Table 1** shows the geographical data of those populations. Voucher specimens were collected from each population and deposited at the Herbarium MEXU. Seeds were aged for nine months in paper bags at room temperature. Three lots of 50 seeds of each population were prepared for the analysis.

2.2. Seed Morphological Features

Weight, length, and width were individually determined, by using a vernier, for 150 seeds of each population.

2.3. Germination

The germination behavior, as a vigor indicator, was evaluated at 25°C ± 2°C (temperature of reference) and at 15°C ± 2°C (stress temperature) for 360 h. Those temperatures were selected because the seeds of *Agave* germinate properly at 25°C [13,14], because 15°C has been reported as a critical temperature for the germination of the species of that genus [15], and because the temperature is the main factor regulating the germination in areas with a marked thermal seasonality [16], as is the case where *A. durangensis* grows. The relative humidity ranged between 98% and 100%. Previous evaluations made by the authors of the present study indicated that the seeds of *A. durangensis* are indifferent to light to germinate; that has been reported for the seeds of other plant species [13,17,18].

Table 1. Collection sites for three natural populations of *Agave durangensis* in Durango, Mexico.

Sample	Number of reference	Latitude N	Longitude W	Altitude (m)
Pino Suárez	D05EPS001	23°47'09.9"	104°25'02.2"	2054
La Parrilla	D16EPA101	24°00'53.5"	103°56'43.6"	2050
Veracruz	D22EVE205	24°00'53.5"	103°56'43.6"	2050

2.4. Rate of Germination

The germination performance was evaluated at 24 h intervals. The germination rate (V) was determined according to Maguire [19] with the Equation (1).
Equation (1):

$$ V = \left[\frac{\text{Total of sprouts at day 1}}{1} \right] + \left[\frac{\text{Total of sprouts at day n}}{n} \right] $$

where V is the rate germination and n is the number of days after imbibition.

2.5. Germinability (% G)

Germinability was determined according to García and Lasa [20], using the Equation (2).
Equation (2):

$$ \% G = \left(\sum_{i=1}^{k} \frac{n_i}{N} \right) [100] $$

where n_i is the number of seeds which germinated on the day i and N is the number of seeds evaluated. Seeds with visible radicle (length > 0.10 mm) were considered as germinated.

2.6. Growth Reduction of Plantets under Cold Stress (15°C)

Seed vigor was also evaluated by the percentage reduction of plantlet growth (representing the distance between the tip of the primary leaf and the primary root tip) under cold stress at 15°C, compared with the growth at 25°C, after 360 h imbibition. This evaluation was made according to Talai and Sen-Mandi [3], using the Equation (3).
Equation (3):

$$ \% \text{ reduction of length at } 15°C = \left(\frac{\left[LES_{op} - LES_{et} \right]}{LES_{op}} \right) (100) $$

where LES_{op} is the plantlet length after 360 h, at 25°C and LES_{et} is the plantlet length after 360 h, at 15°C.

2.7. Extraction of Phenols

The seeds (5 g) of each sampled population were individually grinded in liquid nitrogen. The extraction of phenols was carried out according to Ardekani *et al.* [21], by maceration in 20 mL of a solution containing water-methanol-acetic acid-formic acid (20:40:39:1), at room temperature, darkness, and shaking (120 rpm) for 24 h. The extracts were centrifugated (8000 rpm, 25°C, for

10 min). The supernatants were decanted and concentrated to dryness. The dry extracts were disolved in 2 mL of methanol. Aliquots were used to determine the total phenol levels, the phenol profiles, and the antioxidant activity. The extracts were prepared from three independent seed lots of each population.

2.8. Total Phenols

The concentrations of total phenols were determined with the Folin-Ciocalteu reagement, according to Lozoya-Saldaña *et al*. [22]. The phenol contents were expressed as mg gallic acid equivalents (EAG)/g seeds, using a calibration curve of gallic acid (Abs_{760nm} = 0.0657 [gallic acid] + 0.0159, correlation coefficient r = 0.9874). The absorbances were registered at 760 nm (Jenway Genova spectrophotometer), after 120 min in darkness. All samples were analyzed in triplicate.

2.9. Phenolic Profiles

The phenolic compositions of the extracts (aliquots of 20 µL) were determined by HPLC-DAD, according to a modified gradient method from that of Campos and Markham, [23], by using a Perkin Elmer Series 200 HPLC system and a Perkin Elmer Brownlee Validated C18 (250 × 4.6 mm, 5 µm) column. Solvent A was water acidified with phosphoric acid (pH = 4.0) and the solvent B was acetonitrile. The gradient was: 0 to 24 min, 100% A; 24 to 40 min, 91% A; 40 to 80 min, 68% A; 80 to 84 min, 67% A; 84 to 104 min, 57% A; 104 to 120 min, 57% A, and 120 to 125 min, 100% A. The flow was 0.8 mL/min. The chormatograms were registered at 260 and 340 nm. Spectral data for all the peaks were obtained between 200 to 400 nm, using diode-array detection (Perkin Elmer Series 200). Structural information was obtained by direct comparisons of retention times and UV spectra of resolved compounds with those of standards and according to Mabry *et al*. [24] and Campos and Markham [23].

2.10. Antioxidant Activity

The antioxidant activities of the extracts were determined by the free radical scavenging potential, using a freshly solution of 2,2-diphenil-1-picrilhydracil (DPPH*), according to Campos *et al*. [25]. One hundred microliters of each extract were added to 900 µL of the solution of DPPH* (57.5 µg/ml ethanol). The decrease in absorbance at 523 nm (Jenway Genova spectrophotometer) after 30 min was registered. The percent of DPPH* scavenged by each sample was calculated by the equation (4).

Equation (4):

% DPPH* scavenging activity $= (A_0 - A_1/A_0)100$

where A_0 = absorbance of DPPH* solution, and A_1 =

absorbance of DPPH* solution/sample of extract after 30 min reaction. Ascorbic acid was evaluated in the same manner as reference. Measurements were taken in triplicate.

A lineal regression analysis was done to evaluate the association between the seed phenol contents and the scavenging activity by registering the $A_{523\ nm}$ reduction with increased volumes of seed extracts.

2.11. ADH Activity

The alcohol dehydrogenase (EC 1.1.1.1) activity was registered before seed imbibition and after seed imbibition at 6 h intervals until the radicle profusion, by the reduction of NAD in the presence of ethanol according to Rumpho and Kennedy [26], in extracts prepared from 1.25 mg of embryo and 1.5 mL of a solution containing 10 mM Tris-HCl (pH 7.5), 2 mM EDTA, and 20 mM *β*-mercaptoethanol. Protein assay was performed by the method of Lowry [27], expressed as mg bovine serum albumin equivalents (EBSA equivalents)/mL of extract. A solution of 1 mM NAD and a buffer of 50 mM Tris-HCl were prepared. The reaction mixtures were composed of 400 µL of embryo extract, 200 µL of Tris buffer, and 50 µL of NAD solution. The reaction was initiated by the addition of 50 µL of ethanol. The absorbance was registered at 340 nm (Jenway Genova spectrophotometer) after 2 min of incubation at 25°C and at 15°C. The ADH activity was expressed as µmol NAD^+ reduced/min/mg protein. The value of molar extinction coefficient of 6.22 × 103/M/cm was used to estimate the concentration of NAD^+. Measurements were taken in triplicate.

2.12. Data Analysis

An ANOVA test was used to evaluate differences among samples. Mean comparisons were made by using Statistica 7.0. A principal component analysis (PCA) and a correlation test, using Past 2.12 [28], were carried out from a matrix constructed with the results of all the evaluated parameters. The PCA analysis, grouping the traits, was employed to evaluate the percentage contribution of each trait to the seed vigor variation.

3. Results

3.1. Seed Morphological Characterization

The seeds of the three evaluated populations of *Agave durangensis* were lacrimiform, smooth, and black (**Figure 1**), features that are common to all species of genus *Agave* [29].

The weights and dimensions of the seeds of the populations evaluated are showed in **Table 2**. Significant variations (p < 0.05) in the weight and width among the

Figure 1. Seeds of *Agave durangensis* from three populations of Durango, Mexico ((a) Pino Suárez, (b) La Parrilla, (c) Veracruz).

Table 2. Weight and dimensions of the *Agave durangensis* seeds of three natural populations. The values represent the mean and standard deviation for 150 seeds. Different letters mean significant differences (p < 0.05).

Population	Weight/seed (mg)	Width/seed (mm)	Length/seed (mm)
Pino Suárez	0.68 ± 0.02a	3.99 ± 0.5b	5.56 ± 0.68
La Parrilla	0.99 ± 0.02b	4.62 ± 0.3c	5.92 ± 0.33
Veracruz	1.15 ± 0.01b	3.51 ± 0.52a	5.29 ± 0.60

three kinds of seed samples were observed. Weight varied from 0.68 mg in the seeds of Pino Suárez (p) to 1.15 mg in the seeds of Veracruz (v). The biggest seeds were those of La Parrilla (4.62 × 5.92 mm).

3.2. Germinability

The values of the germination percentage of the analyzed seeds of *A. durangensis* are present in **Tables 3** and **4**. At 25˚C (**Table 3**) the seeds of the three populations began the germination at 72 h. At that time, the highest values of germination were for the seeds of Pino Suárez (17%), whereas those of Veracruz showed the lowest percentage (10%). The seeds of La Parrilla reached 100% of germination at 120 h, those of Pino Suárez at 192 h, and those of Veracruz reached 99% of germination at 216 h. Variability in the proportion of seeds beginning the germination and in the time reaching the highest germinability was observed. However, non significant differences were found after 240 h of imbibition in the germinability values.

At 15˚C, 24% of the seeds from Veracruz (the first ones starting the germination at that temperature) began the germination at 264 h (**Table 4**). That means a gap of 192 h in the beginning of germination compared with the one at 25˚C (**Table 3**); 95% of the seeds of that same population germinated at 15˚C, which was the highest value of germinability at the stress temperature. The seeds of Pino Suárez and La Parrilla started the germination 24 h later, and reached values of 49 and 87%, respectively, at the end of the experiment (360 h). The variations found in the germinability at this temperature

were significant (p < 0.05).

3.3. Rate of Germination (V)

The **Table 3** presents the rates of germination of the different lots of seeds of *A. durangensis* at 25˚C. Those corresponding to 15˚C are present in **Table 4**. Significant differences (p < 0.05) were found between the rates of germination estimated at each condition of temperature, and at 25˚C, significant interpopulation differences were also found. At 25˚C, the seeds of Veracruz and Pino Suárez showed the highest germination rates (48.5 and 47.6 seeds/day, respectively), and at 15˚C, the seeds of Veracruz showed the highest rate (6.77 seeds/day).

3.4. Growth Reduction of Plantets under Cold Stress

The plantlet growth of the three analyzed populations of *Agave durangensis* was sensitive to temperature decrease from 25˚C to 15˚C (**Table 5**). Significant differences (p < 0.05) in the growth reduction were observed among the populations. The plantets of La Parrilla and Veracruz were the most affected, showing a growth reduction of 91.45% and 91.99%, respectively, whereas the ones of Pino Suárez were the least affected having reduced their growth only 86.66%.

3.5. Total Phenols and Antioxidant Capacity

Significant interpopulation variability (p < 0.05) in the phenol contents of the *Agave durangensis* seeds was found (**Table 6**). The highest value corresponded to the seeds of Pino Suárez, with 85 μg/g seeds; however, the seeds of La Parrilla, with a lower phenol content (71 μg/g seeds), and with no significant differences with the seeds of Veracruz, showed the highest antioxidant potential (50.35%). The antioxidant potential of ascorbic acid was significantly higher (65.28% ± 1.04%, p < 0.05) than any of the extracts of seeds of *A. durangensis*.

A linear reduction of DPPH* concentration associated to increasing of the phenol contents in the seed extracts was observed: Pino Suárez seeds: $A_{523nm} = 0.0551 - 0.0003$(extract volume), r = −0.9944; La Parrilla seeds: $A_{523nm} = 0.0421 - 0.0002$(extract volume), r = −0.9860; and Veracruz seeds: $A_{523nm} = 0.0532 - 0.0002$(extract volume), r = −0.9894) (**Figure 2**).

3.6. Phenolic Profile of Seeds

Important differences in the phenol composition, determined by HPLC-DAD, were found in the seeds of the three natural populations of *Agave durangensis*. The retention times (RT) and the UV spectral data for the 14 compounds resolved are shown in the **Table 7**. The seeds of Pino Suárez accumulated four phenolic acids; the

Table 3. Accumulated germination at 24 h intervals, germination percentage (% G), and rate of germination (V), at 25˚C, of seeds of *Agave durangensis* from three natural populations. The values represent the mean and standard deviation for three independent samples. Different letters mean significant differences (p < 0.05).

Population	72 h	96 h	120 h	144 h	168 h	192 h	216 h	240 h	G (%)	V (seeds/day)
Pino Suárez	0.17 ± 0.04	0.45 ± 0.07	0.92 ± 0.01	0.96 ± 0.04	0.99 ± 0.01	1	1	1	100	47.62 ± 1.78b
La Parrilla	0.15 ± 0.06	0.29 ± 0.01	1	1	1	1	1	1	100	48.5 ± 1.06b
Veracruz	0.10 ± 0.09	0.42 ± 0.01	0.75 ± 0.05	0.95 ± 0.05	0.98 ± 0.02	0.98 ± 0.02	0.99 ± 0.02	0.99 ± 0.02	99 ± 0.02	44.0 ± 0.84a

Table 4. Accumulated germination at 24 h intervals, germination percentage (% G), and rate of germination (V), at 15˚C, of seeds of *Agave durangensis* from three natural populations. The values represent the mean and standard deviation for three independent samples. Different letters mean significant differences (p < 0.05).

Population	168 h	192 h	216 h	240 h	264 h	288 h	312 h	336 h	360 h	G (%)	V (seeds/day)
Pino Suárez	0	0	0	0	0	0.26 ± 0.01	0.30 ± 0.01	0.44 ± 0.07	0.49 ± 0.08	49 ± 0.08a	5.17 ± 0.64
La Parrilla	0	0	0	0	0	0.15 ± 0.05	0.21 ± 0.02	0.67 ± 0.07	0.87 ± 0.07	87 ± 0.07b	6.10 ± 0.33
Veracruz	0	0	0	0	0.24 ± 0.01	0.48 ± 0.05	0.72 ± 0.06	0.78 ± 0.06	0.95 ± 0.01	95 ± 0.01c	6.77 ± 0.70

Table 5. Growth reduction of plantets of three natural populations of *Agave durangensis*, after 360 h, due to the temperature reduction of germination. The values represent the mean and standard deviation for three independent samples. Different letters mean significant differences (p < 0.05).

Population	Plantets growth (cm)		Growth reduction (%)
	25˚C	15˚C	
Pino Suárez	5.49 ± 0.31	0.73 ± 0.02	86.66 ± 3.10a
La Parrilla	5.85 ± 0.90	0.5 ± 0.07	91.45 ± 1.48b
Veracruz	5.01 ± 0.08	0.4 ± 0.05	91.99 ± 0.97b

Table 6. Total phenol contents and antioxidant potential of the seeds of three populations of de *Agave durangensis*. The values represent the mean and standard deviation for three independent samples. Different letters mean significant differences (p < 0.05).

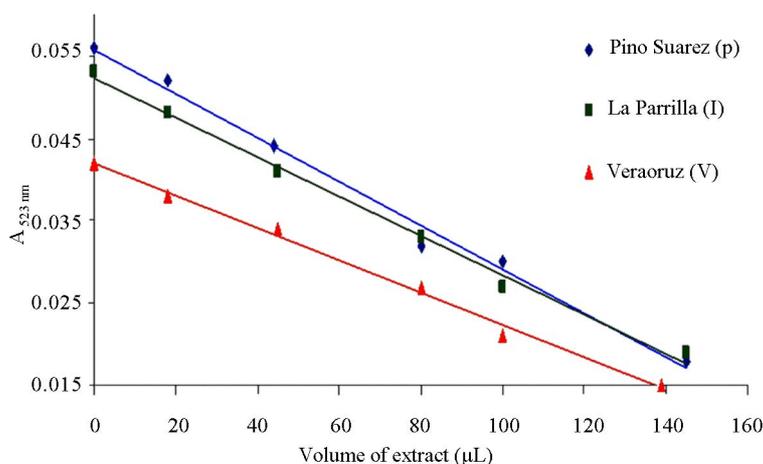| Population	Total phenol content (mg gallic acid equivalents/g seeds)	DPPH* inhibition (%)
Pino Suárez	0.085 ± 0.00b	42.13 ± 3.27
La Parrilla	0.071 ± 0.008a	50.35 ± 6.99
Veracruz	0.078 ± 0.002a	49.39 ± 2.96

Figure 2. DPPH* disappear associated to the increasing in the phenol concentration of the seed extracts of three populations of *Agave durangensis*.

Table 7. Retention time and spectral data of the phenolic compounds found in the seeds of three populations of *Agave durangensis*.

Location	Number of Compound	Type of phenolic compound	λmax (nm)	RT (min)
Pino Suárez	**1**	*O*-coumaric acid derivative	240 sh, 272, 302 sh	70.462
	2	Phenolic acid	272	78.236
	3	Phenolic acid	247	96.393
	4	Phrnolic acid	282 sh, 295	116.956
La Parrilla	**5**	Phenolic acid	235 sh, 290, 320 sh	64.346
	6	Dihydroflavonoid	280, 310 sh	68.556
	7	Flavonol, possible herbacetin derivative	273, 296 sh, 317 sh	70.58
	8	Phrnolic acid	230 sh, 277, 315	74.129
	9	Phenolic acid	240, 305 sh 322	77.4
	10	Flavone, possible scutellarein derivative	272, 330	83.926
	11	Flavone, possible scutellarein derivative	272, 330	95.66
	12	Flavone, possible scutellarein derivative	274, 330	97.405
Veracruz	**13**	Phenolic acid	290, 320	50.824
	14	Dihydroflavonoid	290, 320 sh	61.432
	7	Flavonol, possible herbacetin derivative	273, 296 sh, 317 sh	70.389
	3	Phenolic acid	247	96.276

seeds of La Parrilla, three phenolic acids, one dihydroflavonoid, one flavonol, and three flavones; and the seeds of Veracruz, two phenolic acids, one dihydroflavonoid, and one flavonol. The compound **3**, a phenolic acid, was common to the seeds of Pino Suárez and those of Veracruz, and the compound **7**, one flavonol, possible derivated of the flavonol herbacetin, was common to the seeds of La Parrilla and to those of Veracruz. The respective chromatograms are displayed in **Figure 3**.

3.7. ADH Activity

Significant variability (p < 0.05) in the ADH activity of the seeds, before inhibition, among the three natural populations of *Agave durangensis* was found (**Figure 4**), being the highest for the seeds of Pino Suárez (1100 μmol NAD$^+$/mg protein/min), and the lowest for the seeds of La Parrilla (180 μmol NAD$^+$/mg protein/min).

At 25°C and in imbibitions conditions, after a continuous diminish for 6 h, the ADH activities of the seeds of Veracruz and Pino Suárez, increased to reach a high level (520.2 and 400.9 μmol NAD$^+$/mg protein/min, respectively) at 12 h after imbibition. The seeds of La Parrilla reached latter (18 h after imbibition) their highest level of ADH activity (310.24 μmol NAD$^+$/mg protein/min) (**Figure 4**).

The highest level after imbibitions of the ADH activity at 15°C was observed in the seeds of Veracruz (440.71 μmol NAD$^+$/mg protein/min). Lower activities were estimated for the seeds of Pino Suárez and La Parrilla

(170.74 and 185.62 μmol NAD$^+$/mg protein/min, respectively). The seeds of Veracruz reached the highest ADH activity sooner (at the 12th day after imbibition) than the seeds of La Parrilla and Pino Suárez (both at the 24th day after imbibition) (**Figure 5**). The time taken for the diminution of the ADH activity by the three populations at this temperature was longer than at 25°C (**Figures 4 and 5**), and this gap matched with the delay in the germination at 15°C (**Table 4**).

3.8. PCA and Correlation Analysis

The results of a PCA, based on the genetic variability revealed by the different indicators of seed vigor, are showed in the **Figure 6**. The clear discrimination between the three natural populations of *Agave durangensis* can be observed.

Six principal components accounted for practically 100% of total variance, being the PC1 the mean one, taking 78.9%; this same component had the highest relative discriminating power (eigen value 190774) and the PC6 had the lowest relative discriminating power (eigen value 4.299) (data not shown). The PC1 was mostly correlated with the germinability at 15°C, rate germination at 15°C, weight, and growth reduction; PC2 with the ADH activity at both temperatures; PC3 with the germinability at 25°C; PC4 with the growth reduction; PC5 with the DPPH scavenging; and PC6 with the ADH activity at 25°C.

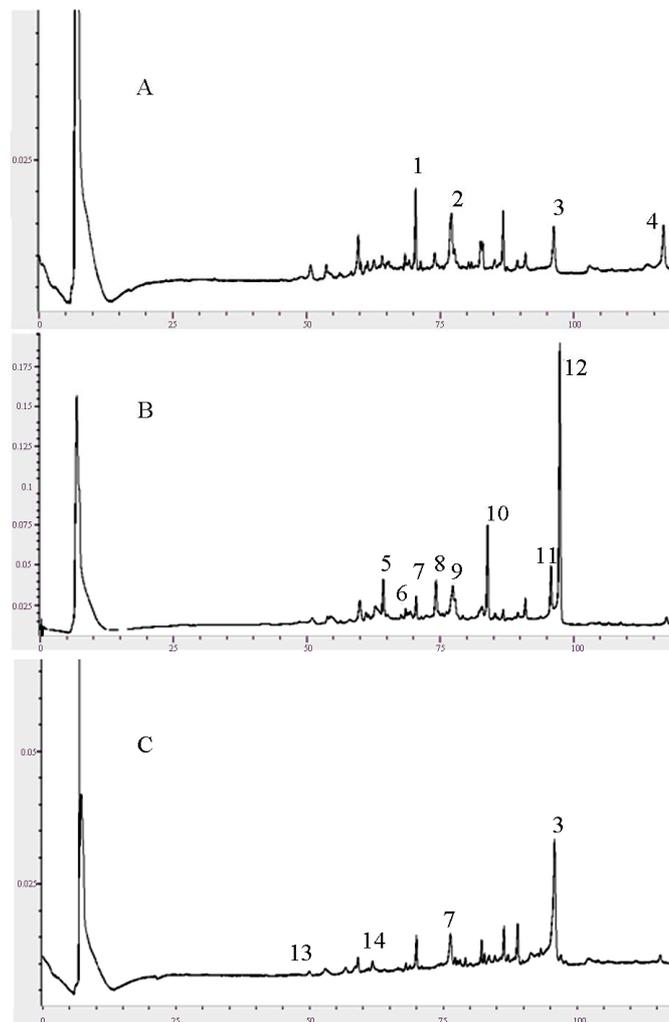The correlation coefficients for the pairs of the seed

Figure 3. HPLC chromatograms of seed extracts of *Agave durangensis* from three populations of Durango, Mexico ((A) Pino Suárez, (B) La Parrilla, (C) Veracruz). The numbers of compounds correspond to those in Table 7.
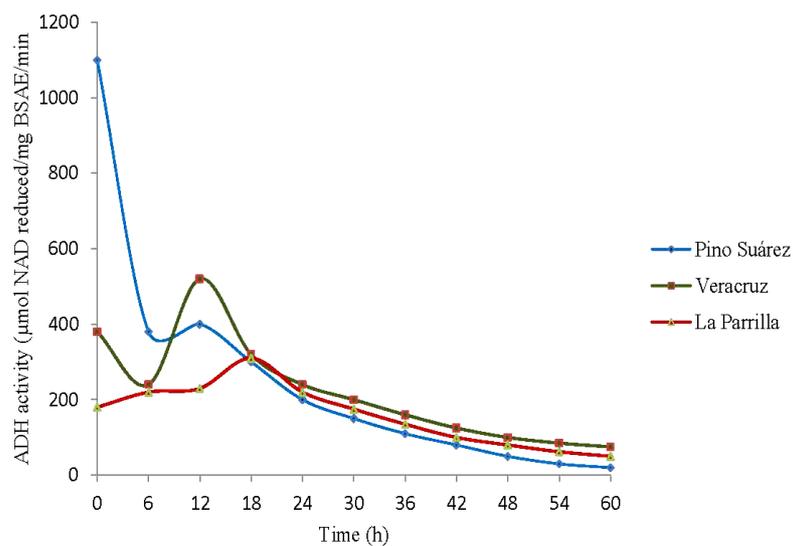

Figure 4. ADH activity at 25˚C in extracts of embryos of seeds of three natural populations of *Agave durangensis*.
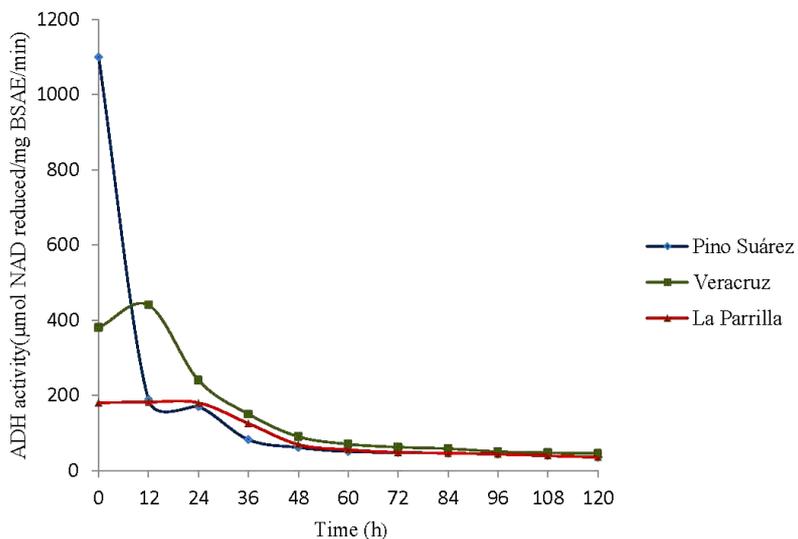
Figure 5. ADH activity at 15˙C in extracts of embryos of seeds of three natural populations of *Agave durangensis*.
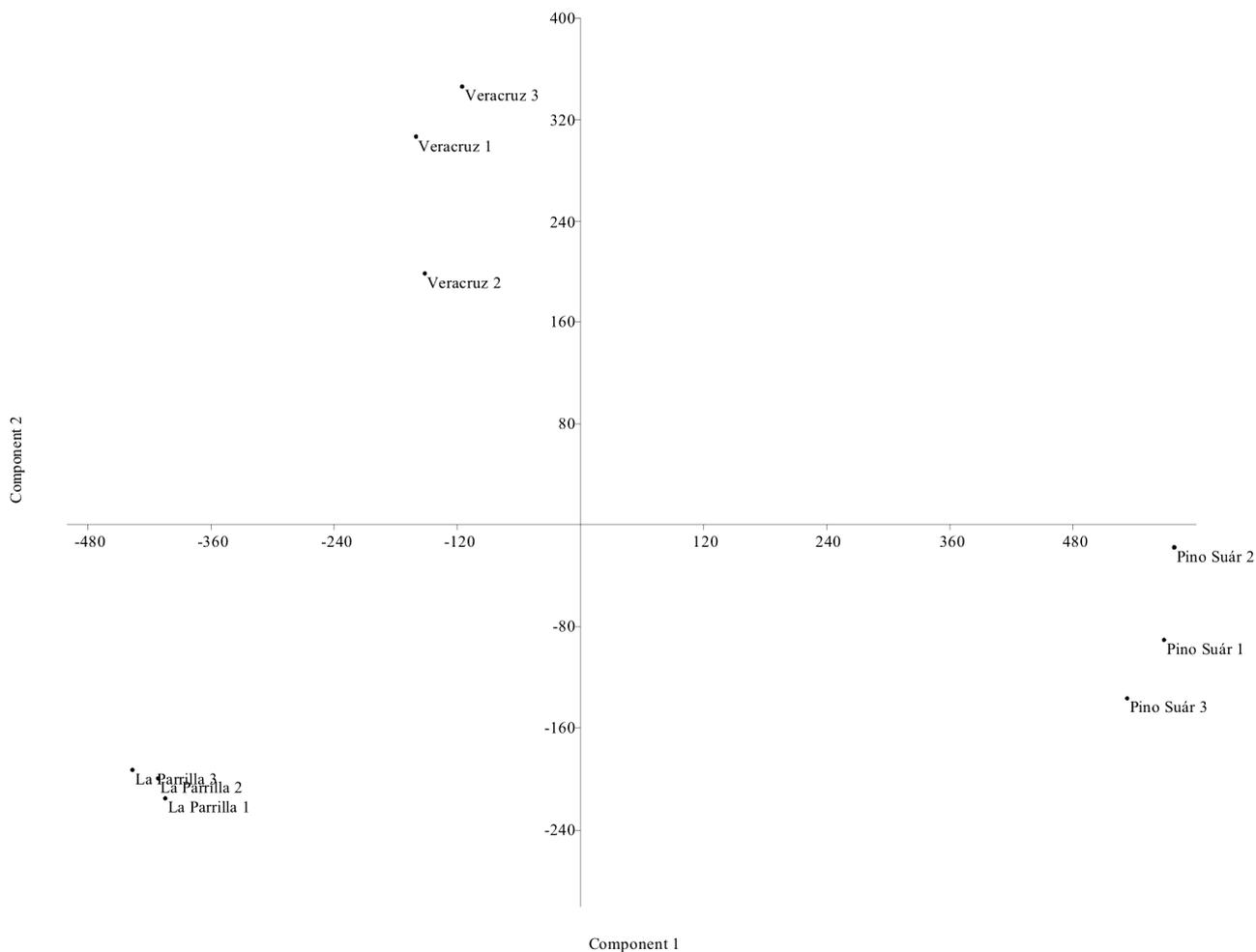

Figure 6. Results of a PCA based on the variation of the weight, and physiological, chemical and biochemical indicators of seed vigor of three lots of three natural populations of *Agave durangensis*.

vigor indicators used to characterize each of natural   population of *A. durangensis* were calculated. The corre-

lation matrix is showed in **Table 8**.

4. Discussion

4.1 Seed Morphological Characterization

Seed weights of 9.50 mg have been reported for *Agave durangensis* [15], a value around nine fold higher than the values reported in the present paper for any of the three natural populations evaluated. This suggests an important intraspecific variation in the seed weight for that species of *Agave*. Some authors have suggested than the morphological variations in the seeds of plants of the same species occurring in adjacent populations can result from interpopulation, and in some cases, even from interspecific hybridization processes [30], can be the result of a processes of adaptation to store high levels of nutrients [31,32], or can be associated to the seed dispersion, the lighter ones favoring the conservation or extending of the natural distribution area of the species with wind dispersed seeds, as is the case of most species of *Agave* [33]. The nutritional conditions of progenitors also can determine the weight of seeds [32,34]. As well the seed moisture content may strongly influence the weight.

4.2. Germinability

The seeds of Pino Suárez and La Parrilla, which had the highest fast germination capacity at 25°C, could have a higher potential than those of Veracruz to take advantage of the favorable temperature and humidity, which are variable over seasons and years; the favorable season for seedling growth is short (four months) in the geographical region of the natural distribution of *A. durangensis*.

The formation of seed banks has been reported as an important strategy of species of unpredicted precipitation environments [35], as those occurring in Durango, Mexico; however, the fast and total germination showed by the seeds of *A. durangensis* at 25°C, happening in a short time (5 to 9 days) suggests that the seeds of that species do not form seed banks. That germination behavior could represent, in natural conditions, a risk in years when the precipitation distribution is irregular and scarce.

Ramírez-Tobías *et al*. [15] reported, for *A. durangensis*, a germination value, at 25°C, of 91%, reached at 150 h. Those results are different from those found in the present study for the same species (**Table 3**); that could be a consequence of the intraspecific variability concerning the germination capability of *A. durangensis*.

The results of the present study indicated that the decrease of temperature caused a delay in the beginning of the germination and a reduction of the germination potential. Ramírez-Tobías *et al*. [15] reported that the germination of *A. durangensis* was inhibited in around 50% at 15°C. Our study revealed that between 49% and 95% of the seeds of the natural populations of *A. durangensis* can germinate at that temperature (**Table 4**), although taking longer than at 25°C.

The temperatures favorable to germination vary much between different species of plants. The boundaries are often narrow for seeds of species adapted to very specific habitats and broader for seeds of species of wide distribution [36]. *Agave durangensis* is a species of reduced distribution [2]; according to the results of the present study, its germination is compatible with a broad interval of temperatures, indicating a high potential of germination response, what is related to its main propagation mechanism, which is by seeds.

Table 8. Correlation coefficients of 11 seed vigor indicators used to characterize the natural populations of *Agave durangensis*.

	G 25°C	G 15°C	Rate of Germ 25°C	Rate of Germ 15°C	Growth reduction	Phenol content	DPPH scavenging	Seed ADH	ADH 25°C	ADH 15°C	Weight
G25°C	1	0.461	0.568	0.690	0.743	0.887	0.642	0.740	0.220	0.765	0.332
G15°C		1	0.353	0.0009	0.012	0.033	0.022	0.0002	0.807	0.135	3.4×10^{-5}
Rate of Germ 25°C			1	0.132	0.703	0.505	0.800	0.878	0.067	0.023	0.175
Rate of Germ 15°C				1	0.067	0.115	0.010	0.012	0.935	0.112	0.001
Growth reduction					1	0.141	0.172	0.013	0.925	0.215	0.010
Phenol contents						1	0.090	0.0009	0.072	0.841	0.106
DPPH scavenging							1	0.042	0.573	0.459	0.079
Seed ADH								1	0.299	0.518	0.004
ADH 25°C									1	0.012	0.706
ADH 15°C										1	0.029
Weight											1

4.3. Rate of Germination (V)

The germination rate is a method to evaluate seed vigor; it considers the number of normal plants that germinate per day in preestablished conditions of germination. A high value of germination rate is related to seeds with high vigor [19].

The results of the present study indicate that the seeds of Veracruz showed the best response of germination under temperature stress conditions (**Table 4**). Several reports about the use of germination rate evaluating the seed vigor of monocotyledonous species [37-39] and of dicotyledonous species [40-42] have been published. Ramírez-Tobías *et al*. [15] reported that the germination rate of *Agave durangensis* at 25˚C was around 0.5% seeds/h. The rates found in the present study for any of the three populations analyzed were higher (also at 25˚C), between 3.66% seeds/h for the seeds of Veracruz to 4.04% seeds/h for those of La Parrilla (values estimated from our raw data to compare with the results of Ramírez-Tobías *et al*. [15]).

4.4. Growth Reduction of Plantets under Cold Stress

According to Talai and Sen-Mandi [3] there is an inverse relation between the growth reduction of plantets under cold stress and the seed vigor. In this frame, the seeds of *A. durangensis* of Veracruz (v), with a growth reduction of 91.9% at 15˚C, would be the less vigorous, and those of Pino Suárez (p), with a growth reduction of 86.6% at 15˚C compared with the growth at 24˚C, would be the most vigorous (**Table 5**). To our knowledge, no reports on the evaluation of this parameter in *Agave* have been published.

4.5. Phenol Composition and Antioxidant Capacity

The germination is related to the antioxidant potential of seeds [43]. Some authors have suggested the use of that potential as vigor indicator [44]; according to those authors the seeds of La Parrilla, with the highest antioxidant potential (50.35%, **Table 6**) would display the uppermost vigor. The antioxidant potential, before and during germination, is determined by the antioxidant enzymatic activity of the embryo cells once these are hydrated [44-46], and by the non-enzymatic antioxidant compounds found in an active way in seeds, independently of the hydratation of seeds [47,48]. Phenolics are among those compounds; actually they are the main responsible in maintaining the growth potential of the embryo at storage conditions (pregerminatory stage) until the imbibition allows the activation of the antioxidant mechanism directed by enzymes [3]. A high correlation between the phenol content and the antioxidant potential

in the seeds of *Agave durangensis* was found in the present study (**Figure 2**), in accordance to the proposal of Talai and Sen-Mandi [3]. The differences between the free radical scavenging activities exhibited by the seeds of each natural population of *Agave durangensis* can be due to the variations in phenol contents (**Table 6**) and the types of phenols accumulated, which were different in the seeds of each population (**Table 7**). The particular phenol profile is a relevant feature to determine the antioxidant properties of any plant tissue or structure [49].

Seed phenols form complexes with carbohydrates and lipids, enhancing the stability of those organic compounds in the endosperm under oxidative stress conditions, avoiding the beginning of a tandem oxidative degradation caused by oxygen reactive species [50]. Seed phenols also regulate the osmoconditioning in the first stage of germination, in which the phenolic levels inside seeds reduce, as a consequence of being moved out the seed tissues [51].

4.6. ADH Activity

The ADH activity results suggest that the seeds of the natural populations of *Agave durangensis* begin the germination *sensu strict* between 12 and 18 hrs after imbibitions. At that stage the polypeptide synthesis is activated, causing the metabolization of the endospermic tissues and the development and growth of the vascular structures of the new plant [52]. The major anaerobiosis conditions are present as a consequence of imbibition and minimum oxygen exchange [53], and then the highest metabolic activity in anaerobic conditions is displayed [54]. All those conditions shoot the metabolic pathways to synthesis of adenosin triphosphate (ATP), with ethanol as a subproduct and using ADH like catalyzer [55].

At 25˚C, the seeds of *Agave durangensis* from Pino Suárez and from Veracruz began a reduction of the enzymatic activity 12 hrs after imbibition, while the seeds from La Parrilla began a reduction 18 hrs after imbibition. At this temperature the seeds of Veracruz showed the highest level of ADH activity (520.2 µmol NAD$^+$/mg protein/min) (**Figure 4**).

At 15˚C and after imbibitions, the seeds of Veracruz showed the highest ADH levels (440.71 µmol NAD$^+$/mg EBSA/min) (**Figure 5**). The time taken by the three populations of *A. durangensis*, for the diminution of ADH activity, was longer at 15˚C than at 25˚C, because, according to Labouriau [56], at 15˚C the seeds are closer to the required thermodynamic limit to start germination.

The ADH activity decrease through the time, at both 15˚C and 25˚C, is due to a change in the permeability of the seed membranes, increasing the oxygen exchange of the embryo [9]. That decrease went on until the emergence of the hypocotile and radicle, when the plantets could carry out the aerobic respiration (**Figures 4** and **5**,

Table 3).

Talai and Sen-Mandi [3] stated that the ADH activity is essential during the anaerobic respiration in the pre-germination and germination of seeds and for that reason the ADH activity is a vigor indicator. According to that, the seeds of *A. durangensis* of Veracruz (v), which showed the highest ADH activity at either 15˚C or 25˚C, represent the lots with the best vigor features.

4.7. PCA and Correlation Analysis

The seed vigor variation of *A. durangensis* has not been well investigated; just a few reports have been published about some issues of germination process [15]. To our knowledge, the present study represents the first attempt to investigate that kind of variation among natural populations of this species. The clear separation of the three populations analyzed, with base on the variation of seed weight and the evaluated physiological, chemical, and biochemical indicators of seed vigor, suggests that worthy alleles can be found from the natural populations of *A. durangensis* to select seeds with high vigor to be used in the establishment of plantations. Among the three populations of *A. durangensis* analyzed, that of Veracruz showed high values in eight (weight, germinability at 25˚C and 15˚C, germination rate at 15˚C, phenol content, scavenging activity, and ADH activity at 25˚C and 15˚C) of the 11 seed vigor features evaluated.

The biochemical indicators (DPPH scavenging and ADH activity) showed high associations with the physiological indicators of seed vigor and with the phenol contents in the seeds (**Table 8**). The results support those previously reported by several authors about the significance of the chemical and biochemical markers as seed vigor indicators [3,44] and emphasize the importance of the antioxidant phenols in the seeds to prevent oxidative damage in the embryos and the essential participation of alcohol deshydrogenase in the anaerobic respiration in the germination of the seeds of *A. durangensis*.

5. Conclusion

Morphometric differences can be found among the seeds of *Agave durangensis* of different natural populations. Variation in some physiological, chemical and biochemical indicators of vigor was detected. According to the results of germination potential, and to biochemical attributes, like ADH activity, phenolic composition, and antioxidant potential, the seeds of Veracruz have the highest vigor. Each of the three natural populations of *A. durangensis* could be typified by their morphological attributes and by their physiological, chemical and biochemical indicators of seed vigor. High correlations between chemical and biochemical markers and the germination markers were found, in such a way that the evalua-

tion of the former ones as indicators of seed vigor can assist in the selection of seed lots with high germination performance. The results of the present study suggest that the variability of the natural populations of *A. durangensis* is an important source of worthy alleles, which can provide relevant support for the genetic improvement of this species of *Agave*.

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