

Germination and emergence of *Agave salmiana* Otto ex Salm-Dyck ‘Xamini’ seeds treated with chitosan

Running head: Effects of chitosan on agave germination

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Abstract

Introduction. Physical, mechanical, and hormonal methods used to enhance germination can, in some cases, cause physiological damage to the seed and endosperm. Chitosan has been used as a germination stimulant in other species; however, its effect has not been evaluated in agave seeds.

Objective. To evaluate the effect of chitosan concentrations on the germination and emergence of *Agave salmiana* Otto ex Salm-Dyck 'Xamini'.

Materials and methods. Treatments consisted of five chitosan concentrations (0.2, 0.5, 0.7, 1.0 and 3.0 g·L⁻¹) and a control (distilled water), arranged in a completely randomized design with four replicates; each experimental unit consisted of 100 seeds. Germination percentages, germination speed index, mean germination time, and emergence percentage and rate were evaluated at 24 h intervals.

Results. Significant differences among treatments were observed for all measured variables ($P \leq 0.0001$). Germination of *A. salmiana* 'Xamini' reached 80 % and 74 % at chitosan concentrations of 0.2 and 0.5 g·L⁻¹, respectively. The optimal dose for both the germination speed index and emergence rate was 0.2 g·L⁻¹. These lower chitosan concentrations improved germination compared to higher doses (1 and 3 g·L⁻¹), which showed the lowest mean values, similar to the control.

Conclusion. The use of chitosan at a concentration of 0.2 g·L⁻¹ represents a natural alternative, free of toxic residues, to enhance the germination and emergence of *A. salmiana* 'Xamini'.

Keywords: Biostimulants, agave, morphometry, germination rate, emergence rate.

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Introduction

The genus *Agave* comprises approximately 200 species; in Mexico, most are distributed across the states of Hidalgo, Tlaxcala, Estado de Mexico, Puebla and San Luis Potosí (Castillo-Quiroz et al. 2020). Agave or maguey is emblematic of traditional Mexican culture and is closely associated with medicine, agroindustry, and gastronomy (García-Mendoza & Comisión Nacional para el Conocimiento y Uso de la Biodiversidad [CONABIO], 2021).

The predominance of vegetative propagation systems in agave (offsets and *in vitro* culture) leads to a significant reduction in genetic variability and increases susceptibility to pests and diseases. It also entails physiological effects that are reflected in the morphometry and functioning of botanical structures, driven by abiotic factors (Enríquez-del Valle et al., 2023). In this context, seed propagation represents an alternative approach for obtaining plants with superior traits that enhance the species' adaptation to both biotic and abiotic factors in each region (Riseh et al., 2024).

Germination and emergence are associated with seed starch reserves and constitute part of the species' strategy to ensure seed survival and persistence (Fajinmi et al., 2021). Germination is inhibited when seeds are exposed to high concentrations of abscisic acid, phenolic compounds, and coumarin, or when the seed coat is impermeable to gases and water (Pham et al., 2019). Methods to enhance germination and emergence include soaking seeds in potable water, mechanical scarification, and soaking in gibberellic acid (GA_3) (Makhaye et al., 2021). However, these methods may, in some cases, cause physical damage to the seed and even alter the biological processes underlying radicle emergence and plumule development (Gutiérrez-Hernández et al., 2020).



Biostimulants or microbial consortia represents an alternative for enhancing plant growth and development, including photosynthetic performance, mitigation of abiotic stress, and nutrient uptake and assimilation (Ahmed et al., 2020). One of these biostimulants is chitosan, the deacetylated form of chitin and one of the most abundant waste-derived biomaterials, primarily obtained from the exoskeletons of insects and crustaceans (Mirbolook et al., 2021).

The use of chitosan is associated with low environmental impact agriculture and has been shown to be compatible with both external and internal cellular tissues of cultivated species (Chagas et al., 2022). According to Riseh et al. (2024), research on chitosan and its effects on cultivated species remains active, with assessments conducted at the levels of soil, radicle, stem, foliage, flower, fruit, and seed. Chitosan is applied as a biostimulant, resistance inducer, biocide, antimicrobial agent, fungicide, nematicide, and bactericide (Chagas et al., 2022). Studies have demonstrated that seeds coated with chitosan show increased germination rates, as well as enhanced growth and vegetative development (Pincay-Manzaba et al., 2021). Chitosan also promotes the activity of plant hormones and nutrient dynamics, reserves degradation processes, and stimulates germination in less than 24 h (El Amerany et al., 2020). In the case of agave, research on the use of chitosan remains scarce, particularly regarding the agronomic management of seed germination and seedling emergence. This approach could accelerate these processes while preserving the genetic characteristics of the species. In this context, the objective of this study was to evaluate the effect of chitosan concentrations on the germination and emergence of seeds of *A. salmiana* Otto ex Salm-Dyck 'Xamini'.

Materials and methods

Experimental site



The study was carried out during the 2024 spring-summer and fall-winter growing seasons at the Agricultural Experimental Unit of the Instituto Tecnológico Superior de Huichapan in El Saucillo, Huichapan, Hidalgo, Mexico. The study site is situated at 20° 19' 09" N and 99° 42' 29" W, at an elevation of 2 172 m above sea level. The mean annual temperature is 15.9 ± 2 °C with an annual precipitation of 520.5 mm. The soils are classified as Haplic Phaeozems, characterized by a dark surface horizon, a C/N ratio of 10-12, and a pH ranging from 5 to 7 (IUSS Working Group WRB, 2015).

Genetic material

Seeds of *A. salmiana* 'Xamini' were collected in El Cardonal del Valle del Mezquital, Hidalgo, Mexico (20° 32' 41" N y 99° 04' 08" W; 2 172 m). The seed lot was validated for genetic, physiological, and physical quality, as well as varietal purity, in accordance with the seed collection program of the Secretaría de Desarrollo Agropecuario of Hidalgo. The genetic material was botanically described by following Vázquez-Díaz et al. (2011). From 1 kg of *A. salmiana* 'Xamini' seed, a total of 2 400 seeds were randomly selected and divided into four lots of 100 seeds each, where each lot represented an experimental unit. The botanical structures of the seeds were weighed before and after treatment using an analytical balance (Citizen CY204®, Citizen Systems, UK). Seed length and width were measured with a Mitutoyo® digital caliper (Mitutoyo Mexicana, Naucalpan, Estado de México). The seeds were labeled and stored at 4 °C in a Criotec CFX-19 refrigeration unit (Mexico) until analysis.



Experimental design and treatments

A total of five Vepinsa® chitosan treatments (Los Mochis, Sinaloa, Mexico) were evaluated, consisting of five concentrations (0.2, 0.5, 0.7, 1.0 and 3.0 g·L⁻¹), equivalent to 6.6, 16.6, 23.3, 33.3 and 100 %, in addition to sterile distilled water as the control treatment (0.0 g·L⁻¹). Treatments were randomized under a completely randomized experimental design. The experimental unit consisted of 100 seeds with three replicates per treatment. Chitosan solutions were prepared in sterile distilled water (500 mL⁻¹) supplemented with 1 % acetic acid, and the pH was adjusted to 5.6 using KOH. Prior to treatment application, *A. salmiana* seeds were disinfected with 2 % NaClO₃ for 1 min, followed by three rinses with sterile distilled water (Gutiérrez-Hernández et al., 2020). Subsequently, the seeds were placed in muslin cloth bags and immersed in the corresponding treatment solution for 72 h. Afterward, the seed were maintained under complete darkness at a constant temperature of 25 °C (Riseh et al., 2024).

Seed management

Following chitosan treatment, the seeds were sown in rigid black polypropylene trays with 50 cavities. Each tray measured 54 cm in length, 28 cm in width, and 9 cm in height, with a thickness of 1 mm, a cavity capacity of 100 cc, and a 10 mm drainage hole diameter. Prior to sowing, the trays were filled with a mixture of vermicompost and tezontle (70:30 v/v). Sowing depth was equivalent to three to five times the size of each seed. Treatments were maintained at field capacity with potable water throughout the evaluation period. The containers remained covered with 600-gauge black polyethylene film for 30 days, and temperature was maintained at 25 °C (Enríquez-del Valle et al., 2023). Evaluations were



conducted at 24 h intervals for 10 days, and seedling emergence was assessed weekly for 30 days. Ambient temperature and substrate moisture were recorded at 7:00, 12:00 and 20:00 h using an Extech 445703® Digital Thermo-Hygrometer (Extech Instruments, FLIR Commercial Systems Inc., Nashua, NH 03063, USA).

Variables assessed

The evaluated variables were based on the botanical description of *A. salmiana*. Seed characterization was conducted using the external morphology of seeds treated with chitosan and water, including seed coat color, shape, contours, compressed elevations, presence of wings and margins, as well as the average seed weight, length, and width before and after treatment application (Vázquez-Díaz et al. 2011).

Germination percentage was determined at the end of the experiment according to the protocol established by the International Seed Testing Association (ISTA, 2016). The germination speed index (GSI) (germinated seeds·day⁻¹) was calculated using the following formula:

$$GSI = \sum_{n=1}^n \frac{Gi}{Ni}$$

where,

G_i = number of germinated seeds

N_i = number of days to germination.

The mean germination time (MGT) indicates the time (D) required to reach 50 % germination of the population and was calculated using the following formula:



$$MGT = \frac{\sum D * n}{\sum nt}$$

where,

D = number of days recorded from the onset of germination

n = number of seeds germinated on day D

nt = total number of seeds sown.

Emergence percentage (EP) was determined when the hypocotyl reached one to three times the size of the seed and emerged above the substrate surface. This variable was calculated using the following formula:

$$EP = \frac{Npe * 100}{Nts}$$

where,

Npe = number of seeds

Nts = total number of seeds.

Finally, the emergence rate (ER) was calculated using the following equation:

$$ER = \frac{n_1}{t_1} + \frac{n_2}{t_2} + \dots + \frac{n_{400}}{t_{40}}$$

where,

n_1, n_2, \dots, n_{400} = number of emerged seedlings

t_1, t_2, \dots, t_{40} = emergence time (days).

Statistical analysis



The data were verified for compliance with the assumptions of parametric tests. Data normality was assessed using the Shapiro-Wilk. Treatment means were compared using one-way ANOVA, and differences among means were determined with the Tukey-Kramer test ($P \leq 0.05$) for multiple pairwise comparisons. Variables expressed as percentages were transformed using the arcsine square-root transformation. Statistical analyses were performed using JMP version 16 (JMP Statistical Discovery LLC., 2026).

Results and Discussion

Botanical structures

A. salmiana seeds treated with chitosan were slightly flattened with obovate contours and showed a black seed coat and teardrop-shaped to ovoid, lenticular, and slightly compressed forms, with narrow wings and keeled, furrowed margins. Under 7x magnification, intense brightness and a convex dorsal surface were observed (Figure 1). The average seed weight was 10 ± 0.3 mg, with an average length of 7.9 ± 0.2 mm and an average width of 5.8 ± 0.4 mm, whereas control seeds showed lower average values. These characteristics are consistent with the descriptions reported by Villanueva-Castillo et al. (2021) for seeds of *A. angustifolia* Haw, *A. karwinskii* Zucc., *A. potatorum* Zucc. and *A. rhodacantha* Trel.

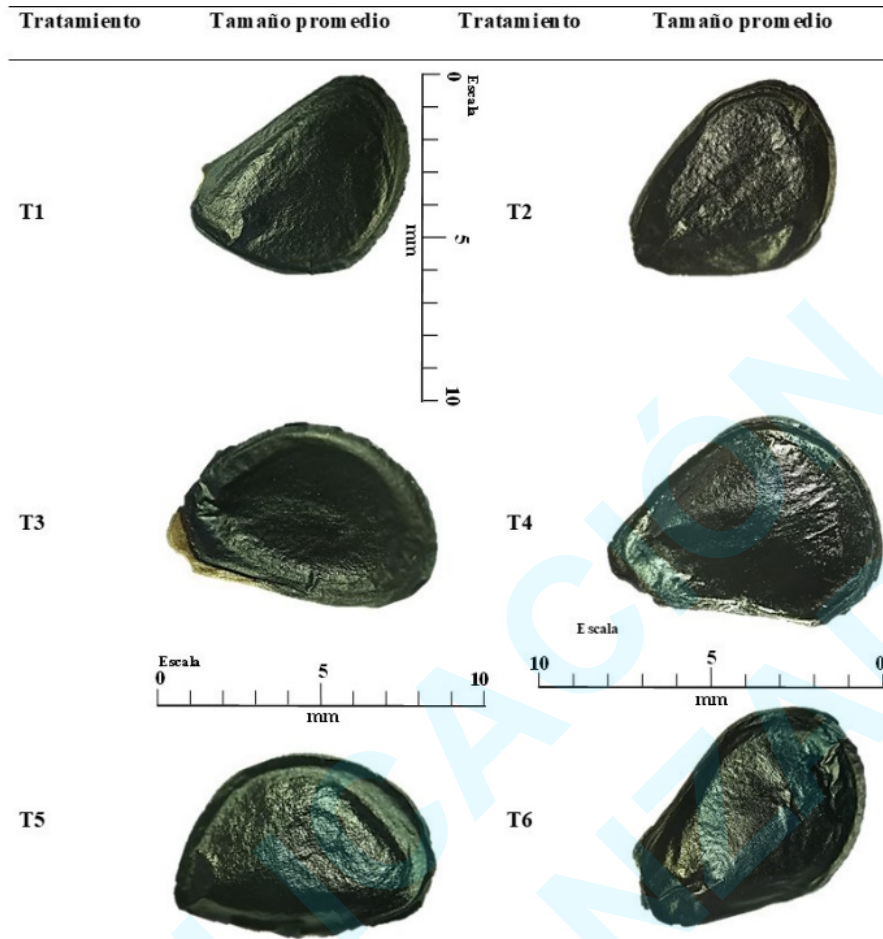


Figure 1. Morphometric characteristics of *Agave salmiana* 'Xamini' seeds treated with different chitosan concentrations (T1 = 0.0 g·L⁻¹, T2 = 0.2 g·L⁻¹, T3 = 0.5 g·L⁻¹, T4 = 0.7 g·L⁻¹, T5 = 1.0 g·L⁻¹ and T6 = 3.0 g·L⁻¹).

Germination and emergence

Table 1 shows the mean values of the evaluated variables. Germination percentage, germination speed index, mean germination time, emergence percentage, and emergence



rate showed significant differences ($P \leq 0.05$) among chitosan treatments and relative to the control. The highest average germination percentage was obtained in seeds treated with $0.2 \text{ g}\cdot\text{L}^{-1}$ chitosan (80 %), while untreated seeds had the lowest values (30 %). GSI showed the highest mean values in seeds treated with 0.2 and $0.5 \text{ g}\cdot\text{L}^{-1}$ chitosan, while the lowest GSI was recorded in untreated seeds ($0.25 \text{ seeds}\cdot\text{day}^{-1}$).

Seeds treated with 0.2 and $0.5 \text{ g}\cdot\text{L}^{-1}$ chitosan showed higher MGT values (5.09 and 4.76 days, respectively) compared with seeds without chitosan (1.83 days). Finally, emergence percentage also showed the highest values with the application of 0.2 and $0.5 \text{ g}\cdot\text{L}^{-1}$ chitosan (80 and 74 %, respectively); however, the emergence rate was higher in seeds treated with $0.2 \text{ g}\cdot\text{L}^{-1}$ chitosan ($13.24 \text{ seedlings}\cdot\text{day}^{-1}$), while seeds from the control treatment showed 30 % emergence with a rate of $5.21 \text{ seeds}\cdot\text{day}^{-1}$.

Table 1. Effect of chitosan concentrations on germination and emergence of *Agave salmiana* 'Xamini'.

Chitosan ($\text{g}\cdot\text{L}^{-1}$)	GP (%)	GSI ($\text{seed}\cdot\text{day}^{-1}$)	MGT (days)	EP (%)	ER ($\text{seedling}\cdot\text{day}^{-1}$)
0	$30.00 \pm 3.55 \text{ c}$	$0.25 \pm 0.03 \text{ d}$	$1.83 \pm 0.20 \text{ d}$	$30.00 \pm 3.55 \text{ c}$	$5.21 \pm 0.66 \text{ e}$
0.2	$80.00 \pm 4.96 \text{ a}$	$0.67 \pm 0.04 \text{ a}$	$5.09 \pm 0.34 \text{ a}$	$80.00 \pm 4.96 \text{ a}$	$13.24 \pm 0.79 \text{ a}$
0.5	$74.00 \pm 2.94 \text{ ab}$	$0.62 \pm 0.02 \text{ a}$	$4.76 \pm 0.18 \text{ a}$	$74.00 \pm 2.94 \text{ ab}$	$12.06 \pm 0.49 \text{ b}$
0.7	$65.00 \pm 1.41 \text{ b}$	$0.54 \pm 0.01 \text{ b}$	$4.14 \pm 0.06 \text{ b}$	$65.00 \pm 1.41 \text{ b}$	$10.76 \pm 0.34 \text{ c}$
1.0	$47.00 \pm 9.25 \text{ c}$	$0.44 \pm 0.02 \text{ c}$	$3.37 \pm 0.15 \text{ c}$	$47.00 \pm 9.25 \text{ c}$	$8.41 \pm 0.33 \text{ d}$
3.0	$48.00 \pm 0.81 \text{ c}$	$0.40 \pm 0.01 \text{ c}$	$3.06 \pm 0.07 \text{ c}$	$48.00 \pm 0.81 \text{ c}$	$7.92 \pm 0.15 \text{ d}$

GP: germination percentage, GSI: germination speed index, TMG: mean germination time, EP: emergence percentage, ER: emergence rate. In the columns, means (\pm standard deviation) followed by different letter are significantly different according to Tukey's test ($P \leq 0.05$).

In this study, chitosan increased the germination percentage of *A. salmiana* seeds, as well as seedling emergence. These effects are consistent with those reported by Peña-Datoli et al. (2016), who stated that chitosan coating, due to its biodegradable and physicochemical properties as a hydrophilic polysaccharide, promotes the activity of plant hormones and nutrients, the redistribution of reserves during degradation processes, and seed germination in cultivated species (Pincay-Manzaba et al., 2021). Similarly, Jasso-de



Rodríguez et al. (2024) highlighted that chitosan stimulates root growth and seedling development, in addition to exhibiting bactericidal, fungicidal, and antiviral effects. In this context, agave producers in Valle del Mezquital, Hidalgo, Mexico, could improve plant health and quality during the early stages of development by establishing production units or perimeter fences using offshoots from mother plants. Likewise, producers could collect seeds and establish seedbeds without concern for potential physical or phytosanitary damage that may alter germination, root development, growth, or induce cellular, and vascular malformations, which may eventually become evident in external structures (El Amerany et al., 2020).

The germination and emergence percentages of *A. salmiana* indicated that seed viability and physiological quality were favorable when seeds were treated with chitosan at 0.2 and 0.5 g·L⁻¹. Peña-Datoli et al. (2016) mentioned that emergence depends on the physiological and biochemical responses of the seed, as well as on biotic and abiotic conditions. In the present study, it is inferred that water combined with chitosan activated the respiratory process of the seed structure and increased germination speed. During the germination process of *A. salmiana* seeds, imbibition is essential for water absorption. According to Pincay-Manzaba et al. (2021), the imbibition process allows seeds to reach similar moisture levels in a shorter period, thereby activating the metabolism associated with the pregerminative process. Costales-Menéndez et al. (2020) reported that in conventional agricultural crops such as tomato and soybean, respiration can be detected in minutes, and the germination and seedlings emergence processes are accelerated compared with agave seeds, in which the process may require several hours (Sánchez-Salas et al., 2017). Likewise, Gutiérrez-Hernández et al. (2020) indicated that chitosan stimulated germination and respiration processes and reduced water loss through transpiration in *A. potatorum* Zucc.

Based on their botanical, morphological, and anatomical structures, the semiplane lacrimiform shape of agave seeds provides buoyancy. In addition, they present a porous seed coat with a hypodermis surrounded by air chambers. According to Villanueva-Castillo et al. (2021), these characteristics facilitate permeability and water uptake during short flooding periods and promote germination; that is, once water is absorbed, the respiratory process begins, cell division is activated at the organelle level, and protein synthesis is



initiated, leading to the formation of new cells and mitochondria (Cruz-Vasconcelos et al., 2020). In the final stage, the seed completes germination and emergence, culminating in seedling development. Jasso-de Rodríguez et al. (2024) reported that tomato seeds imbibed with chitosan can stimulate the activity of ACC synthase, which is involved in ethylene synthesis, thereby promoting root and shoot growth in seedlings.

With the application of 0.2 and 0.5 g·L⁻¹ chitosan, emergence of *A. salmiana* reached 80 and 74 %, respectively, values that differ from those reported by Reyes-Pérez et al. (2020), who observed emergence ranging 55 to 91 % in tomato seeds, but using higher chitosan concentrations (2 g·L⁻¹). However, results are not always favorable; in the case of untreated agave seeds, germination and emergence did not exceed 30 %, in contrast to chitosan-coated seeds, which showed a positive response. Nevertheless, the effect of increasing chitosan concentration in agave seeds is not progressive; therefore, it does not necessarily imply that the response variables (seed germination and seedling emergence) will increase proportionally. In this study, the highest chitosan concentration (3.0 g·L⁻¹) did not produce the highest germination and emergence percentages. In this regard, Reyes-Pérez et al. (2020) observed that both the control and the 3.0 g·L⁻¹ chitosan treatment in tomato seeds showed similar effects on emergence rate, consistent with the present study. This response suggests that optimal chitosan concentrations for seeds should be evaluated and determined based on seed type, structure, seed coat characteristics, and exposure time, to stimulate and enhance germination percentages required for certified seed quality. In a related study, Reyes-Pérez et al. (2021) reported that at low chitosan concentrations, plant tissue responses are favorable for tomato seed germination, as well as for plant development and protection.

In *A. victoriae* Reginae seeds, Sánchez-Salas et al. (2017) reported that germination and germination speed are influenced by treatment type and seed size. Cruz-Vasconcelos et al. (2020) indicated that the CO₂ exchange rate affects the vegetative development of agave seeds and seedlings. GSI was higher in seeds treated with 0.2 g·L⁻¹ chitosan (0.67 ± 0.04) compared with untreated seeds (0.25 ± 0.03).

The emergence rate was higher at the 0.2 g·L⁻¹ dose (13.24 ± 0.79 seedling·day⁻¹), whereas the lowest rate was observed in the control treatment (5.21 ± 0.66 seedling·day⁻¹).



1). The application of chitosan stimulated seed germination and seedling emergence in *A. salmiana*. The concentrations of the active ingredient are important in each of the physiological processes of the seed and, potentially, of the plant. In the case of *A. salmiana*, these results represent the first report using chitosan in seeds; however, recommended doses for commercially cultivated species should be previously evaluated under production site conditions. In this regard, future studies should consider evaluating temperature and substrate interactions with chitosan, as well as seedling morphometric responses.

Conclusions

Germination of *A. salmiana* 'Xamini' using chitosan at concentrations of 0.2 and 0.5 g·L⁻¹ was 80 and 74 %, respectively. The optimal dose for the germination speed index and emergence rate was 0.2 g·L⁻¹. These low chitosan concentrations improve germination compared with higher doses (1 and 3 g·L⁻¹) and may contribute to the conservation and propagation of agave species diversity. This supports the use of chitosan as a natural product, free of toxic residues, thereby reducing the need for synthetic products.

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Conflict of Interest Declaration

The authors declare that they have no economic conflicts of interest or known personal relationships that could have influenced the research presented in this article.



Declaration of Artificial Intelligence (AI) Use

The authors declare that no generative AI or AI-assisted technology was used in the preparation of this manuscript.

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