Imbibition of seeds of romerito (*Suaeda mexicana* Standl.) and purslane (*Portulaca oleracea*) in saline solutions and its effect on germination and initial growth

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ABSTRACT

Objective: To evaluate the effect of salinity sources and concentrations, as well as the interaction of these study factors on the increase in seed mass during imbibition, germination and initial growth of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) seedlings.

Design/methodology/approach: Seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) from the agricultural area of Tláhuac and San Gregorio Atlapulco, Xochimilco, Mexico City were used. They were incubated for 3 h in 15 saline solutions, which resulted from the combination of three sources: NaCl, Na_2SO_4, and $CaCl_2$; and five concentrations: 0, 0.25, 0.50, 0.75, and 1.00 M. The seeds were incubated in Petri dishes at 28 °C in 80% relative humidity. The increase in seed mass gained after the imbibition period, germination percentage, and radicle and shoot length of the seedling were determined.

Results: The 0.25 M NaCl treatment increased germination in romerito; in contrast, doses higher than 0.25 M of $CaCl_2$ and Na_2SO_4 significantly reduced germination, as well as shoot growth. NaCl causes less inhibition of romerito radicle growth than the other sources. In purslane, the 0.25 M $CaCl_2$ treatment significantly reduced germination; but, it stimulated radicle growth. The Na_2SO_4 and NaCl sources at doses starting at 0.5 M inhibited germination in purslane.

Limitations of the study/implications: No certified germplasm was used for this study, since the seed was obtained directly from producers who maintain semi-domesticated native accessions.

Findings/conclusions: Romerito is tolerant to NaCl and Na_2SO_4, but sensitive to $CaCl_2$; this was also observed in purslane, although the ranges of tolerance to the concentration vary between salinity sources.

Keywords: Abiotic stress, growth, halophytes, salinity, seeds.

INTRODUCTION

Glycophyte and halophyte plants respond in a similar way to salinity during germination [1]. In some species, salt stress causes an inhibitory effect on germination, so the development of strategies that allow tolerance of salinity during this phase and in the early stages of growth is essential for plant survival [2]. Facing fluctuations in osmotic potential, salt concentration, and ionic species represents a high energy cost for the plant, which translates into decreased seed germination, prolonged dormancy, and decreased seedling size [3].

The effects and degree of affectation of salinity have been documented to vary from one species to another, according to the type of salinity and its concentrations. In peanut (*Arachis hypogaea*), for example, the increase in salt concentration caused a decrease in seedling emergence, as well as root elongation and plant length, and also decreased dry weight [4]. Similar effects were reported in the germination of pigeon pea (*Cajanus cajan*) seeds [5]. Likewise, in cabbage (*Brassica oleracea* var. *capitata* L.), sugar beet (*Beta vulgaris*), amaranth (*Amaranthus paniculatus*), and pak-choi (*Brassica campestris*), a reduction in germination, root and shoot length was observed [6]. In halophytic plants such as *Crithmum maritimum*, high salt contents delay the germination of their seeds; also, the previous imbibition of these in ascorbic acid improves their germination capacity during saline exposure [7]. In a study carried out on *Suaeda salsa* populations, the seeds belonging to a germplasm bank of an intertidal zone with concentrations of 0.6 to 35.1 ppt NaCl favored the germination of the seeds; however, when the seeds were transferred to a constant saline environment such as a Petri dish, the seeds collected from the same Place were affected by all saline concentrations causing a decrease in germination. The above suggests that even facultative halophyte species such as *S. salsa* are affected by salinity when it is constant [8].

In their natural environment, some seeds take advantage of periods of low salinity and high availability of low-salt water to soak and resist the effects of salinity. This is the case of fenugreek (*Trigonella foenum-graecum*), whose seeds that were soaked in distilled water prior to saline treatments and after being placed in these, where the percentage of final germination, germination speed, and root length increased, compared to those seeds that were not soaked [9]. In three varieties of beans (*Plaseolus vilvaris*) exposed to NaCl and Na_2SO_4, the latter salt decreased germination and inhibited seedling emergence compared to NaCl [10]. Carbonates are salts that also affect seed germination. In purslane (*Portulaca oleracea*), when evaluating the effect of $KHCO_3$, $NaHCO_3$, and $(NH_4)_2CO_3$ on germination and root and stem length, the 50, 100, and 200 mM concentrations of the three salts caused a decrease in the germination percentage as well as the root length and dry weight; while low doses (25 mM) slightly increased the stem length and fresh weight [11].

Although there is a gradient of resistance and adaptation to salinity [12], some plants adopt different seed forms, react differently to dormancy, or may contain different concentrations of abscisic acid (ABA); this combination of factors makes them better adapted to environmental changes and enhances their establishment [13]. In a study on adaptive strategies in the germination phase, it was found that *Suaeda physophora*, *Haloxylon ammodendron*, and *H. persicum* produce seeds without endosperm with a highly developed embryo and covered with a soft testa that facilitates their germination and rapid establishment in saline environments [1].

In Mexico, romerito (*Suaeda mexicana*) is a plant generally found in saline environments, although it can also colonize non-saline sites [14]. It has economic and nutraceutical value since it is part of the diet of Mexicans [15].

Purslane is a halophyte plant with C4 NAD-ME metabolism (NAD-dependent malic enzyme) with economic potential and wide consumption worldwide [16,17,18]. Studies on the effect of NaCl on its germination and early development indicate that the germination of its seeds is not altered at low concentrations ($<$150 mM NaCl), but at higher concentrations, a change in the concentration of several organic compounds such as chlorophyll and proteins [19], flavonoids [20], urea and pinitol [21] is evident, which give the plant a greater response capacity to stress.

Thus, the objective of this research was to compare the germination response of seeds and initial growth of two important halophyte plants in Mexico, romerito and purslane, exposed to different salts and concentrations.

MATERIALS AND METHODS

Plant material

Seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) from collections from the agricultural areas of Tláhuac and San Gregorio Atlapulco, Xochimilco in Mexico City were used. These areas are semiarid with soils of the Terric Salic Anthrosol type [22], with a saturation extract pH between 7.69 and 8.44, which denotes its alkaline character due to the presence of CO_3^{2-} ions in addition to the electrical conductivity value of 34.19 dS m^{-1} [23]. The climate is humid temperate C(w2)(w)b(y'), considered the driest of the subhumid temperate climates with summer rains. Frosts usually begin in October and end in March. Rains occur mainly during summer and autumn; however, due to the relief of the area, there are also two climate subtypes: Cwb (plain and low slope region), and Cwc (temperate with cold winter) which corresponds to the highest areas [24].

Treatment design and experimental design

Two independent experiments were carried out for the species under study: romerito and purslane . In each essay, the main effects of two study factors were evaluated: salinity source ($CaCl_2$, NaCl, and Na_2SO_4) and salt concentration (0, 0.25, 0.50, 0.75, and 1.0 M), as well as their interaction, in a 3×5 factorial experiment with a completely randomized distribution. This resulted in 15 treatments in each experiment. The experimental unit for the treatments with romerito was a Petri dish with 20 seeds; while for purslane, it was a Petri dish with 50 seeds. Each treatment had three replicates. The salinity sources used were reagent grade Meyer® (Mexico City) brand and the solutions were prepared using distilled water.

Prior to applying treatments, groups of 20 romerito seeds and 50 purslane seeds were weighed to obtain their individual weight. Subsequently, the seeds of each experimental unit were incubated for 3 h in 3 mL of the corresponding solution according to the treatment, shaking for 1 min every hour. After the incubation time, the seeds were removed from the flasks, dried, and weighed again, then transferred to Petri dishes with medium-pore filter paper where they were placed, and 10 mL of distilled water was added. The Petri dishes were placed inside an incubation oven (Thermo Scientific, PR205075G, USA), at 28 °C and 80% RH.

Variables evaluated

Percentage increase in initial seed mass due to water absorption. It was determined in each experimental unit before and after imbibition according to Kaymakanova [10], using the formula:

$$PIMAW = \left[(W2 - W1)/W1\right] \times 100$$

where: *PIMAW* is the percentage increase in initial seed mass due to water absorption; $W1$ is the weight of the seeds in each experimental unit before imbibition and $W2$ the weight of the seeds after imbibition.

Germination percentage. The germination period was 120 h (5 d) during which time the seeds were kept at constant temperature and hydration, and seeds were considered germinated when the radicle reached a length of 2 mm [6]. The evaluation was carried out every 24 h.

Stem and radicle length. In romerito, length measurements were made 10 d after the start of germination and in Purslane 8 d after the start of germination. The difference in days was due to the fact that the romerito seeds took longer to germinate.

Statistical analysis

With the data obtained in each species, analysis of variance and comparison of means test (Duncan, $P \leq 0.05$) were performed, using the SAS® software [25].

RESULTS AND DISCUSSION

Water absorption

Halophyte species have different adaptation strategies to salinity, so the germination response can be as diverse as the micro habitats in which they develop. Hence the increase in ionic concentration in the soil is closely linked to the increase in temperature and the decrease in the percentage of humidity [26]. Therefore, most species germinate when such concentrations are reduced, either by a decrease in temperature or by the arrival of rains diluting the concentration of salts.

In this study, the results of water absorption in romerito seeds showed a high coefficient of variation (77.6%), which explains why despite the differences between sources and saline concentrations evaluated, there were no statistical differences (Table 1). The percentage of water absorption in these seeds was 26.5 with NaCl, 18.5 with Na_2SO_4, and 15.3 with $CaCl_2$. Likewise, the percentages of increase in seed weight recorded with concentrations of 0, 0.25, 0.50, 0.75, and 1 M were 10.9, 22.7, 20.8, 25.4, and 20.6, respectively; these data show the positive relationship between salt concentration and water absorption in romerito.

In purslane, neither saline sources nor the interaction of saline source and concentration had any effect on the increase in seed mass due to water absorption during imbibition (Table 1). The increases in seed mass due to water absorption were 8.2, 7.7, and 6.4% in Na_2SO_4, NaCl, and $CaCl_2$, respectively. On the contrary, after a 3-h imbibition period, only the saline concentration significantly influenced water absorption in purslane seeds (Table 1; Figure 1).

Although the effect of the saline source was not significant in either species, the trends observed in the increase in seed mass due to water absorption during the imbibition phase allow us to affirm that in romerito the order of osmotic affectation by the saline source was $CaCl_2 > Na_2SO_4 > NaCl$, while in purslane it was $CaCl_2 > NaCl > Na_2SO_4$.

Contrary to what was observed in romerito, in purslane a negative relationship was observed between the saline concentration and water absorption as shown in Figure 1, with values of 15.3% in the control without salinity and approximately 4.4% with 1 M.

Both romerito and purslane are species that grow in environmental conditions with high abiotic stress [15,21], being exposed to salinity and water stress conditions, possibly one of their adaptive strategies is the production of seeds with permeable testas which facilitate imbibition in rainy conditions, which is combined with the decrease in the saline concentration in the soil. The morphology of the seeds of *Suaeda salsa* and *S.*

Table 1. Main effects of source, salt concentration, and interaction on the increase in seed mass of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) due to water absorption during the imbibition phase.

Species	Salt source (SS)	Salt concentration (SC)	SS×SC
Suaeda mexicana	0.1463 ns	0.3749 ns	0.7710 ns
Portulaca oleracea	0.2828 ns	<0.0001 *	0.7583 ns

*Significant at 5 %; ns: non significant.

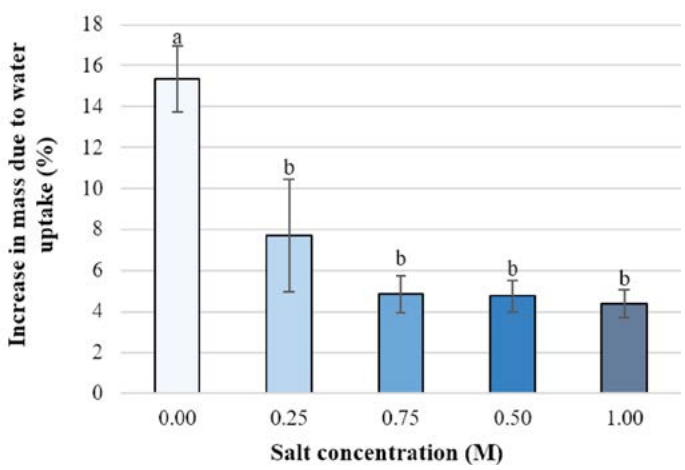

Figure 1. Water absorption in romerito (*Suaeda mexicana*) seeds after 3 h of imbibition in different salt concentrations. Means ± SD with different letters indicate significant statistical differences (Duncan, $P \leq 0.05$).

mexicana is similar, so it is possible that both species develop the same adaptive strategies to salinity in response to short periods of rain [1,8]. On the other hand, *P. oleracea*, being a C4 species that can shit its metabolism to the CAM type [27], has the capacity to better tolerate water stress and salinity, so the permeability of its testa in saline conditions constitutes a representative factor of its adaptation to the environment.

Germination percentage

The germination percentage was evaluated for 120 h at 24-h intervals. In romerito, no significant effects of the interaction between the saline source and its concentration were observed in all evaluations; while, after 48 h, the effect of the saline source was significant and the main effect of the saline concentration was significant in all evaluations. On the contrary, for purslane in all samples, both the main and interaction effects were significant in its germination percentage (Table 2).

The main effects that were significant in romerito are shown in Figure 2. NaCl caused significantly greater germination than the rest of the sources evaluated (Figure 2a). On the other hand, increasing saline doses significantly reduced germination compared to the control, with the exception of the 1 M dose, which in all the samplings followed the control in germination (Figure 2b).

Purslane was less sensitive to sodium salts (NaCl and Na_2SO_4) than to $CaCl_2$; with the latter source, no germination was recorded starting from a concentration of 0.5 M (Table 3). It was also reported that only 50% of the seeds germinated in purslane at a concentration of 106 mM NaCl [28], that with concentrations of 200 mM NaCl germination was significantly reduced [19] and that with an electrical conductivity of 25 dS m^{-1} induced by NaCl, the germination percentage was reduced by up to 60% [29].

Stem and radicle length

Table 4 shows the significance values of the study factors and their interaction on stem

Table 2. Main effects of the source, saline concentration, and their interaction on the germination percentage of seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*).

Species	Germination time (h)	Salt source (SS)	Salt concentration (SC)	SS×SC
Suaeda mexicana	24	0.2218ns	<0.0001 *	0.6491 ns
	48	0.0249 *	<0.0001 *	0.5657 ns
	72	0.0349 *	<0.0001 *	0.5298 ns
	96	0.0121 *	0.0001 *	0.4460 ns
	120	0.0419 *	0.0022 *	0.6591 ns
Portulaca oleracea	24	<0.0001 *	<0.0001 *	<0.0001 *
	48	<0.0001 *	<0.0001 *	<0.0001 *
	72	<0.0001 *	<0.0001 *	<0.0001 *
	96	<0.0001 *	<0.0001 *	<0.0001 *
	120	<0.0001 *	<0.0001 *	<0.0001 *

*Significant at 5%; ns: non significant.

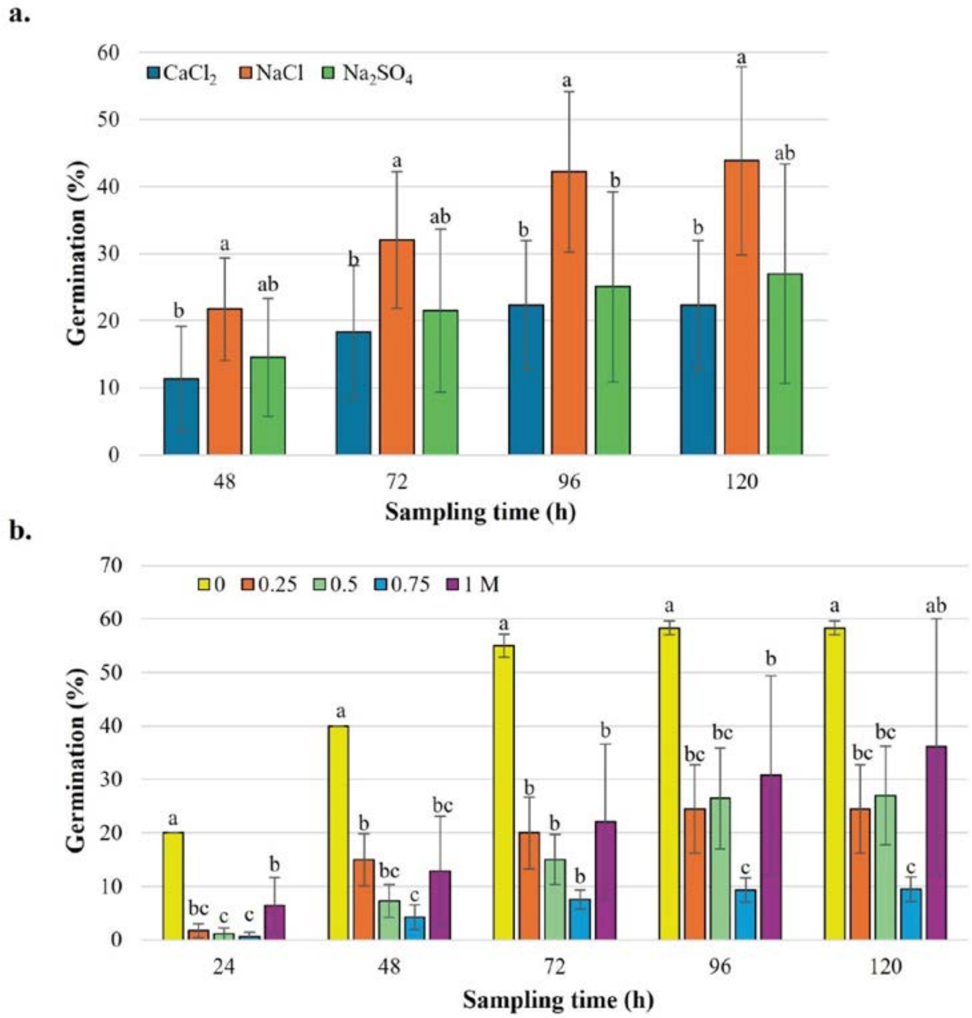

Figure 2. Germination percentage of romerito (*Suaeda mexicana*) seeds incubated for 3 h with different salt sources (a) and different salt concentrations (b). Means ± SD with a different letter in each figure and sample indicate significant statistical differences (Duncan, $P \leq 0.05$).

and radicle lengths in romerito and purslane seedlings. It should be noted that, in both species, the saline concentration had significant effects, while the single effects of the saline source were only significant in purslane. Likewise, the effect of the interaction of the study factors is significant in both species.

Stem length in purslane seedlings was observed to be the variable that experienced the most changes according to their exposure to the evaluated treatments; therefore, it is considered the more sensitive crop (Figure 3). Regarding romerito, the stem growth was statistically lower than the treatment without salinity, with the saline sources $CaCl_2$ and Na_2SO_4 from a concentration of 0.25 M, while with NaCl this occurs from the concentration of 0.5 M (Figure 3a). It should be noted that the same phenomenon was observed in purslane seedlings (Figure 3b).

Figure 4 presents the results obtained for radicle length based on the interaction of the study factors in both species. In general, it was observed that NaCl causes less inhibition of

Table 3. Germination percentage of purslane (*Portulaca oleracea*) seeds incubated for 3 h with different salt sources and concentrations.

Salt source	Salt concentration (M)	Sampling time (h)				
		24	48	72	96	120
$CaCl_2$	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	76.00±1.73 c	80.67±0.58 d	83.33±2.08 c	86.00±1.73 b	86.00±1.73 b
	0.50	0.00±0.00 g	18.00±0.00 g	27.33±4.04 f	33.33±8.02 e	39.33±9.24 e
	0.75	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g
	1.00	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g
NaCl	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	85.00±0.50 b	93.00±0.50 b	93.33±0.29 ab	93.33±0.29 ab	93.33±0.29 ab
	0.50	25.67±0.29 e	25.67±0.29 f	52.67±1.15 e	52.67±1.15 d	53.33±1.53 d
	0.75	3.33±1.44 f	24.67±0.29 f	70.67±0.58 d	93.33±1.15 ab	93.33±1.15 ab
	1.00	0.00±0.00 g	0.00±0.00 h	19.33±6.25 g	24.33±2.02 f	24.33±2.02 f
Na_2SO_4	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	86.67±1.15 ab	96.67±1.15 a	96.67±1.15 a	96.67±1.15 a	96.67±1.15 a
	0.50	48.33±1.44 d	84.00±1.73 c	88.67±1.15 bc	88.67±1.15 b	88.67±1.15 ab
	0.75	26.67±0.58 e	72.67±1.15 e	72.67±1.15 d	72.67±1.15 c	72.67±1.15 c
	1.00	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g

Means ± SD with a different letter in each sample indicate significant statistical differences (Duncan, $P \leq 0.05$).

Table 4. Main effects of the saline source and concentration and their interaction on stem and radicle lengths in romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) seedlings, from seeds incubated in different saline sources and concentrations.

Species	Variable	Salt source (SS)	Salt contentration (SC)	SS×SC
Suaeda mexicana	Stem length	0.0728 ns	<0.0001 *	0.0037 *
	Radicle length	0.0770 ns	<0.0001 *	0.0010 *
Portulaca oleracea	Stem length	<0.0001 *	<0.0001 *	<0.0001 *
	Radicle length	<0.0001 *	<0.0001 *	<0.0001 *

*Significant at 5%; ns: non significant.

radicle growth. Although in particular, it was observed that in purslane the 0.25 M NaCl treatment stimulates it; compared to the rest of the treatments (Figure 4b). In the same way, $CaCl_2$ reduces radicle growth to a greater extent from 0.25 M, compared to the treatment without salt stress for both species (Figures 4a and 4b).

One of the effects of salt stress on seedlings is the reduction of stem length (Figure 3) and radicle length (Figure 4). In this experiment, it was observed that rosemary is more sensitive to the salt sources and concentrations evaluated, given that the inhibition of the growth of the radicle and the stem is of a greater magnitude than that recorded in purslane. Since salinity affects the growth of seedlings and delays the mobilization of reserve substances, cell division tends to be suspended, damaging the hypocotyls [10].

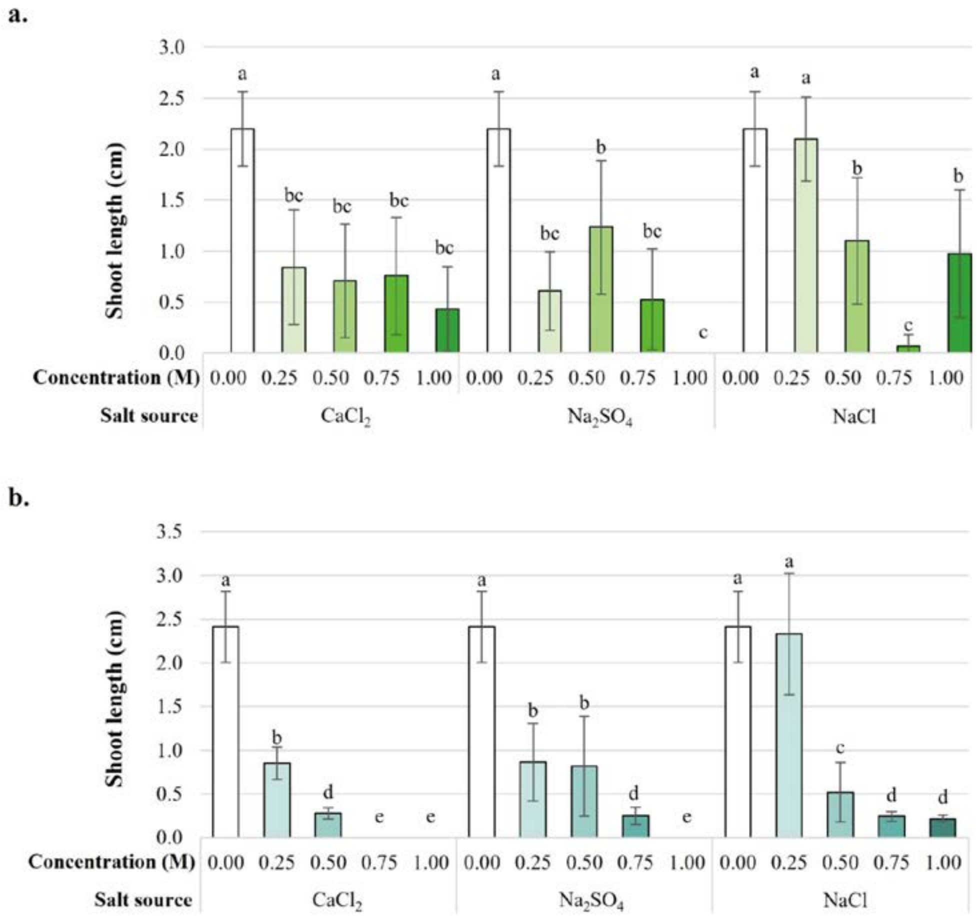

Figure 3. Stem length of romerito [*Suaeda mexicana* (a)] and purslane [(*Portulaca oleracea* (b)] plants from seeds incubated for 3 h with different salt sources and concentrations. Means ± SD with a different letter in each figure indicate significant statistical differences (Duncan, $P \leq 0.05$).

CONCLUSIONS

The germination percentage, and radicle and shoot length are germination variables that respond to the salinity source, concentration, and interaction of the source and concentration. Both romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) have different response mechanisms to the source and concentration gradient of salinity. Purslane is tolerant to sodium salts (NaCl and Na_2SO_4) but sensitive to $CaCl_2$; this is also observed in romerito, although the tolerance ranges vary from one concentration to another and from the type of salt with which it interacts. Both are species that have evolved in semiarid environments; their anatomical and morphological characteristics have allowed them to develop very efficient adaptation mechanisms, which allow their seeds to germinate and the initial growth of seedlings under constant salt concentrations.

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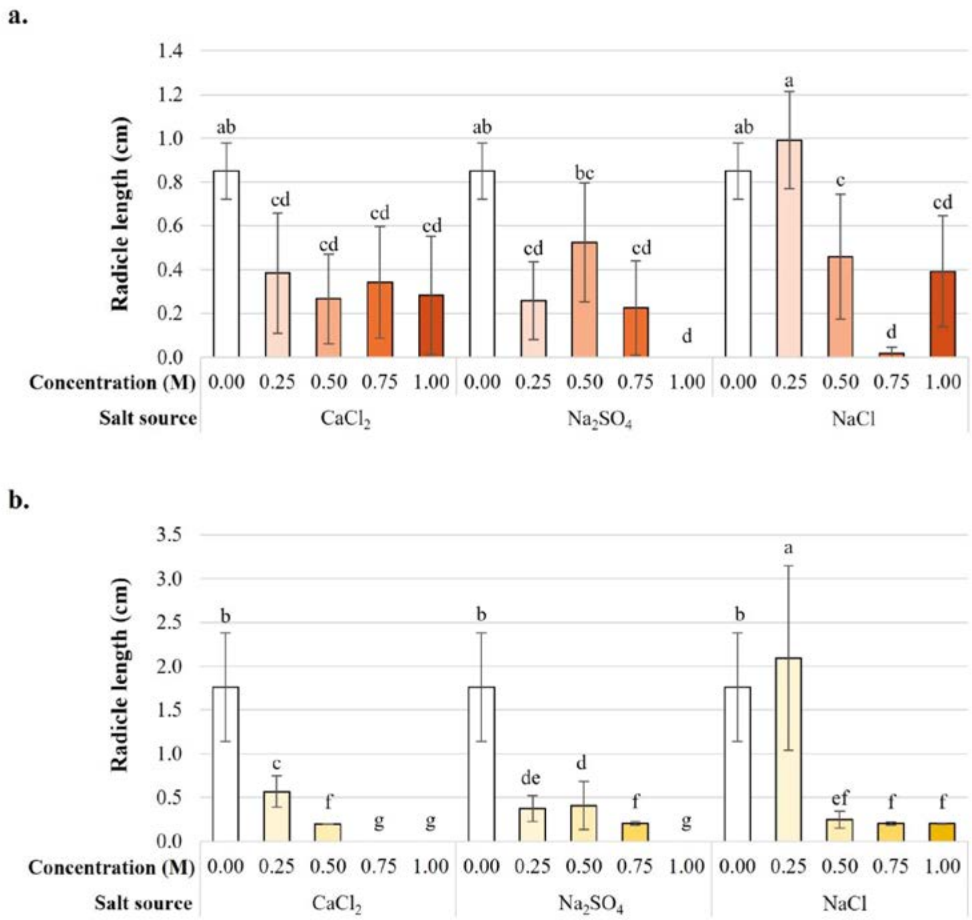

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