Challenges in germination of *Neltuma caldenia* in semi-arid regions: optimization of germination protocols, influence of saline stress and seed quality

Desafíos en la germinación del *Neltuma caldenia* en regiones semiáridas: optimización de protocolos de germinación, influencia del estrés salino y evaluación de la calidad de las semillas

Daniela Dalzotto [1, 2], Sandra Sharry [1, 2, 3, 4], Lucrecia Piñuel [1, 2], Patricia Boeri [1, 2*]

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Abstract

Global climate change presents challenges to arid and semi-arid ecosystems, impacting native species such as *Neltuma caldenia*, endemic to Argentina. This underscores the importance of understanding germination processes for both conservation programs and the restoration of degraded areas. We aimed to evaluate the germination rate of *N. caldenia* seeds from the south Espinal, using various scarification methods (chemical, mechanical and physical), and temperatures (25-30°C). Additionally, we investigate the effects of accelerated aging (0-96 h at 45°C and 100 relative humidity) and different saline solution concentrations during germination (0-0.6 M NaCl). Our results show that all scarification treatments effectively break seed dormancy while temperature significantly affects germination rates. Prolonged storage (0 to 96h) decreased seed viability. Moderate NaCl levels (0-0.2 M) did not affect germination, but higher concentrations inhibited it completely, with a threshold of -1.81 MPa osmotic potential. Understanding the impact of environmental stressors on seed germination can inform the development of effective conservation strategies among these climate change pressures.

Keywords
Fabaceae • *Prosopis* • caldén • dormancy • scarification • optimal temperature

1 Universidad Nacional de Río Negro. Sede Atlántica. Av. Don Bosco y Leloir. Viedma. (C. P. 8500). Argentina. * pboeri@unrn.edu.ar
2 CIT-Río Negro - CONICET. RP N° 1 y Rotonda Cooperación, Viedma (C.P. 8500), Argentina.
3 Universidad Nacional de La Plata. Facultad de Ciencias Agrarias y Forestales. Laboratorio de Investigaciones en Madera (LIMAD). Calle 60 y 119. La Plata. C. P. 1900. Argentina.
4 Sistema Nacional de Investigación. Asociación de Interés Público (SIN AIP). Ciudad del Saber. Clayton. Panamá.

Resumen

El cambio climático global presenta desafíos para los ecosistemas áridos y semiáridos, impactando a especies nativas como *Neltuma caldenia*, endémica de Argentina. Esto resalta la importancia de comprender los procesos de germinación tanto para programas de conservación como para la restauración de áreas degradadas. Nuestro objetivo fue evaluar la tasa de germinación de semillas *de N. caldenia* del sur del Espinal, utilizando varios métodos de escarificación (químico, mecánico y físico) y temperaturas (25-30°C). Además, investigamos los efectos del envejecimiento acelerado (0-96 h a 45°C y 100% de humedad relativa) y de diferentes concentraciones de solución salina durante la germinación (0-0,6 M NaCl). Nuestros resultados muestran que todos los tratamientos de escarificación rompen eficazmente la latencia de las semillas, mientras que la temperatura afecta significativamente las tasas de germinación. El almacenamiento prolongado (0 a 96h) disminuyó la viabilidad de las semillas. Niveles moderados de NaCl (0-0,2 M) no afectaron la germinación, pero concentraciones más altas la inhibieron completamente, con un umbral de -1,81 MPa de potencial osmótico. Comprender el impacto de los factores de estrés ambiental en la germinación de semillas puede informar el desarrollo de estrategias de conservación efectivas ante estas presiones del cambio climático.

Palabras clave
Fabaceae • *Prosopis* • caldén • latencia • escarificación • temperatura óptima

Introduction

Global climate change (GCC), biodiversity loss, and environmental degradation present profound challenges for ecosystems, especially in arid and semi-arid regions (24). These changes exceed the physiological thresholds of many native plant species, including those in the *Neltuma* genus (formerly *Prosopis*), posing severe risks to their survival. Species persistence in such regions relies on traits like seed dormancy, germination temperature, and water stress tolerance. Understanding germination and early survival of plants under these conditions is critical for future conservation. The 'Decade on Ecosystem Restoration' of the United Nations (2021-2030) emphasizes the urgent need to address these environmental challenges. This is especially relevant for species like *Neltuma caldenia* (Burkart) C.E. Hughes & G.P. Lewisthe, known as 'caldén', which is endemic to the Espinal region of Argentina and impacted by GCC and deforestation.

One main constraint for seed germination in arid and semi-arid regions is seed dormancy. In these ecosystems, over 80% of native shrub species have seeds that will not germinate unless dormancy is broken. Dormancy is an adaptive strategy that prevents a viable seed from germinating under favorable conditions until specific triggers are met. This trait may arise from the structures surrounding the embryo, which inhibit germination even when conditions are suitable for non-dormant seeds (36). The presence of seeds with various dormancy levels enables temporal distribution of offspring, offering protection in unpredictable and variable environments, a particularly relevant aspect in arid and semi-arid regions (36). Studies have reported that *N. caldenia* seeds exhibit physical dormancy imposed by the seed coat (31, 36). Germination speed is crucial for species establishment and may vary based on the scarification treatment used. Utello *et al.* (2023) identified several scarification strategies to break dormancy in central Espinal *N. caldenia* seeds, with mechanical and chemical scarification showing the most success. However, physical scarification with boiling water yielded low germination rates, around 20%. Emergence rates for *N. caldenia* vary among studies, depending on scarification method and seed origin (21, 31, 36). Nonetheless, information specific to the La Pampa province, in the southern Espinal region of Argentina, where *N. caldenia* forests predominate, remains scarce. Dormancy type and degree can vary significantly across species within a genus and among populations within a species, influenced by maternal effects during seed formation (11). Thus, studying this trait in local seed provenances is important for understanding germination patterns.

Once dormancy is overcome, temperature becomes a critical factor in the seed germination process, highlighting ideal establishment times. Optimal germination temperatures for other *Neltuma* species from arid regions range from 20 to 40°C, varying by species and origin (4). However, this has not yet been evaluated for *N. caldenia*. Seed banks are vital for preserving genetic material for future conservation and restoration, yet storage conditions also influence germination. Assessing seed vigor, critical for this purpose, requires evaluating seed viability under simulated long-term storage conditions, such as accelerated aging (AA) tests (1). AA testing, though common in agronomic seeds, is less common for native species, despite its relevance for predicting viability thresholds in germplasm conservation (14). Fontana *et al.* (2016) were the first to apply the AA technique to the *Neltuma* genus, suggesting that seed vigor may be influenced by geographic origin and environmental conditions.

Salinity tolerance during germination is crucial for plant establishment in *N. caldenia*-inhabited environments. Soil salinity can hinder germination, particularly during dry years when saline conditions may increase due to GCC (22). Many native and endemic species in arid and semi-arid regions employ strategies to tolerate or avoid environmental filtering at different development stages. Salt stress tolerance has been confirmed in the genus, supporting its potential for restoring soils degraded by salinity (22, 33, 34). While *N. caldenia* has not been studied for salt tolerance, its presence in central-west La Pampa, where saline soils and salt flats are common, suggests it may possess similar tolerance mechanisms (8, 13).

In this study, we aim to evaluate the germination rate of *N. caldenia* seeds from southern Espinal in La Pampa province using various scarification methods and identify optimal germination temperatures. Additionally, we also examine the effects of accelerated aging and salinity on germination quality under laboratory conditions.

Materials and methods

Study area

The sampling zone is situated in the Caldenal district, within the Phytogeographic Province of Espinal, characterized by a temperate and dry climate with predominantly summer rainfall (6, 23). The collection of *N. caldenia* pods took place in La Pampa province (37°24'10.91'' S; 63°40'22.93'' W), where the average annual precipitation is 500 mm and the average temperature is 15°C (17). Permission to access and use native flora was obtained from provincial authorities in compliance with the Convention on Biological Diversity and the Nagoya Protocol.

Seed collection, conditioning and storage

Mature pods from 10 to 20 plants were manually harvested between February and April of 2018 and 2019, following FAO Forest Seed Handling Guide Guidelines (35). Seeds were extracted in the laboratory, using tweezer, selecting only those exhibiting no visible signs of deterioration. Selected seeds were then stored in paper envelopes at room temperature (20±2°C) for 2 to 14 months until the final experiment.

Germination of *N. caldenia*

Various scarification methods were tested and the optimal germination temperature was determined. Before each trial, seeds were disinfected following the protocol for *N. alpataco* (4). Briefly, seeds were soaked in 70% (v/v) ethanol for 15 minutes, followed by a 20 minutes immersion in 30% (v/v) NaClO solutions (48 g of active chlorine/L) and followed by three rinses with distilled water.

Dormancy breaking

Physical (PS), mechanical (MS), and chemical scarification (CS) methods were assessed following guidelines in the FAO Forest Seed Handling Guide (35) and the ISTA International Rules for Seeds Testing (18). PS involved soaking the seeds in water at 100°C until reaching room temperature. For MS, a small incision was made in the seed coat with tweezers avoiding

damage to the embryo. CS required immersing the seeds in sulfuric acid (98%) for 10, 20, 30, and 40 min (CS10, CS20, CS30, and CS40, respectively), followed by rinsing with distilled water, as proposed for other Norpatagonian *Neltuma spp* (4). Based on the obtained results, mechanical scarification was selected for the remaining experiments to be conducted, as it is a more environmentally sustainable methodology and avoids the potential interference of acid with other treatments.

Optimal germination temperature

Seeds were evaluated at temperatures ranging from 25°C to 45°C with a germination chamber, based on previous findings identifying optimal temperatures for *Neltuma spp* (4). The optimal temperature was considered as the one yielding the highest germination percentage in the shortest time.

Determination of seed vigor through accelerated aging (AA) test

The methodology from Fontana *et al.* (2016) for *Neltuma alba* was employed to assess seed vigor. Seeds were exposed to 45°C and 100% relative humidity in a germination chamber for durations of 0 (control), 24, 48, 72, and 96 h. Glass jars containing 100 ml of distilled water with a mesh above the water level were used to support the seeds during this procedure. Then, seeds were mechanically scarified and disinfected (see section 3) for germination.

Effect of salinity on germination

Seeds, previously scarified and disinfected, were arranged for germination in Petri dishes containing moistened cotton and filter paper with NaCl solutions at the following concentrations: 0 (control - T0), 0.05 (T1), 0.1 (T2), 0.2 (T3), 0.4 (T4), and 0.6 M (T5). These were converted to osmotic potential (Ψo) using the van't Hoff relationship (27):

$$\Psi o = (MPa) = - CiRT$$

where
Ψo = the osmotic potential in MPa
C = the concentration in mol/L
i = the dissociation constant of NaCl (*i.e.* 1.8)
R = the gas constant (0.0083 L/atm/mol/K)
T = the temperature in Kelvins.

The obtained osmotic potentials were: 0 (T0), -0.22 (T1), -0.45 (T2), -0.90 (T3), -1.81 (T4), -2.71 (T5) MPa. As temperature can influence osmotic potential it is recommended to perform these tests at the optimal temperature for each species (9); in the case of *N. caldenia* it was 30°C. After 14 days of treatment, the inhibitory effect of salts on the development of surviving seedlings was evaluated by measuring the length of the radicle and hypocotyl (cm). Subsequently, seedling vigor index (SVI), percentage phytotoxicity for roots and hypocotyls (RPT and HPT, respectively) and the tolerance index for roots and hypocotyls (RTI and HTI, respectively) parameters were calculated (26).

Germination conditions

Seeds from all experiments were placed on moistened cotton and filter paper in Petri dishes and then incubated in germination chambers at 30°C, except for the optimal germination temperature assay and the accelerated aging test (see sections 3.2 and 4). All germination experiments were conducted in darkness, as has been done by other authors with *N. caldenia* and other species of the genus (4). The trials were randomized and included 10 replications of 10 seeds each (N=100), with controls. Germination was defined as the emergence of a radicle at least 2 mm long (37), and daily germination counts were recorded for up to a week or until no further germination occurred.

Seed viability

The viability of seeds that appeared healthy but failed to germinate for all experiments was verified using tetrazolium testing (5). A solution of 2,3,5-triphenyl tetrazolium chloride 1% (p/v) in a phosphate buffer at pH 7.4 was prepared. The seeds were immersed in the solution for 24 hours at room temperature and then cut in half to observe viability. Stains were analyzed based on the color patterns described by Craviotto *et al.* (2011).

Germination evaluation

The germination parameters evaluated after one week of daily observations in the various germination assays (scarifications, optimal temperature, accelerated aging, and saline stress) were:

Germination Capacity (GC) (3), which is the total germination percentage at the end of the experiment and it is calculated as shown in table 1, in equation E1, where *G* is the number of germinated seeds at the end of the experiment and *n* is the total number of seeds in the test.

Table 1. Equations used to calculate the germination parameters evaluated in this study.
Tabla 1. Ecuaciones utilizadas para calcular los parámetros germinativos evaluados en este estudio.

The table presents the equations for Germination Capacity (GC), Mean Germination Time (MTG), germination speed index (GSI), Velocity Coefficient (VC), and Uniformity Factor (U), along with their respective units of measurement.

La tabla presenta las ecuaciones para la Capacidad Germinativa (GC), el Tiempo Medio de Germinación (MTG), el índice de velocidad germinativa (GSI), el Coeficiente de Velocidad (VC) y el Factor de Uniformidad (U), junto con sus respectivas unidades de medida.

Equation	Formula	Units of measure
E1	$GC = \sum\left(\frac{G}{n}\right) * 100$	Percentage (%)
E2	$MGT = \sum(n_i t_i)/\sum n_i$	Days
E3	$GSI = \frac{G1}{AG1} + \frac{G2}{AG2} + \ldots \frac{Gn}{AGn} = \sum(Gi/AGi)$	Number of seeds per day
E4	$VC = \frac{\sum n_i}{\sum(n_i * t_i)} * 100$	-
E5	$U = \frac{\sum(g - \sum t_i)^2 n_i}{\sum n_i - 1}$	-

Mean Germination Time (MTG) with the formula proposed by Martínez-Gonzáles *et al.* (2022), (E2):

where

T = germination time

ti = number of days of assay

ni = number of seeds germinated on day *i*

Germination Speed Index (GSI) or Maguire's Index (19), which is expressed as the number of germinated seeds per day (E3):

where

G1 = the number of seeds that germinated on day 1 (not cumulative)

G2 = the number of seeds that germinated on day 2 (not cumulative)

Gn = the number of seeds that germinated on day *n* (not cumulative, end of the experiment)

AG1 = the cumulative number of germinated seeds on day 1

AG2 = the cumulative number of germinated seeds on day 2

AGn = the cumulative number of germinated seeds on day *n* (end of the experiment).

Germinative energy (GE)(4) is the percentage of daily cumulative germination at the highest germination rate.

Specifically, for defining an efficient scarification protocol in terms of velocity and uniformity, the following parameters were also assessed:

Velocity Coefficient (VC) (31) is an index based on the number of germinated seeds inversely related to the time and the number of seeds germinated per day. It is a measure of the distribution of germination over time in relation to the number of germinated seeds and is expressed in the equation E4:

where

VC = velocity coefficient

n = number of seeds germinated on day *i*

t = number of days since sowing.

Uniformity Factor (U) (31) is proposed as a measure of the variance in germination time or the germination over time (E5):

where

U = uniformity factor

g = mean germination time

ti = number of days after sowing

ni = number of seeds germinated on day *i*.

Additionally, the time to reach the maximum accumulated germination (Tmax) was considered, which indicates the day from which no further germinations occurred.

Statistical treatment of data

The study employed a completely randomized experimental design. Data analysis was conducted using the open-source statistical analysis software InfoStat (30). Treatment differences were assessed using ANOVA with Tukey's test, or the non-parametric Kruskal-Wallis test when assumptions were not met (*i.e.* optimal germination temperature, accelerated aging test and effect of salinity on germination). Results were presented as mean ± standard error (SE) of the replications. The Pearson Correlation Coefficient assessed the relationship between germination parameters in the dormancy interruption assay (see section Dormancy interruption) following reference ranges by Schober *et al.* (2018): very low correlation for r^2 less than 0.00, low for 0.10-0.39, moderate for 0.40-0.69, strong for 0.70-0.89, and very strong for 0.90-1.

Results and discussion

Seed germination protocol optimization

Dormancy breaking

In the control group of *N. caldenia* seeds, 56% did not germinate but were viable based on the tetrazolium test, indicating dormancy. Conversely, 42% germinated, and 2% did not germinate and were non-viable. Scarification enabled germination of all viable seeds, regardless of treatment, confirming physical dormancy imposed by the seed coat, as reported by Utello *et al.* (2023).

The evaluated germination parameters revealed a strong to very strong positive correlation between GC and GSI parameters (r^2=0.99 and 0.80, respectively). The correlation indicates that treatments improved both the germination percentage and speed (table 2, page XXX). This improved germination uniformity, with the CS10 and MS treatments exhibiting less dispersion, is consistent with previous findings (31). Furthermore, due to rapid germination, the obtained Germination Energy (GE) values were similar to those of CG and thus were excluded from further analysis.

Scarification with sulfuric acid for up to 30 minutes (CS30) resulted in a GC greater than 95%, but longer exposure negatively affected germination. However, the GC values obtained were considerably higher than those reported in seeds from other provenance, which ranged between 75% and 30% (31, 36). Although acid treatments (CS20, CS30, CS40) did not significantly affect the GC, they impacted GSI, MGT and Tmax. A negative or low correlation was observed among these parameters (r^2= -0.83 to 0.53). This suggests that while a high GC was maintained, the temporal efficiency of the process was reduced.

Table 2. Germinative parameters evaluated in scarification of *N. caldenia* seeds.
Tabla 2. Parámetros germinativos evaluados en las escarificaciones de las semillas de *N. caldenia*.

Treatment	GC (%)	MGT (days)	T max (days)	GSI (n°seeds/day)	VC	U
CS10	98.0±1.3^{a}	1.1±0.1^{a}	1.5±0.2^{a}	9.3±0.3^{a}	92.1±3.6^{a}	7.5±0.3^{a}
CS20	96.0±1.6^{ab}	1.9±0.1 ^{bc}	3.3±0.3^{c}	6.3±0.2^{c}	54.1±4.1^{ed}	25.9±1.8^{c}
CS30	95.0±2.2^{ab}	1.6±0.2 ^{b}	2.8±0.4^{bc}	7.3±0.41^{bc}	68.1±4.1^{dc}	26.9±2.0^{c}
CS40	89.0±3.1^{b}	2.1±0.1 ^{c}	3.9±0.2^{d}	5.2±0.4^{d}	46.7±2.0^{e}	24.4±1.5^{bc}
PS	100.0±0.0^{ab}	1.2±0.1 ^{a}	2.6±0.2 ^{bc}	8.2±0.2^{b}	76.3±3.8^{bc}	13.9±0.4^{b}
MS	100.0±0.0 ^{a}	1.2±0.1 ^{a}	2.0±0.0^{a}	9.2±0.2^{a}	87.1±2.9^{ab}	6.0±0.1^{a}
Control	42.0±5.6 ^{c}	1.3±0.1 ^{a}	2.0±0.3^{ab}	3.5±0.5^{e}	71.2±9.6^{bc}	17.5±2.9^{b}
p-value*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.003

Germinative Capacity (GC), Mean Germination Time (MGT), Time of maximum germination (Tmax), Germinative Speed Index (GSI), Velocity Coefficient (VC) and Uniformity Factor (U) for chemical scarification treatments with sulfuric acid for 10 (CS10), 20 (CS20), 30 (CS30) and 40 (CS40) min, physical scarification (PS), mechanical scarification (MS) and the control.

Results were expressed as the mean ± standard error (SE) of the repetitions.

*different letters are not significantly different (*p*-value > 0.05).

Capacidad Germinativa (GC), Tiempo Medio de Germinación (MGT), Tiempo máximo de germinación (Tmax), Índice de Velocidad Germinativa (GSI), el Coeficiente de Velocidad (VC) y el Factor de Uniformidad (U) para los tratamientos de escarificación química con ácido sulfúrico durante 10 (CS10), 20 (CS20), 30 (CS30) y 40 (CS40) min, escarificación física (PS), escarificación mecánica (MS) y el control.

Los resultados se expresaron como la media ± error estándar (EE) de las repeticiones.

*letras distintas son significativamente diferentes (*p*-valor > 0,05).

Prolonged acid immersion decreased GSI and increased MGT and Tmax. This led to a more dispersed germination pattern, indicated by a decrease in VC (<92) and an increase in U (>7.5). Utello *et al*. (2023) used sulfuric acid scarification for 15 minutes on *N. caldenia* seeds from another province in the central Espinal region. Their results exhibited similar germination speed and duration values to our study at the longest exposure times (CS30 and CS40). The same trend was observed in the control seeds analyzed by Utello *et al*. (2023), with a Tmax five times higher than that of the control seeds in our study, despite a similar GC. This difference may stem from seed morphology, storage conditions, chemical composition, or seed coat thickness, influenced by regional environmental conditions (4, 21). Longer acid exposure than 10 minutes led to oxidative stress and reduced radicle elongation, consistent with Utello *et al.* (2023). The decline in germination rates may result from acid infiltration into seed tissues, raising temperatures and potentially harming the embryo (36).

Both mechanical and physical scarification treatments effectively broke dormancy in *N. caldenia* seeds, with no significant difference in MGT. MS was more efficient for germination speed, resulting in more uniform germination with increased VC, comparable to CS10. Utello *et al.* (2023) reported a 90% improvement in *N. caldenia* germination using a mechanical method. However, their physical scarification yielded germination rates approximately four times lower than those in our study, with a GSI 2.5 times higher. Zeberio and Pérez (2020) observed no germination when applying a combination of mechanical and physical scarification to *N. caldenia* seeds from the northern Monte region. In contrast, our study yielded significantly higher germination rates by applying similar scarification methods separately.

Timing, speed, homogeneity, and synchrony of germination are essential for understanding seed vigor and stress performance. Homogeneous germination supports synchronized seedling establishment, which benefits agriculture and restoration. While varied timing aids survival in wild populations, synchronized germination in managed environments promotes consistent and resilient growth (15). In this regard, the shorter duration chemical scarification method (CS10) and mechanical scarification were statistically more efficient than the other treatments. Furthermore, mechanical treatments for seed germination represent an effective and sustainable approach.

Optimal germination temperature

Table 3 (page XXX), summarizes the evaluations of germination parameters at different temperatures. Germination rates remained near 100% up to 40°C but decreased at 45°C, where no seeds germinated. The tetrazolium staining indicated that these seeds were non-viable. GSI values showed significantly faster germination at 30 and 35°C. Thus, the

optimal temperature range for *N. caldenia* in the south-central region of Espinal was between 30 and 35°C. Similar ranges have been reported for related species. Boeri *et al.* (2019) found an optimum germination temperature of 30°C for *N. alpataco*, while Villagra *et al.* (2017) suggested 35°C for both *N. alpataco* and *N. argentina*. In this sense, the optimal germination temperature varies according to species and geographic distribution.

Table 3. Optimal germination temperature.
Tabla 3. Temperatura óptima de germinación.

Treatment	GC (%)	MGT (days)	GSI (n° seeds/day)
25°C	100.0±0.0 [a]	1.26±0.0 [a]	9.2±0.2 [a]
30°C	99.0±1.0 [a]	1.04±0.0 [b]	10.6±0.6 [b]
35°C	100.0±0.0[a]	1.05±0.0 [b]	9.8±0.1 [ab]
40°C	98.0±1.3 [a]	1.7±0.15 [c]	6.6±0.3 [c]
45°C	0.0 [b]	0.0 [d]	0.0 [d]
p-value*	<0.0001	<0.0001	<0.0001

Values of Germinative Capacity (GC), Mean Germination Time (MGT) and Germinative Speed Index (GSI) obtained for *N. caldenia* germinations from 25 to 45°C.

The results were expressed as the mean ± standard error (SE).

* different letters are not significantly different (*p*-value > 0.05).

Valores de Capacidad Germinativa (GC), Tiempo Medio de Germinación (MGT) e Índice de Velocidad Germinativa (GSI) las germinaciones de *N. caldenia* de 25 a 45°C.

Los resultados se expresaron como la media ± error estándar (EE).

* letras distintas son significativamente diferentes (*p*-valor > 0,05).

In the study region, the highest precipitation occurs during the warm semester (October to March), accounting for 69% of the annual total. During these months, average maximum temperatures range between 28 and 36°C (29). In this sense, the optimal germination temperature of *N. caldenia* coincides with the period of highest precipitation in the region. However, climate change has led to a significant increase in temperature amplitude, which may alter the optimal conditions for germination and seedling survival.

Seed vigor

Accelerated aging (AA) of *Neltuma caldenia* seeds significantly reduced germination rates over time (table 4, page XXX). The highest GC occurred within the first 24 hours of AA. This was followed by a 15% decline between 48 and 72 hours compared to the control. MGT remained stable for up to 72 hours. However, at 96 hours, GC decreased by 50%, accompanied by a twofold increase in MGT. Fontana *et al*. (2016) applied this method to *N. alba* seeds from northern Argentina and observed 50% lethality within 48 hours of storage. This suggests that *N. caldenia* seeds may demonstrate greater resilience to high-temperature and humidity conditions, potentially due to higher vigor. Seed vigor can vary by species and is influenced by environmental factors such as light, temperature, soil moisture, and nutrients (15). GSI decreased significantly with longer AA durations, resulting in germination rates 2.17 times lower than the control (table 4). This decline indicates potential physiological and biochemical changes, such as reduced plasma membrane integrity, molecular alterations in nucleic acids, decreased enzymatic activities during seed senescence, and delayed germination (25).

Effect of salinity on germination

The inhibitory effects of salinity on germination, due to ionic toxicity and osmotic stress impeding water uptake by the embryo, are well documented in various *Neltuma* species. Table 5 (page XXX), summarizes these effects on *N. caldenia* germination. Germination capacity remained unaffected at osmotic potentials up to -0.90 MPa (T1-T3). However, it decreased significantly under higher osmotic stress, with total inhibition at the most severe level (T5) and no viable seeds according to the tetrazolium test. The germination response of *N. caldenia* under saline conditions resembles that of salt-tolerant plants, halophytes, showing resistance up to a critical concentration followed by a sharp decline.

Similar patterns were observed in *N. alpataco*, with reduced germination at comparable osmotic potentials (32). However, studies on *Strombocarpa strombulifera* and *N. alba* report a higher saline tolerance, with GC above 80% at -1.2 and -2.2 MPa, respectively (22). Additionally, *N. chilensis* showed 56% germination at -2.7 MPa, highlighting species-specific adaptations to salinity within the genus (34). This variation underscores the diverse salinity responses within *Neltuma*, illustrating the complex nature of salinity adaptation.

Table 4. Accelerated Aging (AA) test.

Tabla 4. Prueba del envejecimiento acelerado (AA).

Treatment (h)	GC (%)	MGT (days)	GSI (n° seeds/day)
0	100.0±0.0 [a]	1.2±0.3 [a]	9.8±0.1 [a]
24	100.0±0.0 [a]	1.3±0.1 [a]	9.2±0.2 [a]
48	88.0±3.9 [ab]	1.3±0.0 [a]	7.3±0.2 [b]
72	85.0±4.0 [b]	1.1±0.1 [a]	8.1±0.5 [ab]
96	50.0±8.2 [c]	2.3±0.1 [b]	2.4±0.4 [c]
p-value*	<0.0001	<0.0001	<0.0001

Germinative Capacity (GC), Mean Germination Time (MGT) and Germinative Speed Index (GSI) obtained for the AA test of *N. caldenia* seeds for 0, 24, 48, 72 and 96 h.

Results were expressed as the mean ± standard error (SE) of the repetitions.

*different letters are not significantly different (*p*-value > 0.05).

Capacidad Germinativa (GC), Tiempo Medio de Germinación (MGT) e Índice de Velocidad Germinativa (GSI) obtenidos para el ensayo de AA de las semillas de *N. caldenia* durante 0, 24, 48, 72 y 96 h.

Los resultados se expresaron como la media ± error estándar (EE) de las repeticiones.

* letras distintas son significativamente diferentes (*p*-valor > 0,05).

Table 5. Values of Germinative Capacity (GC), Mean Germination Time (MGT) and Germinative Speed Index (GSI) obtained for seeds subjected to treatments T0, T1, T2, T3, T4 and T5 with different osmotic potentials (Ψo) induced by NaCl.

Tabla 5. Valores de Capacidad Germinativa (GC), Tiempo Medio de Germinación (MGT) e Índice de Velocidad Germinativa (GSI) obtenidos para las semillas sometidas a los tratamientos T0, T1, T2, T3, T4 y T5 con diferentes potenciales osmóticos (Ψo) inducidos por NaCl.

Ψo (MPa)	GC (%)	MGT (days)	GSI (n° seeds/day)
0 (T0)	99.0±3.0[a]	1.0±0.0[a]	9.8±0.4[a]
-0.22 (T1)	98.0±1.3 [ab]	1.1±0.0 [ab]	9.2±0.1 [ab]
-0.45 (T2)	97.0± 0.6 [ab]	1.0±0.0 [a]	9.3±0.2 [ab]
-0.90 (T3)	98.0±2.0 [ab]	1.4±0.1[bc]	8.3±0.5 [bc]
-1.81 (T4)	83.0±4.9 [b]	2.7±0.2 [c]	3.0±0.1 [c]
-2.71 (T5)	0.0±0.0	0.0±0.0	0.0±0.0
p-value*	<0,0001	<0,0001	<0,0001

The results were expressed as the mean ± standard error (SE) of the repetitions.

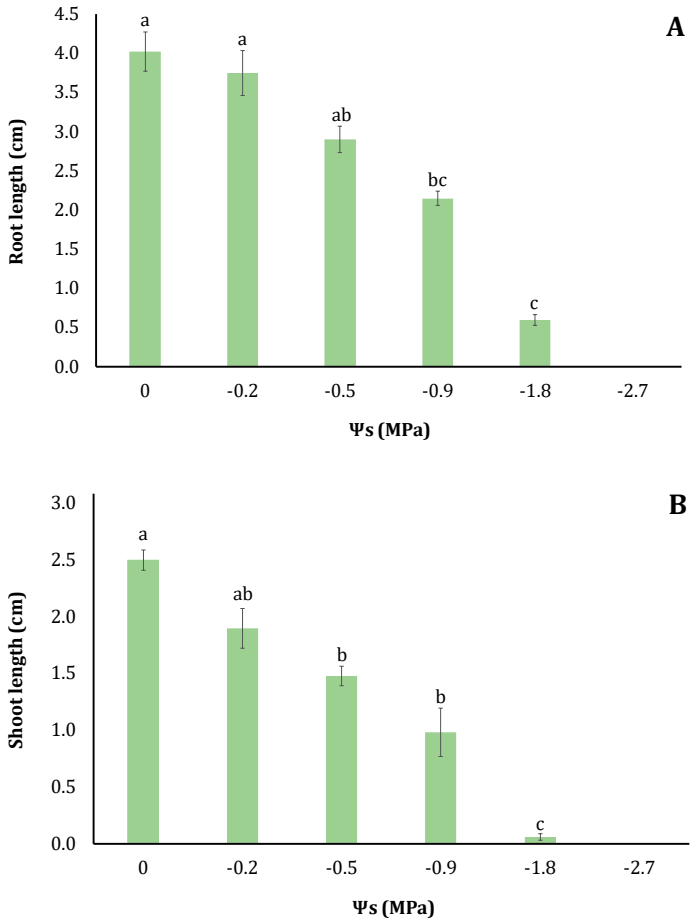
* different letters are not significantly different (*p*-value > 0.05).

Los resultados se expresaron como la media ± error estándar (EE) de las repeticiones.

* letras distintas son significativamente diferentes (*p*-valor > 0,05).

MGT and GSI were unaffected at lower salinity levels (T1, T2). However, they decreased significantly at higher salinity (T3, T4), likely due to delayed seed imbibition from low water potential, as observed in *N. alba* (22). Westphal *et al.* (2015) reported a similar germination delay in *N. chilensis* under saline conditions (NaCl 450-600 mM), requiring 5 days longer than controls to reach maximum germination. Similarly, *N. caldenia* seeds needed 4 days to reach maximum germination at high NaCl levels (T4), twice the duration of the control.

To quantify the impact of the osmotic pressure on seedling growth, both radicle and shoot lengths were measured. Increased salinity significantly reduced root and shoot lengths (figure 1, page XXX). Root length showed no significant reduction at -0.22 and -0.45 MPa but declined beyond T3, reaching 6.7 times less than control length at -1.81 MPa (figure 1A, page XXX). Shoot length declined from the lowest NaCl concentration, approaching minimal values at -1.81 MPa (figure 1B, page XXX). These results indicate that NaCl inhibits shoot growth more than root growth, potentially due to endogenous abscisic acid (ABA), a phytohormone that reduces shoot growth and moderates root elongation under osmotic stress (2). Similar findings in *S. strombulifera* showed increased ABA levels and reduced shoot growth under high humidity and NaCl (12).



The results are expressed as the mean, and the bars indicate the standard error (SE) of the repetitions. Means with the same letter are not significantly different (p-value > 0.05).

Los resultados se expresaron como la media y las barras indican el error estándar (EE) de las repeticiones. medias con letra común no son significativamente diferentes (p-valor > 0,05).

Figure 1. Influence of osmotic treatments induced by NaCl (0 to -2.7 MPa) on the development of roots (A) and hypocotyls (B), recorded in cm of *N. caldenia*.

Figura 1. Influencia de los tratamientos osmóticos inducidos por NaCl (0 a -2,7 MPa) en el desarrollo de las raíces (A) y de los hipocótilos (B) de *N. caldenia*, registrado en cm.

The reduction in shoot and root growth led to a decline in SVI (table 6, page XXX), which remained statistically similar to the control up to an osmotic pressure of -0.22 MPa. From treatment T1 onwards, SVI progressively decreased, with vigor dropping below half at -1.81 MPa. Root and shoot tolerance indices showed similar patterns, exceeding 50% up to -0.45 MPa, while salinity at T3 and T4 induced more severe phytotoxic effects on shoots. Total toxicity was observed for roots and shoots at T5.

Given that *N. caldenia* inhabits saline soils and salt flats during wet seasons, it may possess adaptive mechanisms to salinity, as our findings indicated. However, tolerance at the germination stage does not ensure similar tolerance in seedling growth (8, 13). While seeds tolerated up to -0.90 MPa during germination, 14-day-old seedlings were more sensitive, showing toxic effects at -0.45 MPa. This heightened sensitivity could limit seedling recruitment and survival in variable salinity environments. Beyond tolerable salinity, reductions in radicle and seedling growth are likely due to NaCl toxicity and impaired nutrient absorption (7). As soil salinity fluctuates with precipitation, it is essential to consider both germination capacity and salinity effects on seedling growth to inform effective conservation and restoration strategies.

Table 6. Seedling Vigor Index (SVI), Root Phytotoxicity (RPT), Hypocotyl Phytotoxicity (HPT), Root Tolerance Index (RTI) and Hypocotyl Tolerance Index (HTI) obtained for seedlings subjected to treatments T0, T1, T2, T3, T4 and T5 with different osmotic potentials (Ψo) induced by NaCl.

Tabla 6. Índice de Vigor de Plántula (SVI), Fitotoxicidad de la raíz (RPT) y de hipocótilo (HPT), Índice de Tolerancia de la raíz (RTI) e índice de Tolerancia del hipocótilo (HTI) obtenidos para las plántulas sometidas a los tratamientos T0, T1, T2, T3, T4 y T5 con diferentes potenciales osmóticos (Ψo) inducidos por NaCl.

The results were expressed as the mean ± standard error (SE) of the repetitions.

*means with common letter are not significantly different (*p*-value > 0.05).

Los resultados se expresaron como la media ± error estándar (EE) de las repeticiones.

*medias con letra común no son significativamente diferentes (*p*-valor > 0,05).

Treatment (MPa)	SVI	RPT (%)	HPT (%)	RTI (%)	HTI (%)
0 (T0)	646.4±26.4 [a]	16.4±2.9 [a]	7.4±2.5 [a]	100.00±3.5 [a]	100.0±6.2 [a]
-0.23 (T1)	553.3±42.8 [ab]	29.3±5.4 [ab]	28.3±6.4 [ab]	70.62±5.4 [ab]	72.1±6.6 [ab]
-0.45 (T2)	432.7±25.1 [bc]	44.4±3.2 [bc]	43.7±3.2 [bc]	55.54±3.1 [bc]	56.2±3.2 [bc]
-0.91 (T3)	303.9±18.5 [cd]	59.4±1.7 [cd]	63.2±7.6 [c]	40.6±1.7 [cd]	37.1±8.0 [c]
-1.81 (T4)	57.2±9.5 [d]	88.5±1.2 [d]	97.6±1.1 [d]	11.4±1.2 [d]	2.4±1.1 [d]
-2.72 (T5)	0.0±0.0	100.0±0.0	100.0±0.0	0.0±0.0	0.0±0.0
p-value*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Conclusions

This study demonstrates the effectiveness of scarification techniques in promoting *N. caldenia* seed germination, with both mechanical and chemical methods successfully breaking seed dormancy. The seeds showed high vigor, with germination rates strongly affected by temperature, although prolonged storage reduced vigor, especially after accelerated aging. These findings underscore the need for appropriate storage practices to preserve seed viability. Additionally, *N. caldenia* seeds displayed salinity tolerance levels during germination comparable to or greater than those of other salt-tolerant species within its genus. Optimizing germination protocols and understanding the effects of salinity are essential steps toward formulating robust conservation and management strategies. Optimizing germination protocols and understanding salinity impacts are key to developing effective conservation and management strategies. Addressing these factors supports environmental restoration and habitat preservation, contributing to the sustainable use of *N. caldenia*, a notable species of the Espinal ecosystem under significant environmental pressure.

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