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Research article

Germinación de semillas en *Agave potatorum* Zucc.

Seed Germination in *Agave potatorum* Zucc.

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Abstract

The study of seeds includes tests that allow characterizing and defining the suitability of germplasm to conserve or propagate species. This study aimed to evaluate the effect of different pre-germination treatments on the germination rate of *Agave potatorum*. A completely randomized design with a 5×3×2 fixed-effect factorial arrangement was utilized; five pre-germination treatments were assessed: T1: 500 ppm gibberellic acid (AG_3); T2: 1 000 ppm AG_3; T3: 50 % inoculation of mycorrhizae (23 spores gram^{-1}) (*Glomus intraradices*); T4: 100 % mycorrhizae (46 spores gram^{-1}) and T5: Control (distilled water), applied in three seed sizes (small, medium and large) and two dates of monitoring: D1=10 and D2=15 days; the assessed variable was germination rate (*GR*, %). Tukey tests (α=0.05) were applied when statistical differences between factors, levels, and interactions were obtained. The results indicated that germination of *A. potatorum* differs statistically between dates (p=0.0004) and treatments (p=0.0005), but not between seed sizes (p=0.3335). The application of 500 ppm AG_3 registered a *GR* of 75 %. This pre-germination treatment speeds germination, improves seedling production, and maximizes the potential of the seed lot. *Ex situ* conservation is essential to promote sustainable use and revalue the potential of germplasm, both in its ecological and productive functions.

Keywords: Gibberellic acid, Agavoideae, Asparagaceae, germination, *Tobalá* agave, mycorrhizae.

Resumen

El estudio de las semillas incluye ensayos que permiten caracterizar y definir la aptitud del germoplasma para conservar o propagar especies. El objetivo de este estudio fue evaluar el efecto de diferentes tratamientos pregerminativos sobre el porcentaje de germinación de *Agave potatorum.* Se utilizó un diseño completamente al azar con arreglo factorial 5×3×2 de efectos fijos; se evaluaron cinco tratamientos pregerminativos: T1: 500 ppm de ácido giberélico (AG_3); T2: 1 000 ppm de AG_3; T3: 50 % de inoculación de micorriza (23 esporas gramo^{-1}) (*Glomus intraradices*); T4: 100 % de micorriza (46 esporas gramo^{-1}) y T5: Testigo (agua destilada), aplicados en tres tamaños de semilla (chica, mediana y grande) y dos fechas de monitoreo: F1=10 y F2=15 días; la variable evaluada fue la germinación (*GE*, %). Se aplicaron pruebas de *Tukey* (α=0.05) cuando se obtuvieron diferencias estadísticas entre factores, niveles e interacciones. Los resultados indicaron que la germinación

de *A. potatorum* es estadísticamente diferente entre fechas (p=0.0004) y tratamientos (p=0.0005), pero no en tamaño de semillas (p=0.3335). La aplicación de 500 ppm de AG_3 registró una *GE* de 75 %. Este tratamiento pregerminativo hace más rápida la germinación, mejora la producción de plántulas y maximiza el potencial del lote de semilla. La conservación *ex situ* es fundamental para promover el uso sostenible y revalorizar el potencial del germoplasma, tanto en sus funciones ecológicas como productivas.

Palabras clave: Ácido giberélico, Agavoideae, Asparagaceae, germinación, maguey tobalá, micorriza.

Introduction

Agave potatorum Zucc. (Verschaffelt agave) belongs to the family Asparagaceae and subfamily Agavoideae (APG, 2016; García-Mendoza et al., 2019); it is distributed from the eastern end of the Balsas River basin, through the Tehuacán-Cuicatlán Valley, to the lower parts of the Mixtec *Sierra* and central mountains of *Oaxaca* (García-Mendoza, 2010). It is a non-timber species utilized to produce "*Tobalá*" mezcal, which has a high market potential (Barrientos *et al.*, 2019).

In *Oaxaca*, its cultivation is incipient (García *et al.*, 2004) and the *acaule* or agave heart requires between 8 and 12 years for its utilization (Martínez-Ramírez *et al.*, 2013); it is used before flowering, which interrupts the process of sexual reproduction, limits the production of seeds, which is a source of germplasm for more effective propagation (García-Mendoza, 2010). Asexual reproduction is nonexistent, because it does not produce vegetative propagules (Torres *et al.*, 2015).

According to the International Seed Testing Association, ISTA (ISTA, 2021), standard germination evaluation is the most common procedure to know the physiological quality of a seed lot, this is performed under controlled conditions for seeds to carry out the stages or phases of germination (García-López *et al.*, 2016).

In *A. potatorum*, germination is epigeous, oleoproteaginous, and recalcitrant (Gutiérrez-Hernández *et al.*, 2020). Ortiz-Hernández *et al.* (2018) suggest assessing the germination rate 10 and 15 days after sowing (dds).

In other species like *Agave lechuguilla* Torr., *Agave asperrima* Jacobi, *Agave salmiana* Otto *ex* Salm-Dyck, and *Agave striata* Zucc., the physiological quality of the seed, the effect of the temperature gradient and water potential (ψ) on germination have been evaluated (Ramírez, 2010). Peña-Valdivia *et al.* (2006) observed a germination rate of 95 % for *A. salmiana*, 70 % for *A. mapisaga* Trel., and 50 % for *A. angusifolia* Haw. subsp. *tequilana* (F. A. C. Weber) Valenz.-Zap. & Nabhan (Ramírez *et al.*, 2016).

In germination processes, arbuscular mycorrhizal fungi (AMF) influence the softening of the testa (Quiñones-Aguilar *et al.*, 2016) and the production of phytohormones such as gibberellins and indolacetic acid in the seed (Alcántara *et al.*, 2019). Likewise, AMF form symbiotic associations with plants and influence phosphorus uptake; in agaves, their effect has been evaluated in the growth of *Agave inaequidens* K. Koch (Quiñones-Aguilar *et al.*, 2016) and *Agave cupreata* Trel. & A. Berger (Trinidad-Cruz *et al.*, 2017).

Agave potatorum is a strategic phytogenetic resource with economic value inextricably linked to the culture of the communities of *San Miguel Piedras*, *Nochixtlán*, *Oaxaca*, Mexico, where there is a need to promote its sustainability. Within this context, the objective was to assess the effect of different pre-germination treatments on the germination rate, to generate a propagation and multiplication strategy.

Materials and Methods

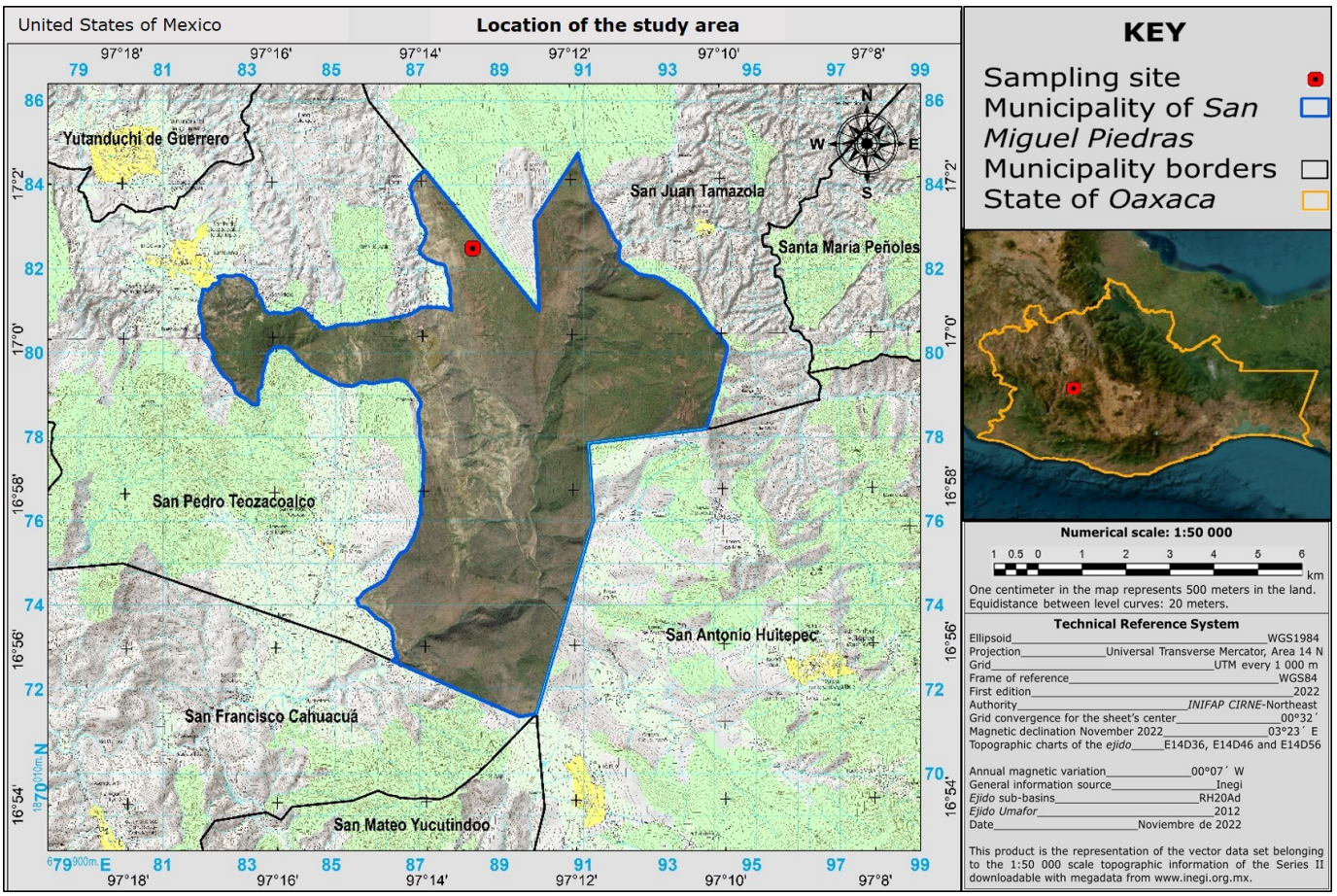
Study species

Agave potatorum is a perennial, monocarpic plant 30 to 60 cm high and 34 to 60 cm in diameter; it blooms from August to November, fructifies from November to March, and produces seeds only once in its life cycle; it reaches sexual maturity at approximately six years of age; it is acaulescent (does not generate tillers), and its leaves are succulent, ovate, 15 to 35 cm long and 5 to 10 cm wide, whitish to green in color, arranged in a rosette (Gentry, 1982; García-Mendoza, 2010). It is distributed within an altitude range of 1 240 to 2 400 meters above sea level, in association with pine-oak vegetation, low deciduous forest, and xerophilous scrub with *Quercus* L. It grows on flat or gently sloping sites, on sandy soils derived from limestone rocks (Gentry, 1982; Morales *et al.*, 2017; Gutiérrez-Hernández *et al.*, 2020).

Seed collection and study area

The collection site is located in the *ejido Guadalupe Victoria*, municipality of *San Miguel Piedras*, *Nochixtlán*, *Oaxaca*, Mexico, between the coordinates 17°01'7.62" N and 97°13'19.27" W, at an altitude of 1 832 m (Calderón de Rzedowski and Germán, 1993); its climate type is semi-warm sub-humid (A)C(w_1) (García *et al.*,

2004), with mean annual temperature of 16.5 °C, average annual precipitation of 842.2 mm; its soil type is Litosol (Inegi, 2018) (Figure 1).



Yutanduchi de Guerrero = Municipality of *Yutanduchi de Guerrero*; *San Juan Tamazola* = Municipality of *San Juan Tamazola*; *Santa María Peñoles* = Municipality of *Santa María Peñoles*; *San Pedro Teozacoalco* = Municipality of *San Pedro Teozacoalco*; *San Antonio Huitepec* = Municipality of *San Antonio Huitepec*; *San Francisco Cahuacua* = Municipality of *San Francisco Cahuacua*; *San Mateo Yucutindoo* = Municipality *of San Mateo Yucutindoo*.

Figure 1. Location of the study area in the *ejido Guadalupe Victoria*, municipality of *San Miguel Piedras*, *Nochixtlán*, *Oaxaca*, Mexico.

In February 2021, 80 mature capsules of *A. potatorum* were collected from adult plants and selected based on their height (cm), diameter (cm), and rosette conformation (Garcia-Mendoza *et al.*, 2019). The fruits were placed in clear, airtight plastic bags (10×10 cm) at room temperature (25 °C).

Pre-germination treatments

The seeds were kept for one year under refrigeration at 4 °C and a relative humidity of 5 %; germination tests were carried out in the Plant Tissue Culture laboratories of the *Saltillo* Experimental Field *CIRNE-INIFAP* and at the Center for Training and Development in Seed Technology (*CCDTS*) of the "*Antonio Narro*" Autonomous Agrarian University (*Universidad Autónoma Agraria Antonio Narro*).

Pure seeds (310.27 g) were used in the study with a viability of 71 %, determined with the 0.5 % 2,3,5-triphenyl tetrazolium chloride (Sigma-Aldrich®, USA) test (ISTA, 2021). The pure seed was separated by size with a model CFY-II South Dakota® blower, with an opening of 3 cm 1 min^{-1}, to obtain three samples: small (186.15 g), medium (77.56 g), and large (46.56 g) seed according to the classification of Vázquez *et al.* (2011).

Five treatments were assessed for the three seed sizes (small, medium, and large): T1: 500 ppm gibberellic acid (Sigma-Aldrich®, USA) (AG_3); T2: 1 000 ppm AG_3 (base solution of 100 mg of AG_3 in 100 mL of distilled water); T3: 50 % mycorrhiza inoculation (23 spores g^{-1}) (*Glomus intraradices* N. C. Schenck & G. S. Sm. 1982); T4: 100 % mycorrhiza (46 spores g^{-1}) (base solution 1 g of mycorrhiza in 100 mL of distilled water); and T5: Distilled water (control).

The germination test was carried out based on ISTA (ISTA, 2021) with the modification in the number of seeds in each experimental unit, due to the reduced amount of pure seeds collected. 20 seeds of each size were considered as an experimental unit, and nine repeats per treatment were established. Rao *et al.* (2007) and Di Sacco *et al.* (2020) have suggested that, for species with regeneration problems, especially wild taxa, 100 to 300 seeds constitute a good sample size for statistical analysis.

Each experimental unit was placed in a Petri dish on Whatman #1 filter paper, previously soaked with 3 mL of the corresponding treatment. The nine repeats per treatment of the three seed sizes were placed in a model UMF500 Hoffman® brand incubation chamber at a controlled temperature (28±1 °C), with a photoperiod of 8 h light and 16 h dark (ISTA, 2021).

For each treatment, two evaluation dates were considered: D1=10 dds and D2=15 dds (Ortiz-Hernández *et al.*, 2018). For each seed size, 900 were evaluated for a total of 2 700.

Evaluated variables and statistical analysis

The number of germinated seeds was determined to obtain the standard germination rate (*GR*) by treatment and seed size, based on the classification of AOSA (1983); germination was the dependent variable. The data were subjected to an analysis corresponding to a completely randomized design with a 5×3×2 factorial arrangement of fixed effects with the following model:

$$Y_{ijkl} = \mu + D_i + S_j + \tau_k + (DS)_{ij} + (D\tau)_{jk} + (S\tau)_{ik} + (DS\tau)_{ijk} + \varepsilon_{ijkl} \; ; \; k = 1, \ldots r$$

Where:

Y_{ijkl} = Response variable

μ = Overall mean

D_i = i^{th} level of the date factor, $i = 1,2$

S_j = j^{th} level of the seed size factor, $j = 1,2,3$

τ_k = k^{th} treatment effect, $k = 1,2,\ldots 5$

$(DS)_{ij}$ = Interaction of the factor date *i* and seed size *j*

$(D\tau)_{jk}$ = Interaction of factor date *i* and treatment *k*

$(S\tau)_{ik}$ = Interaction between the seed size and treatment factors, at levels *i* and *k*

$(DS\tau)_{ijk}$ = Interaction of factor date *i*, seed size *j*, and treatment *k*

ε_{ijkl} = Experimental error, $\sim NI(0,\sigma^2)$: *i. e.*, follows a normal/independent distribution, with mean 0 and σ^2 variance

The assumptions of homogeneity of variance (Barlett and Levene for interactions), normality (Shapiro-Wilk), independence (test of spurts) and non-additivity of the model (Tukey) were verified to ensure that they were met. Since the variable is expressed in percentage units, the arcsine transformation was used. When statistical differences existed between factors, levels, and interactions, Tukey tests were applied (α=0.05). For the statistical analysis, the following libraries were used:

'tidyverse' and 'agricolae' of the R® statistical software version 3.5.3 (R Core Team, 2020).

Results and Discussion

Compliance with the assumptions of the analysis of variance

Barlett's homogeneity for the germination variable was constant for Date (p=0.4823), Size (p=0.5799) and Treatments (p=0.2353). Levene's test for the interactions (Date×Size×Treatments) did not reject the Ho hypothesis (p=0.9938). The residuals were normally distributed (p=0.3955), were independent (p=0.2015), and the model was additive (Tukey p=0.5162).

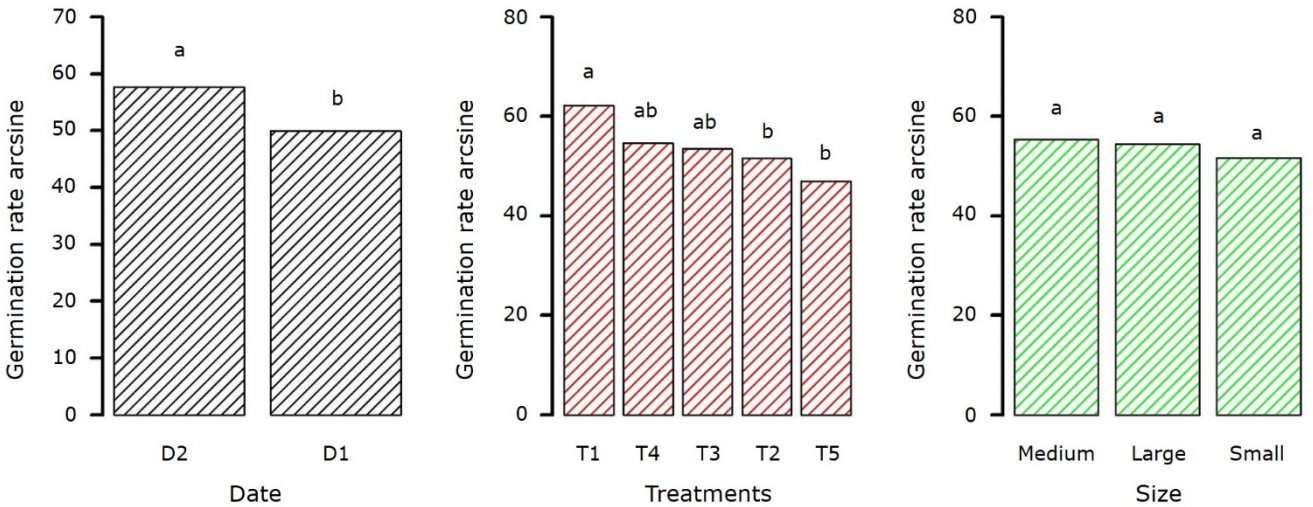
Germination of *Agave potatorum* seeds

There are significant differences in the germination race of *A. potatorum* between: Dates (p=0.0004), Treatments (p=0.0005); and in the interactions: Date×Treatments (p=0.0011) and Size×Treatments (p=0.0012) (Table 1, Figure 2). A Coefficient of variation of 18.12 % was obtained and the model explained more than 63 % of the variability of germination, resulting in a good model.

Table 1. Analysis of variance of *Agave potatorum* Zucc. germination tests.

Variation source	D. F.	S. S.	M. S.	*F* value	*Pr*(>*F*)	Significance
Date	1	1 359.90	1 359.87	14.32	0.0004	***
Size	2	212.40	106.20	1.12	0.3335	
Treatments	4	2 232.00	558.01	5.88	0.0005	***
Date×Size	2	405.60	202.82	2.14	0.1270	
Date×Treatments	4	1 978.80	494.71	5.21	0.0011	**
Size×Treatments	8	2 871.90	358.99	3.78	0.0012	**
Date×Size×Treatments	8	742.90	92.86	0.98	0.4620	
Residuals	60	5 697.20	94.95			

D. F. = Degrees of freedom; S. S. = Sum of squares; M. S. = Mean squares; *Pr*(>*F*) = Probability; ** = Significant; *** = Highly significant.



Letters above the bars indicate the groups according to Tukey's test at 95 %. D1 and D2 = Germination evaluation dates at 10 and 15 days after planting; T1 = 500 ppm gibberellic acid (AG_3); T2 = 1 000 ppm gibberellic acid (AG_3); T3 = 50 % *Glomus intraradices* N. C. Schenck & G. S. Sm. 1982; T4 = 100 % *G. intraradices*; T5 = Distilled water (control).

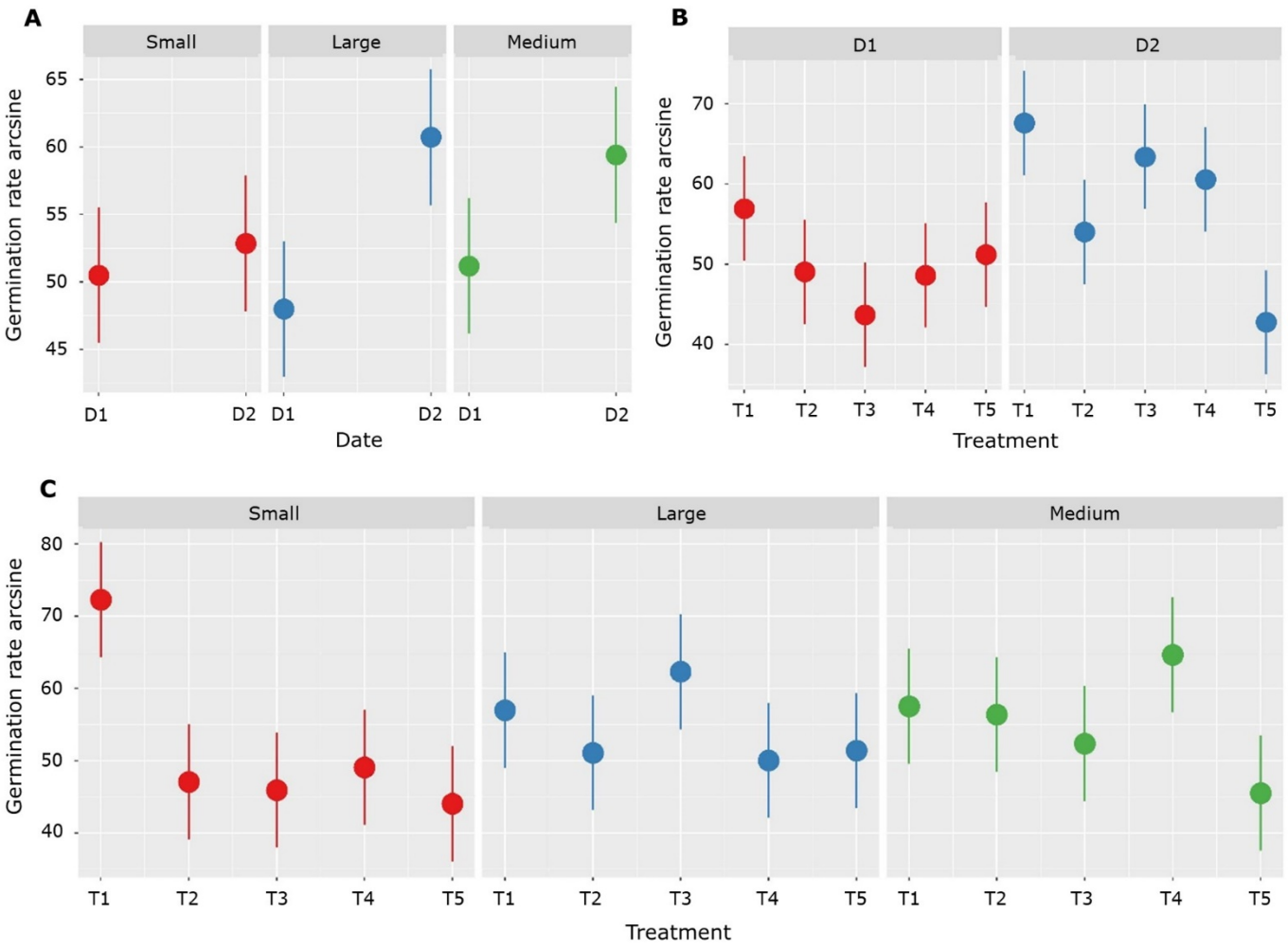
Figure 2. Effect of the date, pre-germination treatments, and seed size on germination in *Agave potatorum* Zucc.

Evaluation dates. The highest germination rate (69.00 %) of *A. potatorum* was registered at 15 dds (Table 2; Figure 3A). However, it differs from the findings of Ortiz-Hernández *et al.* (2018), who, under the same germination conditions, estimated a *GR* of 82 to 85 % with seeds from *Coixtlahuaca* (*Mixteca* region) and *Zaachila* (Central Valleys) of *Oaxaca*, respectively. The two sites exhibit differences in altitude (2 500 masl and 1 650 masl), precipitation (600 to 700 mm and 800 to 900 mm), and temperature (5 to 25 °C and 10 to 30 °C), with respect to the collection site of the present study, which shows the phenotypic plasticity of the species and the influence of the environment on the germination capacity.

Table 2. Descriptive statistics of germination (%) of *Agave potatorum* Zucc. in original units.

Factor/level	*n*	Min.	Max.	Median	I. Q. R.	Mean*	S. D.	S. E.	C. I.	C. V.
				Date						
D1	2 700	30.00	100.00	60.00	30.00	57.33 a	18.02	2.69	5.41	31.42
D2	2 700	30.00	100.00	70.00	40.00	69.00 b	18.82	2.81	5.65	27.27
				Size						
Small	900	30.00	100.00	60.00	35.00	59.33 a	19.99	3.65	7.46	33.69
Large	900	30.00	100.00	60.00	30.00	64.00 a	19.23	3.51	7.18	30.04
Medium	900	30.00	90.00	70.00	30.00	66.17 a	18.46	3.37	6.89	27.90
				Treatments						
T1	540	30.00	100.00	80.00	17.50	75.00 a	19.78	4.66	9.84	26.37
T2	540	30.00	90.00	60.00	27.50	60.56 b	18.30	4.31	9.10	30.22
T3	540	30.00	100.00	60.00	20.00	62.22 ab	19.87	4.68	9.88	31.93
T4	540	40.00	90.00	65.00	30.00	65.00 ab	18.55	4.37	9.23	28.54
T5	540	30.00	90.00	50.00	11.25	53.06 b	14.26	3.36	7.09	26.88

n = Number of observations; Min. = Minimum value; Max. = Maximum value, I. Q. R. = Interquartile range; Mean* = Equal letters indicate that the groups are statistically equal at 95 % according to Tukey's test; S. D. = Standard deviation; S. E. = Standard error; C. I. = Confidence interval of the mean; C. V. = Coefficient of variation (%). D1 and D2 = Germination evaluation dates 10 and 15 days after planting; T1 = 500 ppm gibberellic acid (AG_3); T2 = 1 000 ppm gibberellic acid (AG_3); T3 = 50 % *Glomus intraradices* N. C. Schenck & G. S. Sm. 1982; T4 = 100 % *G. intraradices*; T5 = Distilled water (control).



Between: A = Dates×Seed size; B = Date×Treatments; C = Seed size×Treatments. D1 and D2 = Germination rate assessment dates 10 and 15 days after sowing; T1 =

500 ppm gibberellic acid (AG_3); T2 = 1 000 ppm gibberellic acid (AG_3); T3 = 50 % *Glomus intraradices* N. C. Schenck & G. S. Sm. 1982; T4 = 100 % *G. intraradices*; T5 = Distilled water (control). The horizontal overlap of the vertical lines (within each figure, A, B, or C) indicates that the means are statistically equal (95 %) according to Tukey's test.

Figure 3. Germination interactions of *Agave potatorum* Zucc.

A close relationship between seed size and germination rate has been registered (Vázquez *et al.*, 2011) due to reserve content and embryo size; however, the seed size of *A. potatorum* did not influence the germination rate (p=0.3335, Table 1).

Effect of treatments. The highest germination rate in *A. potatorum* (75 %) was obtained with the application of gibberellic acid (AG_3) at 500 ppm (T1); while the rest of the treatments (T2-T5) registered statistically the lowest germination rates (Figure 3B); this is also the case with *Agave lechuguilla*, *A. asperrima*, *A. salmiana*, and *A. striata* (Ramírez, 2010). The effect of this treatment shows the need to apply a pre-germination treatment to improve germination and optimize the seed lot of *A. potatorum*.

Ortiz-Hernández *et al.* (2018) recorded a germination rate of 4 % for the same species without AG_3 application, a result that corroborates the need to assess the physiological quality of the seed and its response to different pre-germination treatments.

Although AG_3 has been documented to enhance germination in some species (Kaya and Kulan, 2020), in *A. potatorum*, 1 000 ppm AG_3 had an inhibitory effect, similar to that observed in other cultivars (Vásquez *et al.*, 2019), as it affects the synthesis of α-amylases and hydrolytic enzymes (Ho *et al.*, 2003). Although there is no information on the mycorrhizal effect on the germination of this species, the inoculation registered a difference of only 12 % concerning the Control (T5), which

exhibited the lowest germination rate (53 %); the application of *G. intraradices* may influence the mechanical resistance of the testa (Quiñones-Aguilar *et al.*, 2016) and the stimulation of the production of phytohormones such as gibberellins and indoleacetic acid (Alcántara *et al.*, 2019).

Date×Treatment interaction. The Anova showed that treatments recorded different germination rates of *A. potatorum* between Dates (p=0.0011) (Table 1); only certain interactions were statistically significant (p<0.05, Table 3C, Figure 3B). This is consistent with previous studies (Constantino *et al.*, 2010; Quiñones-Aguilar *et al.*, 2016; Alcántara *et al.*, 2019; Cruz-Cárdenas *et al.*, 2021), as the difference depends on the physiology of each species.

Table 3. Statistically significant Tukey's multiple contrasts of the *Agave potatorum* Zucc. germination rate.

Contrast	Difference	Lower limit	Upper limit	Adjusted *p* value	Means*	
		Date of assessment (A)				
D2-D1	7.7742	2.4549	13.0935	0.005	69.00	57.33
		Treatments (B)				
T5-T1	-15.280	-25.645	-4.915	0.001	53.06	75.00
T2-T1	-10.746	-21.111	-0.381	0.038	60.56	75.00
		Date: Treatments (C)				
D2:T5-D2:T1	-24.861	-41.955	-7.768	0.000	46.11	83.33
D1:T3-D2:T1	-23.930	-41.023	-6.836	0.001	47.78	83.33
D2:T5-D2:T3	-20.639	-37.733	-3.546	0.007	46.11	76.67
D2:T3-D1:T3	19.708	2.614	36.801	0.012	76.67	47.78
D1:T4-D2:T1	-18.999	-36.093	-1.906	0.018	55.56	83.33
D1:T2-D2:T1	-18.579	-35.673	-1.486	0.022	56.67	83.33
D2:T5-D2:T4	-17.813	-34.907	-0.720	0.034	46.11	74.44
D2:T4-D1:T3	16.881	-0.212	33.975	0.056	74.44	47.78
		Seed size: Treatments (D)				
T5:Small-T1:Small	-28.251	-51.846	-4.657	0.006	48.33	86.67
T5:Medium-T1:Small	-26.765	-50.360	-3.170	0.012	50.83	86.67
T3:Small-T1:Small	-26.371	-49.965	-2.776	0.015	51.67	86.67

T2:Small-T1:Small	-25.221	-48.816	-1.626	0.025	53.33	86.67
T4:Small-T1:Small	-23.214	-46.809	0.381	0.058	56.67	86.67

D1 and D2 = Germination assessment dates 10 and 15 days after planting; T1 = 500 ppm gibberellic acid (AG_3); T2 = 1 000 ppm gibberellic acid (AG_3); T3 = 50 % *Glomus intraradices* N. C. Schenck & G. S. Sm. 1982; T4 = 100 % *G. intraradices*; T5 = Distilled water (control). Means* in original units, respectively corresponding to the variables in column 1.

Size×Treatments interaction. The results of Tukey's mean contrasts showed that the highest germination rate (86.67 %) of *A. potatorum* occurred with T1, in small seeds (Table 3D, Figure 3C). No statistically significant differences were observed in the germination rate of *A. potatorum* among the treatments applied to large seeds.

The results indicate that the use of AG_3 significantly improves germination of *A. potatorum* seeds; however, high doses inhibit the hormonal action of the seeds. The inhibitory effect may also be due to an alteration of other phytohormones such as cytokinins, polyamines, and jasmonic acid, that are also involved in the embryonic and root development during germination (Alcántara *et al.*, 2019).

It has been observed that the large seeds, having a larger and heavier embryo, exhibit a germination capacity that allows them to denature certain proteins, synthesize nutrients, and perform lipid hydrolysis (Baskin and Baskin, 2001; Finch-Savage and Leubner-Metzger, 2006), therefore producing healthy seedlings (Ramírez, 2010).

Conclusions

Applying 500 ppm of gibberellic acid (AG_3) 15 days after sowing is the most effective treatment to improve the germination of *A. potatorum* seeds; therefore, its use in propagation practices is suggested. A higher dose of AG_3 and the use of mycorrhizae

inhibit germination. Small seeds respond better to germination treatments with 500 ppm AG_3, while the size of medium and large seeds does not influence the germination of this species. The results support the *ex situ* conservation and sustainable utilization of *A. potatorum*, as they provide a solid basis for future research and propagation practices.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

Contribution by author

Eulalia Edith Villavicencio-Gutiérrez: execution, research and analysis supervision, interpretation of results, and drafting of the manuscript; Ma. Alejandra Torres-Tapia: methodological design and revision of the manuscript; Jorge Méndez

González: statistical analysis; Carolina Curiel-López: germplasm selection and collection; Félix Sánchez-Pérez: verification and statistical analysis of the results.

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