

Silicon increases seed weight and initial seedling growth of maize under non-stress conditions, and improves the index of velocity of germination under salt stress conditions

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Abstract

Salinity is one of the most critical factors affecting agriculture worldwide. The application of beneficial elements like silicon (Si) is one of the alternatives to mitigate its effects. In this research, we evaluated the effect of Si applied during seed imbibition on mitigating the negative effects caused by salinity during the germination and initial growth phases of maize (*Zea mays* L.) SB-308 seedlings. Seed pre-treatment during the imbibition was made with 0.0-, 1.5- and 3.0-mM Si. Afterwards, seeds that were imbibed were placed in plastic containers and treated with 0, 80, 160, and 240 mM NaCl. The evaluated concentrations of Si and NaCl gave rise to 12 treatments. Pre-treated seeds with 3 mM Si had an increase of weight after imbibition, 5.1% higher than the control. The treatments obtained from combining NaCl and Si levels did not affect the total and relative germination. The radicle length increased by 13.6% with 3 mM Si compared to the control. Conversely, it was lower with increasing salinity. These trends were observed in plant height. The interaction of the study factors produced an increase in the radicle length in the interval from 0 to 160 mM NaCl, when the Si dose was increased. However, there were no significant differences among equal levels of salinity without Si. It is concluded that Si increased the absorption of water during the imbibition and raised the index of velocity of germination under salinity, except in the dose 240 mM NaCl. Likewise, the pre-treatment of seeds with Si tends to increase radicle length under saline conditions.

Keywords: abiotic stress; beneficial elements; osmotic stress; salinity; *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is a crop that is grown in a wide variety of soil types, as well as in different climatic conditions. This species is moderately sensitive to salinity, therefore, high concentrations of salts in soils are a serious problem for the production of this and other staple crops cultivated worldwide (Farooq *et al.*, 2015).

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Salinity is one of the abiotic factors that severely restrict crop growth and compromise the development of sustainable agriculture worldwide (Zhu and Gong, 2014). In its initial phase, salt stress decreases the water absorption capacity of the root system, and accelerates the loss of water from the leaves due to the osmotic stress generated by the high accumulation of salts in the soil and in the plant. For these reasons, salinity is considered a hyperosmotic stress (Munns, 2005). In its second phase, salinity causes salt damage and this occurs in senescent leaves, which die due to the rapid increase in the concentration of salts in the cell walls or cytoplasm, when the vacuoles can no longer store excess salts (Munns, 1993). Furthermore, salinity causes adverse effects on the germination of many crop species, since it generates a decrease in the osmotic potential outside the seed, inhibiting water absorption due to the toxic effect of Na⁺ and Cl⁻ ions (Khajeh-Hosseini *et al.*, 2003).

In particular, in the border area between the southwestern United States and northern Mexico, maize is grown in arid and semi-arid environments, with high temperatures and saline soil conditions; also, in these areas, water resources are declining and saline or brackish irrigation water represents a serious problem (Pratt *et al.*, 2022). In maize, salinity stress affects plant growth, development, and production. However, plant response varies depending on the stress level, the cultivar and its stress tolerance capacity, and the phenological stage (Xue *et al.*, 2009). In germination tests it has been shown that various maize cultivars tolerate soil electrical conductivity levels of up to 10 dS m⁻¹ (Maas *et al.*, 1983). However, seedling development is drastically reduced when irrigation water is applied with salt concentrations above 1 dS m⁻¹ (Favaro *et al.*, 2007). To counteract the effects of salinity, sustainable strategies have been implemented, such as the application of biostimulants of both an organic and inorganic nature, as in the case of silicon (Si) (Dell' Aversana *et al.*, 2020).

Silicon is the second most abundant element in the Earth's crust after oxygen, yet its essentiality in plant growth and development continues to be debated, since plants differ widely in their ability to absorb Si (Sommer *et al.*, 2006; Yamaji *et al.*, 2012). The beneficial effects of Si are associated with its high accumulation in plant tissues (Carneiro *et al.*, 2010; Ma and Yamaji, 2015). Species belonging to the Poaceae family respond positively to Si supply (Epstein, 1999; Ma *et al.*, 2007), but many other dicotyledons, including species of the Fabaceae, Cucurbitaceae, and Solanaceae families, may also exhibit Si-responsiveness (Ma, 2004; Fauteux *et al.*, 2005; Trejo-Téllez *et al.*, 2020; Trejo-Téllez *et al.*, 2022). This beneficial element can mitigate the negative effects caused by biotic and abiotic stress factors, including salinity (Ma and Yamaji, 2008; Dhiman *et al.*, 2021). Si significantly increases the growth of many plant species through increased photosynthetic activity, leaf area, and chlorophyll content, and it improves chloroplast structure in salt-stressed plants (Soundararajan *et al.*, 2013). Leaf application of Si can increase the firmness of the fruit and the angle of tonality of the fruit, when it is cultivated in a sodic soil (González-Terán *et al.*, 2020). The objective of this research was to evaluate the effect of Si applied during seed imbibition on mitigating the negative effects caused by salinity during the germination and initial growth phases of SB-308 hybrid white maize seedlings.

Materials and Methods

Plant material and study conditions

In the present study, SB-308 hybrid white maize (*Zea mays* L.) seeds from the Berentsen commercial company (Celaya, Mexico) were used. This hybrid is one of the most used by producers in the State of Mexico, as it grows well in high valleys and has a low acquisition cost in the market. The present study was performed in the laboratory. During the first 48 h, seeds were placed in the plastic containers under controlled conditions using a growth chamber (Thermo Scientific Precision; Waltham, MA, USA) at a temperature of 25 °C and 50% of relative humidity in the dark. Subsequently, seeds were removed from the growth chamber and placed on the bench of the laboratory (temperature 25 to 30 °C, 60 to 65% of relative humidity, and a photoperiod of 12 h (100 μmol m⁻² s⁻¹).

Treatment design and experimental design

A 3 × 4 factorial experiment was established, where the study factors were Si and salinity induced by NaCl. The Si source was SiO₂ (Sigma-Aldrich®; Darmstadt, Germany) and the levels evaluated were 0.0, 1.5, and 3.0 mM Si. The doses of Si were based on the results of previous research carried out in our work group with Si and salinity (González-Terán *et al.*, 2020; Trejo-Téllez *et al.*, 2020). The different Si treatments were evaluated during the imbibition phase of the seeds, which lasted 12 h. Salinity was induced with NaCl (Meyer®; Mexico City); applied at 0, 80, 160, and 240 mM, added after the imbibition phase. Sodium chloride (NaCl) doses were defined based on the results obtained by Ahmed *et al.* (2017), who concluded that 80 mM NaCl concentration was found acceptable for germination and early seedling growth of maize. The combination of Si and NaCl levels resulted in 12 treatments, each having four replicates. The experimental unit consisted of a 12 x 11 x 7 cm polyethylene (PET) box with 10 seeds on filter paper. The number of seeds per experimental unit was defined based on the size of the boxes, to ensure sufficient space for germination and initial growth of the seedlings.

Application of treatments

The seeds were weighed and divided into three groups to be subjected to imbibition in the different SiO₂ concentrations for 12 h. Given the low solubility of SiO₂, during imbibition the SiO₂ suspensions were kept on racks with magnetic stirring (Corning, PC-620D; Corning, NY, USA). Subsequently, the imbibed seeds were placed in groups of 10 on filter paper inside polyethylene boxes, which constituted the experimental unit.

Each experimental unit received 25 mL of the solutions corresponding to the NaCl treatments and introduced into a germination chamber (Thermo Scientific Precision), at 25 °C in the dark, with 50% of relative humidity. After 48 h, the boxes were removed from the germination chamber and kept at room temperature on the bench of the laboratory.

Evaluated variables

After the imbibition period, the seeds were weighed again to estimate their percentage increase in weight. Germination evaluations were performed every 24 h for 5 days (120 h) and the following variables were evaluated:

Seed germination was recorded every 24 h and until its value was constant in each treatment. The seeds were considered germinated when the radicle emerged approximately 2 mm (Sharma and Sharma, 2010).

The total germination percentage (TG) considered the maximum germination value reached in germinated seeds, using the following formula (Al-Mudaris, 1998):

$$TG = \left[\frac{(\text{Germinated seeds})}{(\text{Total seeds})} \times 100 \right]$$

The index of velocity of germination (IVG) was estimated using the following formula, described by Maguire (1962):

$$IVG = [\text{Number of germinated seeds per box}/A_1T_1+A_2T_2+\dots+A_5T_5]$$

Where A is the number of seeds emerged in a particular number of days, the numbers 1, 2, ... and 5 are the respective number of seeds germinated for each respective day after the start of incubation.

The relative germination percentage (RG) was calculated using the following formula (Tam and Tiquia, 1994):

$$RG = \left[\frac{(\text{Seeds germinated in the treatment})}{(\text{Seeds germinated in the control})} \right] \times 100$$

The control is the treatment without salinity and without Si. After 5 days, shoot height and root length were measured using an image analysis program, “ImageJ” (ImageJ, 2017).

Data analysis

With the results obtained, analyses of variance and Duncan’s means comparison tests (Multiple range test) (0.05) were performed with the SAS software (SAS Institute, 2011).

Results and Discussion

The first phase of seed germination is imbibition, which comprises the initial process of water absorption by the seed (Jorgensen and Chesser, 2000). At the end of the imbibition period, the percentage of weight gain of the seeds in the 3 mM Si treatment was higher than that registered in seeds of the control treatment (Figure 1). The increased water absorption during imbibition is particularly due to the activation of aquaporins (intrinsic membrane proteins), which positively regulate the entry of water into the cell (Liu *et al.*, 2013). Si can participate in hydraulic signalling through the expression of aquaporins, achieving greater efficiency in water use (Rios *et al.*, 2017).

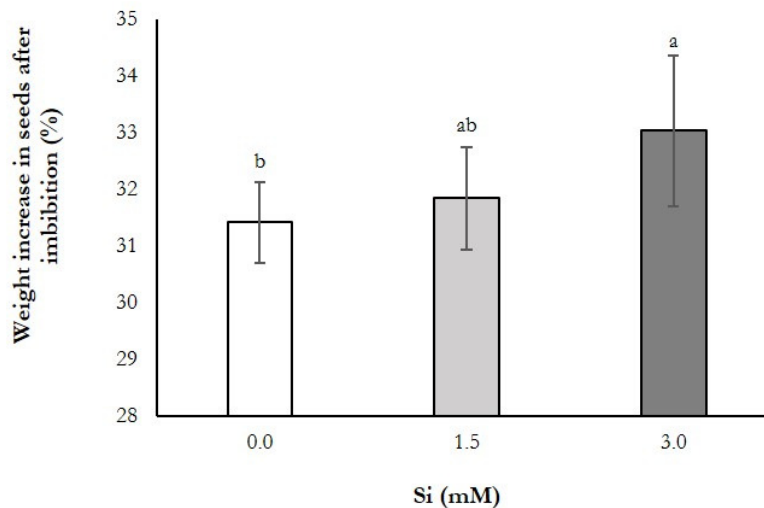


Figure 1. Percentage increase in the weight of maize (*Zea mays* L.) seeds after 12 h of imbibition in solutions with different Si concentrations

Means \pm SD with different letters is statistically different (Duncan, $\alpha = 0.05$)

Percentage of total germination (TG) and relative germination (RG)

The percentages of total germination (TG) and relative germination (RG) were not affected by the evaluated levels of Si and NaCl (Table 1). In tomato (*Solanum lycopersicum* L.) under water deficit stress simulated by 10% (w/v) polyethylene glycol (PEG-6000), the application of 0.5 mM $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ did not cause significant effects on the evaluated treatments (Shi *et al.*, 2014), which coincides with the results obtained here. On the other hand, the evaluation of six levels of NaCl (0, 50, 100, 200, 400, and 600 mM) in three plants tolerant to salinity (*Limonium sinense* Kuntze, *Glycine soja* Sieb., and *Sorghum sudanense* Stapf.), recorded a negative relationship between germination and salinity level (50, 100, 200, 400 and 800 mM) (Li, 2008), which differs from what was observed in maize in this study. Similarly, another study reported a decrease in the total

germination percentage of *Momordica charantia* seeds as the NaCl level increased; and within the salinity treatments, an increase in TG was observed with the 2 mM Si dose (Wang *et al.*, 2010).

Table 1. Total and relative germination of maize (*Zea mays* L.) seeds in response to the main effects of Si treatment during the imbibition phase and of NaCl during the germination phase

Si (mM)	Total germination (%)	Relative germination (%)
0	99.4 ± 1.3 a	101.9 ± 1.3 a
1.5	99.4 ± 1.3 a	101.9 ± 1.3 a
3.0	97.5 ± 2.2 a	100.0 ± 2.3 a
NaCl (mM)	Total germination (%)	Relative germination (%)
0	98.3 ± 1.9 a	100.9 ± 2.0 a
80	100.0 ± 0.0 a	102.6 ± 0.1 a
160	98.3 ± 1.9 a	100.9 ± 2.0 a
240	98.3 ± 1.9 a	100.9 ± 2.0 a

Means ± SD with the same letter in each study factor and variable, are not statistically different (Duncan, $\alpha = 0.05$)

The treatments resulting from the combination of NaCl and Si levels had no effect on total germination and relative germination (Table 2). In *Glycyrrhiza uralensis*, the positive effects of Si were found to be dependent on the salinity level (Zhang *et al.*, 2015). It has also been observed that the benefits of Si application under salt stress are observed at different doses depending on the plant species (Ali *et al.*, 2009).

Table 2. Total and relative germination of maize (*Zea mays* L.) seeds in response to the interaction effects of Si treatment during the imbibition phase and of NaCl during the germination phase

Si (mM)	NaCl (mM)	Total germination (%)	Relative germination (%)
0	0	97.5 ± 2.5 a	100.0 ± 2.6 a
	80	100.0 ± 0.0 a	102.6 ± 0.0 a
	160	100.0 ± 0.0 a	102.6 ± 0.0 a
	240	100.0 ± 0.0 a	102.6 ± 0.0 a
1.5	0	97.5 ± 2.5 a	100.0 ± 2.6 a
	80	100.0 ± 0.0 a	102.6 ± 0.0 a
	160	100.0 ± 0.0 a	102.6 ± 0.0 a
	240	100.0 ± 0.0 a	102.6 ± 0.0 a
3.0	0	100.0 ± 0.0 a	102.6 ± 0.0 a
	80	100.0 ± 0.0 a	102.6 ± 0.0 a
	160	95.0 ± 2.9 a	97.4 ± 2.9 a
	240	95.0 ± 2.9 a	97.4 ± 2.9 a

Means ± SD of the interaction factors with the same letter in each column are not statistically different (Duncan, $\alpha = 0.05$)

Index of velocity of germination (IVG)

The IVG did not have significant effects in the different doses of Si evaluated (Figure 2A); while it was reduced as the NaCl concentration increased (Figure 2B). Depending on the level of salinity, delays in the germination of maize seeds are observed, which is attributed to the increase in osmotic pressure caused by salinity, which in turn delays water absorption (Aliu *et al.*, 2015; Machado and Serralheiro, 2017).

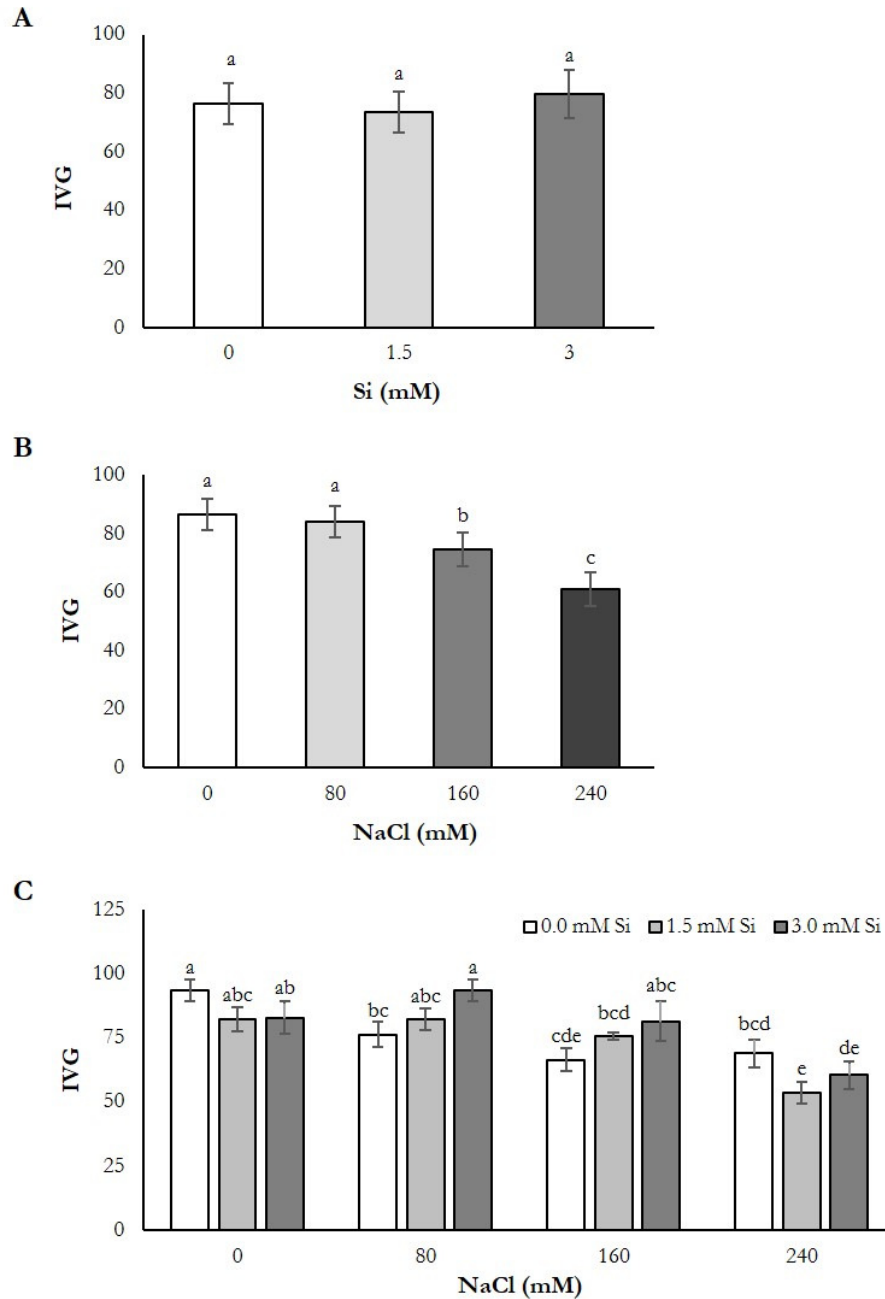


Figure 2. Index of velocity of germination (IVG) of maize (*Zea mays* L.) seeds imbibed with different concentrations of silicon for 12 h (A), NaCl during germination (B), and both (Si × NaCl) (C) Means ± SD with different letters in each figure are statistically different (Duncan, $\alpha = 0.05$)

At high salinity levels (80 and 160 mM NaCl), the IVG was higher in seeds pre-treated with 3 mM Si, compared to those not treated with Si and those treated with 1.5 mM Si (Figure 2C). These results show the regulation that Si exerts on the expression of aquaporins, which increase water absorption in the plant (Liu *et al.*, 2013). This increase in Si-mediated water absorption mitigates osmotic stress caused by salt stress (Sidari *et al.*, 2007).

Radicle length and shoot height

Seedlings from seeds treated with 3 mM Si had a root length 13.6% greater than those from the control. However, this difference was significant even though the high variability in this parameter between replicates, probably due to the complexity involved in *in vivo* measurement of small roots under these conditions (Figure 3A). Contrary to the trend observed here, in *Borage officinalis* L. silicon doses greater than 1.5 mM reduced root length (Torabi *et al.*, 2012).

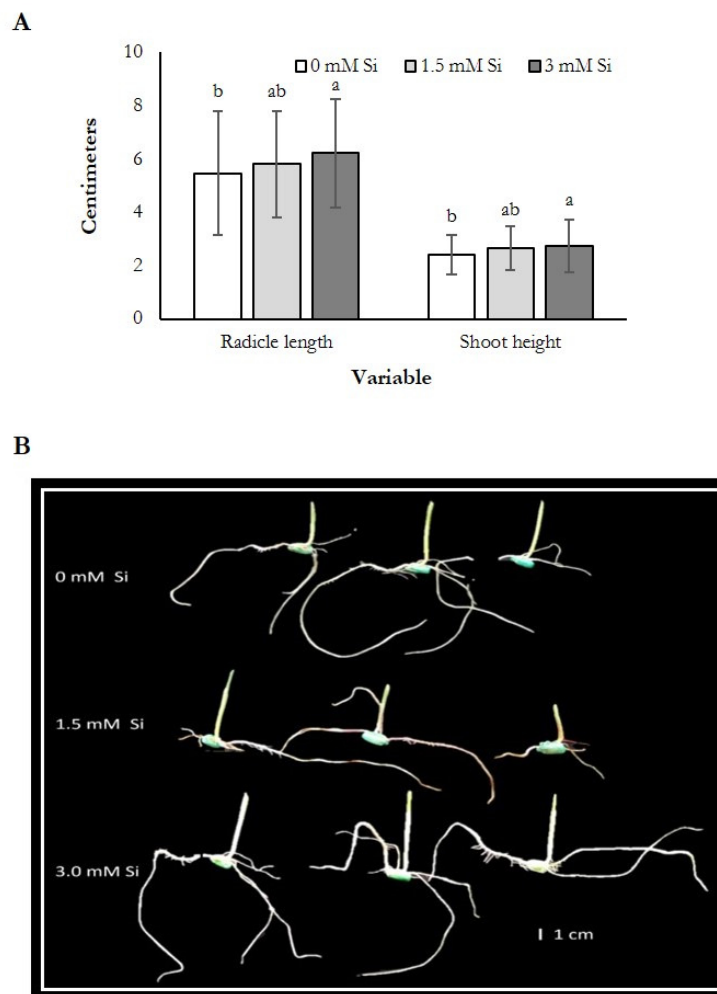


Figure 3. Effects of Si pretreatment on maize (*Zea mays* L.) seeds on early seedling growth. (A) Radicle length and shoot height from seeds treated for 12 h in the imbibition phase with different Si doses. (B) Appearance of 5d maize seedlings, from seeds treated for 12 h in the imbibition phase with different Si doses

Means \pm SD with different letters in each variable are statistically different (Duncan, $\alpha = 0.05$)

Likewise, Figure 3A shows that shoot height was positively related to Si dose. Treatments with 1.5 and 3.0 mM Si increased height by 9.5 and 13.7%, respectively, compared to the control. Similarly, in wheat plants (*Triticum aestivum* L.) treated with 52 mM H₄SiO₄, an increase in plant height was also observed (Abro *et al.*, 2009). In soybean [*Glycine max* (L.) Merrill], the application of 30, 60, 90, and 120 g Si 100 kg⁻¹ seed, increased the weight of dry shoot biomass in an evaluated cultivar (de Carvalho *et al.*, 2019). However, in coffee (*Coffea arabica* L.) seedlings, the application of 2 mmol Si L⁻¹ for 33 d significantly reduced the dry biomass of shoots and leaves, compared to the control (Cunha *et al.*, 2012).

Figure 3B shows the positive effect that the imbibition of seeds with Si had on the growth of maize seedlings. In rice (*Oryza sativa* L.), Si increased seedling height, root length, as well as fresh and dry biomass weights of shoots and roots during initial growth (Ramírez-Olvera *et al.*, 2019).

Radicle length is negatively related to NaCl levels (Figure 4A). The reduction in the radicle length of maize seedlings under salt stress conditions is related to various physiological factors, such as osmotic stress and ion toxicity, caused by the accumulation of salts in the plant (Sidari *et al.*, 2007). In maize, a significant decrease in radicle length has been reported when increasing salt concentrations from 25 to 200 mM NaCl (Sozharajan and Natarajan, 2014). Likewise, reductions of 10.6, 20.0, and 90.6% have been observed in the dry biomass of roots of maize seedlings treated with 20, 40, and 320 mM NaCl, respectively, compared to the control (Khodarahmpour *et al.*, 2012). In tomato cv. BINA-10, the radicle growth evaluated after 5, 7, 9, and 11 days of treatment was significantly reduced, and was totally inhibited with applications of 150 mM NaCl (Rofekuggaman *et al.*, 2020).

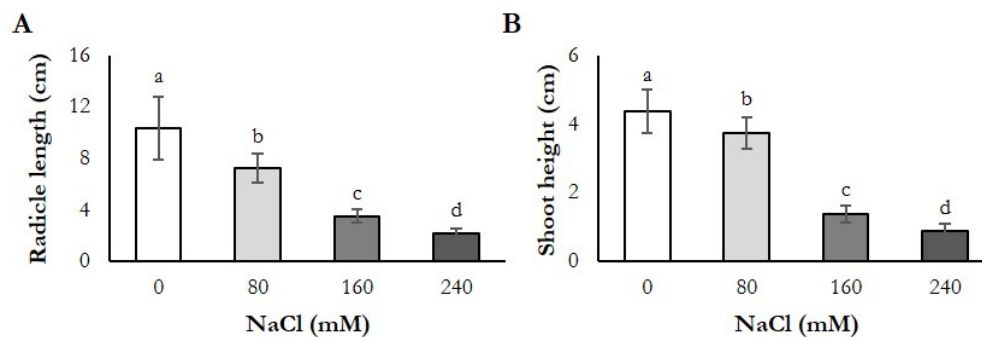


Figure 4. Effects of NaCl treatment on radicle length (A) and shoot height (B) of maize (*Zea mays* L.) seedlings

Means \pm SD with different letters in each figure are statistically different (Duncan, $\alpha = 0.05$)

Shoot growth was inhibited by NaCl, with height decreases of 14.4, 68.3, and 80.1% with doses of 80, 160, and 240 mM NaCl, compared to the control (Figure 4B). The reduction in height of maize seedlings under salinity conditions is attributed to low water availability, sodium chloride toxicity, or a combination of both factors (Munns and Teser, 2008). Evaluations of the effect of salinity on maize crops have shown a significant decrease in seedling height under salt stress, which is why it is argued that maize is a salinity-sensitive crop (Farsiani and Ghobadi, 2009). Hassan *et al.* (2018) obtained a reduction in shoot length in the BARI Maize 5 variety with high salinity (200 mM). Likewise, a decrease in seedling length in summer savoury (*Satureja hortensis*) is reported with the application of 160 mM NaCl (Saberali and Moradi, 2019). Salinity-induced growth reduction in maize is due to inhibition of the initiation, expansion, and growth of leaf internodes (Farooq *et al.*, 2015).

The length of the radicle was greater as the Si dose increased from 0 to 160 mM NaCl. However, there are no statistically significant differences between equal salinity levels without Si (Figure 5A). In soybean (*Glycine max* L.) Si was found to increase the size of the radicle, but as the salinity dose increases, the growth of the radicle is inhibited (Lee *et al.*, 2010).

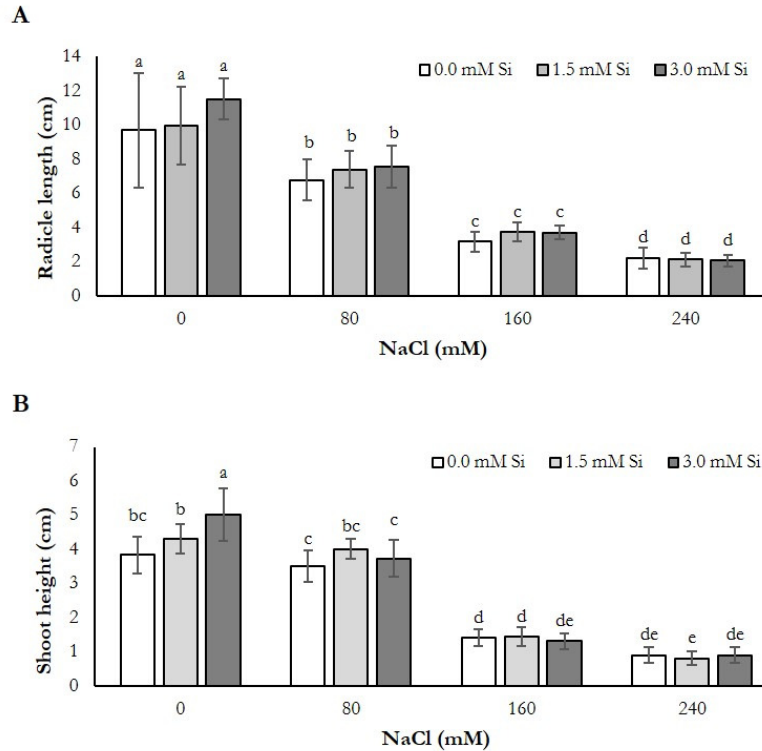


Figure 5. Effects of the interaction of study factors Si and NaCl on radicle length (A) and shoot height (B) of maize (*Zea mays* L.) seedlings. Means \pm SD with different letters in each figure are statistically different (Duncan, $\alpha = 0.05$)

The highest seedling height was recorded in the treatment with 3 mM Si without salt stress, while the lowest height was recorded in the three treatments with 160 and 240 mM NaCl, regardless of the Si dose (Figure 5B). The increase in seedling height due to Si is attributed to biochemical and morphological changes in the plant, when it is not subjected to a stress factor (Epstein, 1999). In tomato, a decrease in shoot size was recorded under the influence of salinity and silicon. These results coincide with those obtained here, since the joint effect of Si and NaCl was not positive for seedling height (Haghighi *et al.*, 2012).

Higher plants display different responsiveness to Si treatments, and the accumulation patterns of Si in plant tissues greatly vary among species. In roots, Si is taken up by influx Lsi channels, and translocated within the plant by efflux Lsi channels. Additionally, siliplant1 proteins (Slp1) are involved in the process of biosilicification. The abundance and enzymatic activity of Lsi and Slp1 proteins may ultimately determine Si metabolism and responsiveness to Si (Gómez-Merino *et al.*, 2020; Trejo-Télez *et al.*, 2022). Hence, we are currently developing novel approaches to unveil the biochemical and molecular machinery that may explain the responsiveness of maize to Si under saline conditions.

Conclusions

In this research, a beneficial effect of silicon application on seed germination and initial growth of maize (*Zea mays* L.) seedlings was proven. The imbibition of seeds with 3 mM Si increased the percentage of seed weight significantly compared to the control. In the absence of salinity, Si promoted radicle growth. Salinity significantly delayed germination. The application of NaCl had negative effects on the initial growth of maize seedlings. In the interaction of study factors, the fact stands out that in seeds treated with 80 mM NaCl, Si at a dose of 3 mM increases the index of velocity of germination.

Authors' Contributions

Conceptualization, L.I.T.T. and F.C.G.M.; methodology, D.N.R. and M.G.P.S.; validation, D.N.R. and G.A.G.; formal analysis, L.I.T.T. and O.T.S.; research, D.N.R.; resources, L.I.T.T., F.C.G.M. and G.A.G.; writing - original draft preparation, D.N.R. and L.I.T.T.; writing - proofreading and editing, F.C.G.M., M.G.P.S. and O.T.S.; supervision, L.I.T.T. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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