



Respuesta de la germinación de semillas forrajeras a soluciones salinas en condiciones controladas
Response of forage seed germination to saline solutions under controlled conditions

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Data of the Article

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Resumen

La salinidad es uno de los factores limitantes que afectan negativamente la germinación, emergencia y desarrollo de pastos en la parte baja de la subcuenca del Río Lauca - Oruro, reduciendo la disponibilidad de forrajes para la ganadería. Con el propósito de identificar especies forrajeras tolerantes a la salinidad, se evaluó la capacidad germinativa en soluciones salinas de ocho especies: *Agropyron elongatum* (Host) P. Beauv., *Hordeum muticum* J. Presl., *Bromus catharticus* Vahl., *X. Triticosecale* Wittmarck, *Hordeum vulgare* L., *Atriplex cristata*, *Trifolium amabile* (L.) Kunth., y *Suaeda foliosa* Moq. La investigación, se realizó en laboratorios de la Facultad de Ciencias Agrícolas y Naturales – Universidad Técnica de Oruro; a las semillas se aplicó soluciones salinas con cuatro concentraciones de NaCl por un litro de agua destilada: 2.56, 5.12, 7.68 y 10.54 g, y un testigo sin salinidad. Los resultados indican que *X. Triticosecale* Wittmarck y *H. vulgare* tienen mayor tolerancia a las soluciones de extrema salinidad y poco afectadas en su capacidad germinativa. Las semillas de *A. elongatum*, *B. catharticus* y *S. foliosa* reducen significativamente la germinación, sin embargo, logran germinar en todos los niveles de salinidad. Las semillas de *A. cristata* y *T. amabile*, son las más sensibles a la afectación salina, la germinación se reduce significativamente en todos los niveles de salinidad, incluso se anula en la concentración más alta. Se concluye que estas especies pueden utilizarse como forrajeras para el aprovechamiento de suelos salinizados, en función a la tolerancia máxima determinada.

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Abstract

Salinity is one of the limiting factors that negatively affect the germination, emergence, and development of pastures in the lower part of the Lauca - Oruro River sub-basin, reducing the availability of forages for livestock. With the purpose of identifying salinity tolerant forage species, the germination capacity in saline solutions of eight species was evaluated: *Agropyron elongatum* (Host) P. Beauv., *Hordeum muticum* J. Presl., *Bromus catharticus* Vahl., *X. Triticosecale* Wittmarck, *Hordeum vulgare* L., *Atriplex cristata*, *Trifolium amabile* (L.) Kunth., and *Suaeda foliosa* Moq. The research was carried out in laboratories of the Faculty of Agricultural and Natural Sciences - Technical University of Oruro; Saline solutions with four concentrations of NaCl per one liter of distilled water were applied to the seeds: 2.56, 5.12, 7.68, and 10.54 g, and control without salinity. The results indicate that *X. Triticosecale* Wittmarck and *H. vulgare* have greater tolerance to solutions of extreme salinity and little affected in their germination capacity. The seeds of *A. elongatum*, *B. catharticus* and *S. foliosa* significantly reduce germination, however, they manage to germinate at all salinity levels. The seeds of *A. cristata* and *T. amabile* are the most sensitive to saline affectation, germination is significantly reduced at all salinity levels, even at the highest concentration. It is concluded that these species can be used as forage for the use of salinized soils, depending on the maximum tolerance determined.



Keywords:

Germination,
saline solutions,
forage species,
salinity,
sodium chloride.

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Introduction

The seeds germinations is a critical stage of the plants development, they are exposed to adverse factors, salinity and water stress¹⁻³, mainly the decrease in the availability of water in the soil^{4,5}. Most species are sensitive to salinity during germination and emergence stages, that in its growth and development^{6,7}. Germination depends on the availability of water, under conditions of water stress cause poor or no germination the seeds^{1,8,9}. The increase in salinity in soils causes a decrease in water potential, inducing toxic effects on germination^{10,11}. The excessive accumulation of ions, of Cl⁻ or Na⁺ in the soil solution, could be toxic for most species¹²⁻¹⁴. If have the ability to control the transport and absorption of Na⁺ to the photosynthetic tissue, have the ability to tolerate salinity^{15,16}, mainly in the germination stage, but also it is crucial in the beginning of development and growth^{17,18}. Many species have tolerance to salts by polygenic inheritances, which have the ability to withstand osmotic and ionic stress at cellular level¹⁹⁻²³. The salts are not a stimulus in the seeds germination stage, if not, they act as a toxic in early stages of plants. The toxic action of the cation or anion can exceed the effect produced on osmotic pressure²⁴. Salinity reduces the power seeds germination and reduces the development of plants²⁵. Salinity is an important cause for the poor development of forage grasslands and main problems of life systems in the lower basin Lauca River ecosystems, a region the ancestral nation Uru Chipaya (Department of Oruro). The present research was carried with the purpose of identifying forage species with tolerance to different concentrations of NaCl in the germination stage of eight native and naturalized forage species in laboratory.

Materials and methods

The research was carried from October to December 2017 and January 2018 at the Facultad de Ciencias

Agrarias y Naturales of the Universidad Técnica de Oruro (FCAN-UTO), ciudadela universitaria to the south Oruro city, with altitude 3735 m above sea level.

Seed. seeds of eight forage species that have tolerance to salinity, which three species come from commercial certified stores: alkar (*Agropyron elongatum* (Host) P. Beauv.), Triticale (*X. Triticosecale* Wittmarck) and barley (*Hordeum vulgare* L.). The five species are native to the highlands and were collected in the Saucarí province of the Department of Oruro: cola de raton (*Hordeum muticum* J. Presl), cebadilla nativa (*Bromus catharticus* Vahl.), Livi livi (*Atriplex cristata*), layu layu (*Trifolium amabile* (L.) Kunth.) and qauchi (*Suaeda foliosa* Moq.). Seeds of each species were cleaning of physical impurities, no pre-germination or disinfection treatment was applied.

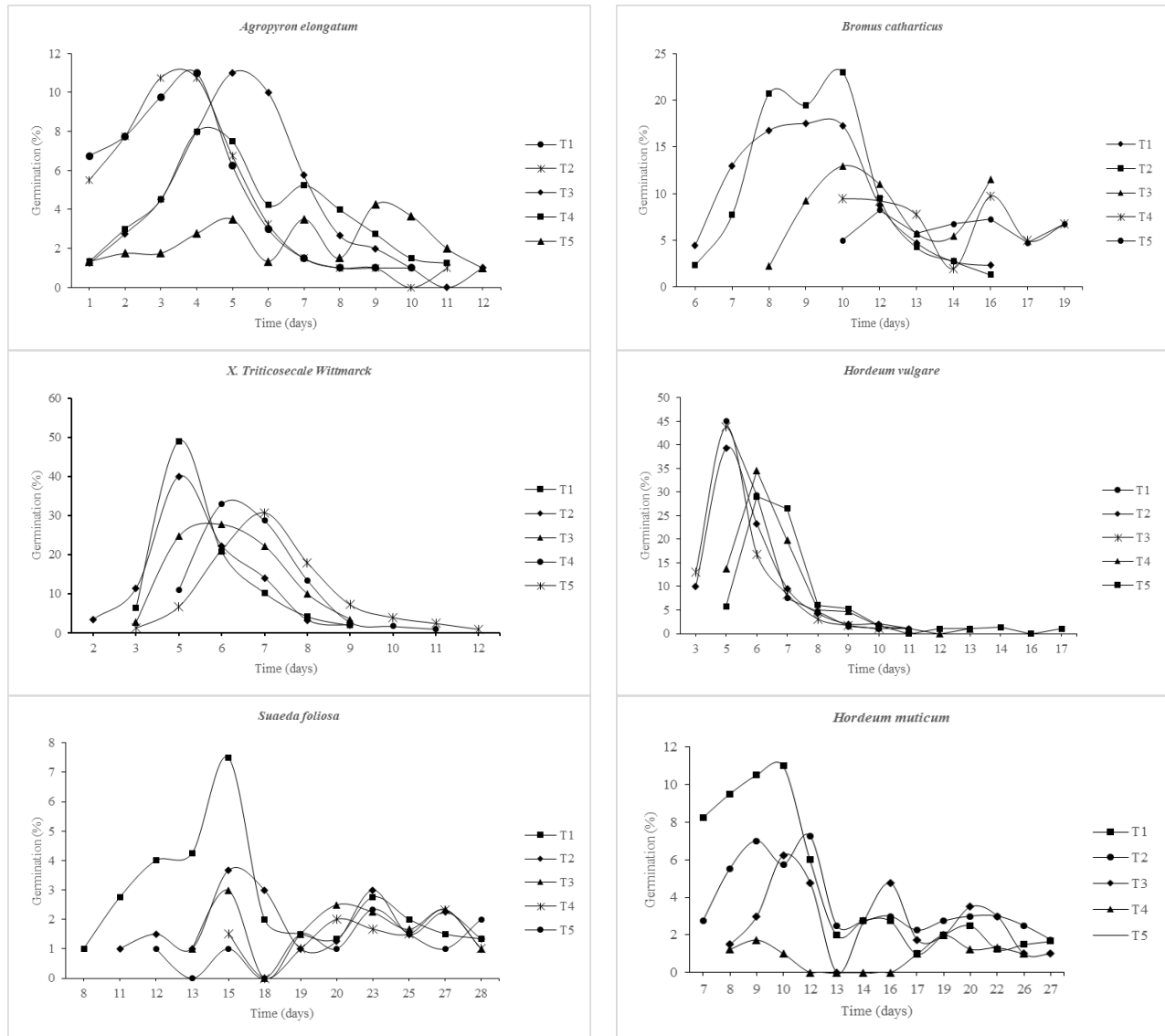
Saline solutions. Saline solutions were prepared in 1 L of distilled water with different levels of NaCl: T₁=distilled water control (without salinity), T₂=2.56 g of NaCl, equivalent to 4 dS m⁻¹ (light salinity), T₃=5.12 g of NaCl (8 dS m⁻¹) (medium salinity), T₄=7.68 g of (12 dS m⁻¹) (strong salinity) T₅=10.54 g of NaCl (16 dS m⁻¹) (extreme salinity)^{26,27}. The variables evaluated were: daily germination and germination capacity. The first count was evaluated at 24 h after sowing and was evaluated daily for 30 days.

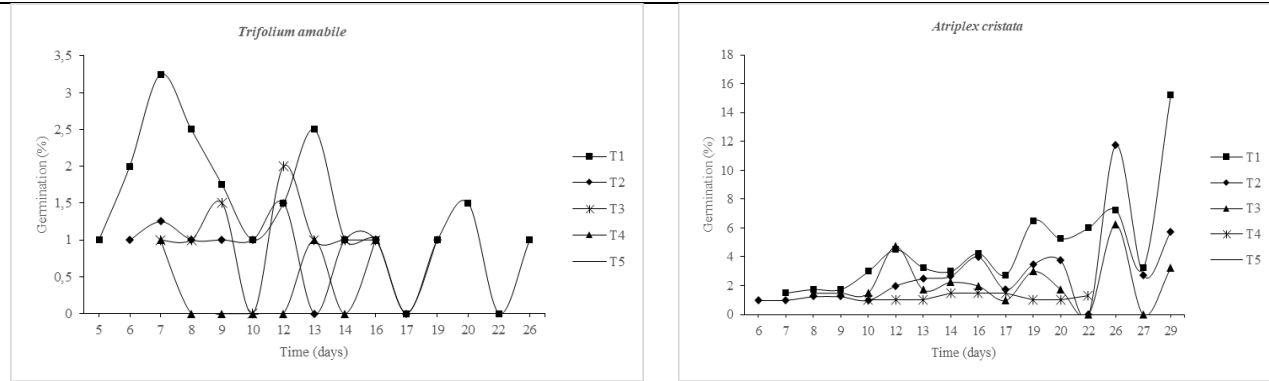
Incubation for seed germination. In 9 cm diameter Petri dishes with filter paper, 100 seeds of each species were sown, the plates were moistened with 5 mL of each NaCl saline solution and kept in an automatic incubator under 25 °C temperature, 80% relative humidity, 12 h light/darkness for 30 days^{28,29}.

Experimental design. The completely randomized experimental design (DBCA) was used with four repetitions. The data were analyzed using the analysis of variance (ANOVA) and the Tukey test was used with $P < 0.05$ ³⁰⁻³².

Results

Figure 1 Daily germination of eight forage species at different concentrations of NaCl saline solutions

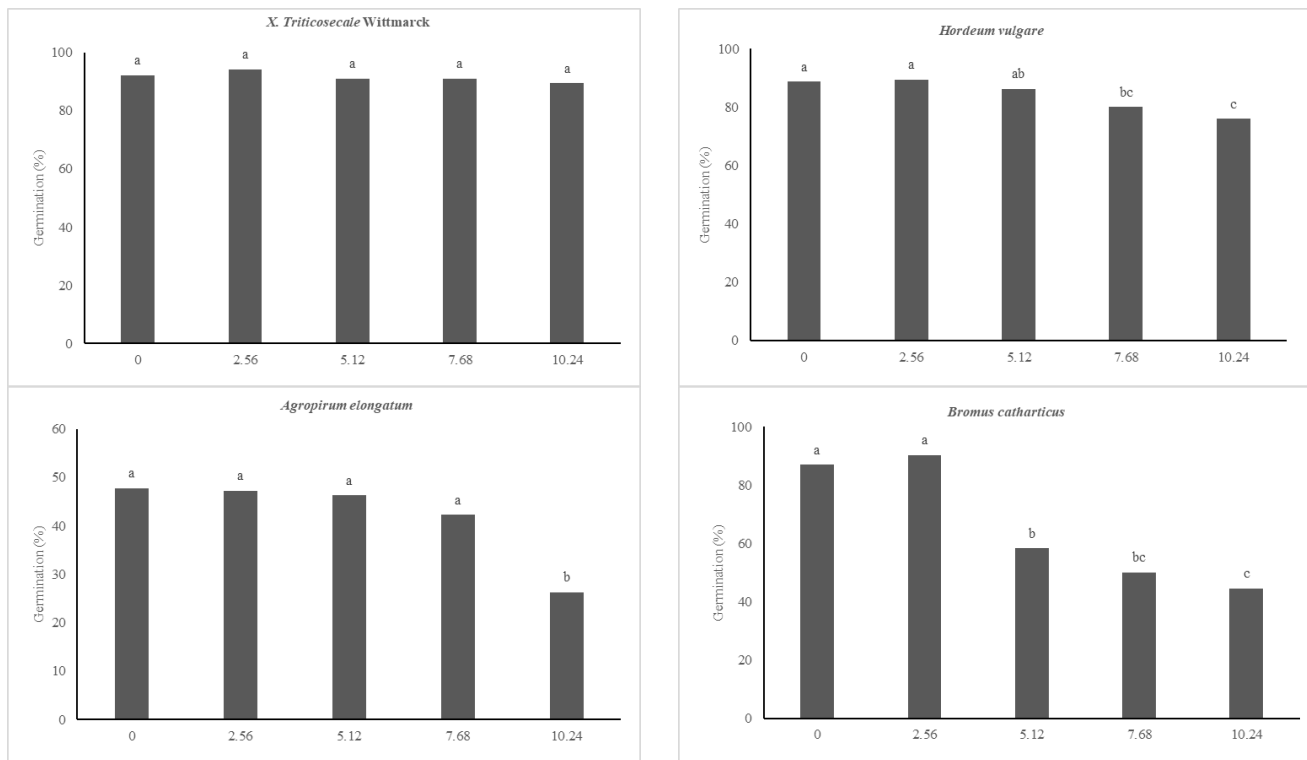


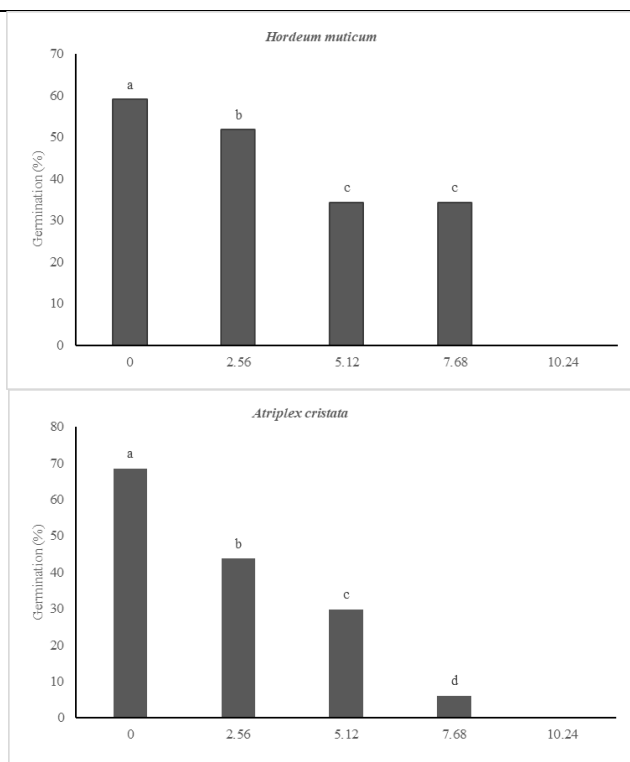
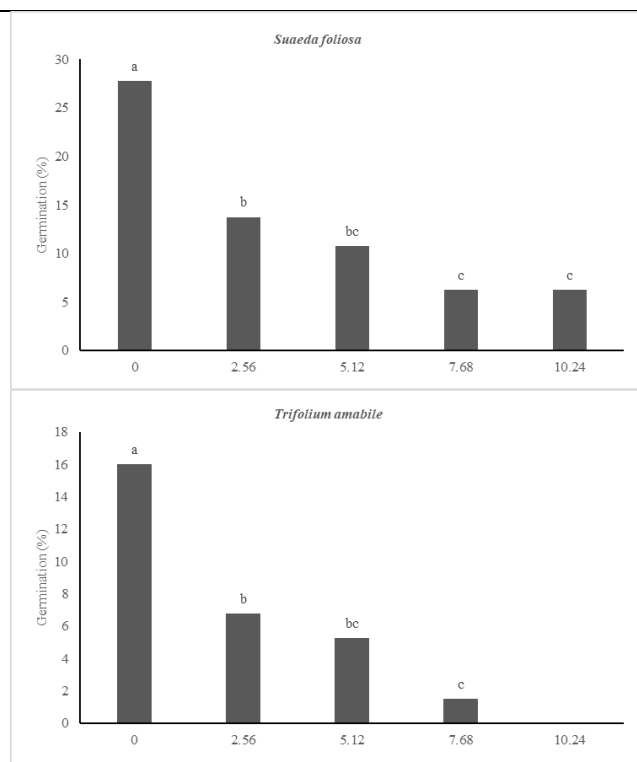


Daily germination. *A. elongatum* (Host) P. Beauv in all treatments started germination on the first day, the duration of the germination process was 10 days in conditions without salinity (T₁), 11 days with light salinity (T₂) and 12 days for solutions of mod-

erate (T₃), strong (T₄) and extreme salinity (T₅). The maximum number of germinated seeds per day (germination peak) occurred on day 4 for T₁ and T₂, and on day 5 for T₃, T₄ and T₅, figure 1.

Figure 2 Comparison of means of the germination capacity under saline stress of eight seeds evaluated at different NaCl concentrations





B. catharticus Vahl., germination started on day 6 for T₁ and T₂, on day 8 for T₃ and on day 10 for T₄ and T₅. The duration of the germination process was 10 days for T₁, T₂, T₄ and T₅, while for T₃ it was 9 days. The germination peaks occurred at 9 days for T₁, at 10 days for T₂, T₃, T₄, and at 12 days for T₅.

X. Triticosecale Wittmarck., germination started on day 2 for T₂, day 3 for T₁, T₃, T₅, and day 5 for T₄, 7 days was the duration of germination for T₁, T₃ and T₄, 8 days for T₂ and 10 days for T₅. The germination peaks occurred on day 5 for T₁ and T₂, on day 6 for T₃ and T₄, and on day 7 for T₅.

H. vulgare L. the start of germination was on day 3 for T₂ and T₃, on day 5 for T₁, T₄ and T₅, while the last germinated seed was recorded after 7 days for T₁, 8 days for T₃, 9 days for T₂ and T₄, and 13 days for T₅. The maximum number of germinated seeds per day was recorded at 5 for T₁, T₂ and T₃, and on day 6 for T₄ and T₅.

S. foliosa Moq., germination started on day 8 for T₁, day 11 for T₂, day 12 for T₅, day 13 for T₃ and day 15 for T₅, it lasted up to 20 days for T₁, 17 days for T₂, 16 days for T₅, 15 days for T₃ and 15 days for

T₄. Germination peaks were recorded on day 15 for T₁, T₂ and T₃, day 20 for T₄ and day 23 for T₅.

H. muticum J. Presl the beginning of germination was recorded on day 7 for T₁ and T₂, day 8 for T₃ and T₄, T₅ did not germinate. Germination lasted 20 days from the beginning for T₁ and T₂, 19 days for T₃ and 18 days for T₄. The germination peaks occurred on days 9 (T₁), 10 (T₃) and 11 (T₂), T₄ did not present a clear germination peak.

T. amabile (L.) Kunth., It started on day 5 (T₁), day 6 (T₂), and day 7 for T₃ and T₄, this process lasted until 21 days later for T₁, 13 days for T₂, and 9 days for T₃ and T₄. The germination peak is only perceptible in T₁ at 7 days; in the rest there is no outstanding day.

A. cristata germination started on day 6 for T₂, day 7 for T₁, day 8 for T₃, day 10 for T₄, while T₅ failed to germinate, the duration of this process was 22 days for T₁, 23 days for T₂, 21 days for T₃ and 12 days for T₄. The germination peaks occurred at 12 days for T₁ and T₃, and 16 days for T₂, T₄ does not have an outstanding peak

Germination capacity or accumulated germination. germination capacity of the seeds of eight forage species moistened with saline solutions, these species were grouped into three: In group 1 are the *X. Triticosecale* Wittmarck and *H. vulgare* L. in which the salinity levels have low germination affectation from 92.5% to 89.5% and from 88.75% to 76% respectively at the extreme salinity level T₅, figure 2.

In group 2 there are species whose germination are significantly affected with high levels of salinity, *A. elongatum* (Host) P. Beauv., Its germination capacity decreases from 47.75% to 26.25% with T₅, while that *B. catharticus* Vahl reduces germination with T₃, T₄ and T₅, and finally there is *S. foliosa* Moq., that germination is significantly affected with T₂, T₃, T₄ and T₅.

In group 3 are *H. muticum* J. Presl whose germination is affected with T₃ and T₄, as well as *T. amabile* (L.) Kunth. and *A. cristata* where the levels T₂, T₃ and T₄ significantly reduce their germination capacity. In both species, germination is canceled under conditions of extreme salinity (T₅).

Discussion

The Department of Oruro is located in the central Bolivian highlands, sub-basins endorheic territory of the Desaguadero, Poopó and Coipasa are located, which have gradually been degrading the soils by different degrees of salinization in an area estimated at 254.26 km². Soils with extreme salinity 63 dS m⁻¹(33). Soil salinity is one of the abiotic factors of high impact, soil degradation³⁴, it could in future loss of productive capacity by high salt concentrations.

Several methods have been developed for the use or recovery of salinized soils (physical, biological, hydrotechnical and chemical), however, its application in large areas is not viable by to high costs. The forage species implementation is one of the best

alternatives in these conditions, since they allow the extraction of salts from the soil, but also produce forage for livestock³⁵.

The present study identified forage species with great tolerance to salinity, in phenological germination stage in laboratory, since germination and the first stages of plant growth are phenological stages most sensitive to any situation of stress, mainly the decrease in water availability caused by salinity^{18,34,35}.

The results indicate that the germination of the seven species is affected in different degrees by the increase in NaCl concentrations, which is consistent with several studies on the impact of salts on the germination process³⁶⁻³⁸. Only *X. Triticosecale* Wittmarck resisted a salinity and not suffer a significant decrease in seed germination.

Previous studies indicate that plant species do not respond equally to the effects of salts^{17,34,39,40}, the adverse effects of salinity in forage species vary according to the genetic hereditary character⁴¹⁻⁴³.

Lastiri-Hernández et al.⁴⁴ determined that tolerance to salinity depending on the permeability of the seed, composition of lipid structure and cytoplasmic viscosity, key factors for the preservation of the integrity of the seeds plasma membrane, as is the specific case of *H. vulgare*.

The effects of salinity on seed germination, several investigations indicate that salinity decreases the water potential of the soil solution, causing osmotic retention of water, decreasing the availability of water for the seed and generates toxicity ionic effects^{34,35,45-49} therefore, the germination does not occur by the embryo not reach the necessary turgor to break the seminal covers¹⁸.

The seeds reduction germination capacity is gradual as the salt concentrations increase, to a level that can totally inhibit this process⁵⁰⁻⁵². In saline conditions the seed requires greater amounts of energy⁵³

to absorb water a capacity that not all plant species have.

In the specific case of NaCl saline solutions, Ruiz & Terenti¹⁸ determined that they have a combined effect on the seeds: produces the osmotic effect and causes water stress in the seeds and it creates an ionic effect. By the entry and/or accumulation of ions in the seeds causes toxicity and according with Lastiri-Hernández et al.⁴⁴ ionic toxicity affects the functions of the membrane and cell wall of the embryo, resulting from the reduction in the permeability of the plasma membranes, the increase in the influx of external ions and the reflux of cytosolid solutes. Another salinity effect in seeds is the delay of the start and the germination process, an aspect that was registered in seven species, except *A. elongatum* that did not suffer this affectation, this result also coincides with the general trend of several studies in halophytic and glycophyte plants, caused by the decrease in the water absorption capacity and the seeds speed imbibition^{18,44,54,55}.

The speed and uniformity of seed germination is one of the success factors for the development of forage species in saline conditions, therefore the delay of the germination process reduces the species reproduction possibilities¹⁸, however, Lastiri-Hernández et al.⁴⁴, the reduction of germination in salinity conditions increases their latency and dormancy state, two mechanisms that help seeds to germinate in conditions of reduced salinity, therefore, it can be considered as an adaptation to salinity and maximize the chances of survival species⁵⁶⁻⁵⁸.

the results for each species, *A. elongatum* significantly reduces germination in extreme salinity conditions, coinciding with the reports of Ruiz & Terenti¹⁸, Terrazas⁸ and Jauregui et al.⁵⁹ that, in decrease of germination, mention the decrease in germination speed in close relatives of this species.

H. vulgare, it is affected by extreme salinity solution (EC 18 dS m⁻¹), its germination is higher 76%, and similar to reports of Lastiri-Hernández et al.⁴⁴ that indicate that EC levels of 18.25 and 35.3 dS m⁻¹ reduce germination in 33.33 and 76% respectively.

S. foliosa, shown low germination percentage under normal conditions (28%), with the increase in salt concentration its germination capacity decreases significantly to 6%. These data are similar to Morón-Rios³³ with 19% germination in normal conditions, however, it indicates that with extreme salinity solutions, germination it is null by presence of the perianth, impermeable and semi-hard epispem, and also determines that the retardation of the germination process in this species extends to the seventh week. In studies of other species of genus Suaeda, they also record a decrease in germination and delay in the process between 2 to 7 days compared to the control⁶⁰.

The seeds of *A. cristata*, the increase in saline concentrations decrease germination capacity and cancellation in conditions of extreme salinity, results that are similar to the reports of Morón-Rios³³.

In conclusion, species can be used as forage for the use of salinized soils depending on the maximum tolerance. *X. Triticosecale* Wittmarck and *H. vulgare* have tolerance to extreme salinity and can be planted in it conditions. The species *A. elongatum*, *B. catharticus* and *S. foliosa* can be sown in soils with extreme salinity, however, to reach acceptable levels of germination, it is recommended to quintuple the sowing density.

Finally, the species *A. cristata* and *T. amabile* are the most sensitive to salinity and not suggested sow in extreme salinity soils.

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Conflicts of interest

The authors declare no conflicts of interest.

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Ethical aspects

This document was approved by the Dirección de Investigación Científica and Tecnológica of the Universidad Técnica de Oruro.

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