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Response of two pepper species (*Capsicum chinense* Jacq. and *Capsicum frutescens* L.) to salt stress at germination stage in Northeast Brazil

Respuesta de dos especies de ají (*Capsicum chinense* Jacq. and *Capsicum frutescens* L.) al estrés por salinidad en la etapa de germinación en el Nordeste de Brasil

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ARTICLE DATA

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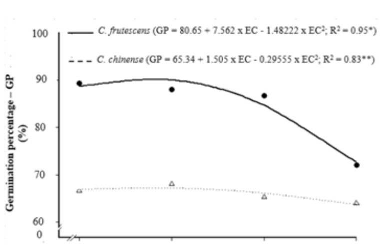


Germination percentage of two pepper species subjected to salt stress.

ABSTRACT

Salinity is one of the striking problems in agricultural production in many parts of the world. Seed germination and seedling growth are two critical stages for the establishment of crops generally most sensitive to salt stress. The present study aimed at evaluating the germination and initial growth of pepper seedlings produced from seeds under different soaking times in NaCl solutions. The experiment was carried out in a completely randomized design, in a 2 × 4 × 5 factorial scheme using two pepper species (*Capsicum chinense* Jacq. and *Capsicum frutescens* L.), four levels of electrical conductivity (EC) of solutions (1.5, 3.0, 4.5, and 6.0dS m^{-1}) and five times of seed soaking in the solutions (2, 4, 6, 8, and 10h), with three replications. The traits evaluated were the number of germinated seedlings, percentage of germination, seedling height, and root length. The results showed that *C. frutescens* pepper was more tolerant to different times of soaking in saline solutions prepared with NaCl compared to *C. chinense*. Thus, the results suggest that depending on the pepper species, it is recommended to use seeds primed in saline solutions with salinity levels compatible with those under field conditions (in saline soils and/or irrigation with saline waters).

Keywords: Electrical conductivity; cultivation establishment; initial growth; seedling emergence; salt stress.

RESUMEN

La salinidad es un problema de interés en la producción agrícola en muchas partes del mundo. La germinación de semillas y el crecimiento inicial de las plántulas son dos aspectos críticos para el establecimiento de cultivos, especialmente los sensibles al estrés por salinidad. El presente estudio tuvo como objetivo evaluar la germinación y crecimiento inicial de las plántulas de ají producidas a partir de semillas en diferentes tiempos de imbibición en soluciones de NaCl. El experimento se realizó en un diseño completamente al azar, en un arreglo trifactorial de 2 × 4 × 5 utilizando dos especies de ají (*Capsicum chinense* Jacq. y *Capsicum frutescens* L.), cuatro niveles de conductividad eléctrica (CE) de soluciones (1,5; 3,0; 4,5 y 6,0dS m^{-1}) y cinco tiempos de imbibición de semillas en las soluciones (2, 4, 6, 8 y 10h), con tres repeticiones. Las variables evaluadas fueron el número de plántulas germinadas, porcentaje de germinación, altura de las plántulas y longitud de la raíz. Los resultados mostraron que *C. frutescens* fue más tolerante en soluciones salinas preparadas con NaCl en diferentes tiempos de imbibición en comparación con *C. chinense*. Así, los resultados sugieren que dependiendo de la variedad de ají, se recomienda el uso de semillas impregnadas en solución salina con niveles de salinidad compatibles con los de campo (en suelos salinos y/o riego con aguas salinas).

Palabras clave: Conductividad eléctrica; establecimiento de cultivos; crecimiento inicial; emergencia de plántulas; estrés salino.

INTRODUCTION

Pepper plants species are a perennial shrub, belonging to the genus *Capsicum*, originated in Topical America. It is one of the oldest plant species (Dinu *et al*., 2018) and is usually cultivated worldwide for its adaptation to different climatic regions and its wide variety of forms, sizes, colors, and levels of pungency in the fruit (Bojórquez-Quintal *et al*., 2014; Pérez-Gutiérrez *et al*., 2017). There are about 30 species of this genus, six of which are more known and widely cultivated, as *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum pubescens* Ruiz & Pav. and *Capsicum praetermissum* Heiser & P.G.Sm (Zamljen *et al*., 2020).

Pepper crops have significant economic importance in Brazilian agribusiness and have undergone major transformations seeking to meet the demands of the consumer market (Barroca *et al*., 2015). Peppers grow in virtually all Brazilian regions and are one of the best examples of family farming and small farmer-agroindustry integration because more than 100 wages per hectare are required to harvest (Caldas *et al*., 2016). Brazilian Northeast has potential for pepper crops due to its favorable soils and climate (Oliveira *et al*., 2014). There, the most used species are *C. chinense* and *C. frutescens*.

In some regions, soil and water salinity are a growing problem for agriculture under irrigation (Oliveira *et al*., 2019; Yuan *et al*., 2019; Jan *et al*., 2020). Information about the cultivation of pepper varieties is scarce, especially related to salt tolerance (Sá *et al*., 2019).

Salinity limits plant growth through the negative effect of osmotic potential (Steiner *et al*., 2019; Vendruscolo and Seleguini, 2020). The increase of salt concentration in the root zone makes water less available to plants (Shihab and Hamza, 2020). This leads to changes in water relations in plant cells (García-Caparrós and Lao, 2018) and accumulation of ions (predominantly Na^+ and/or Cl^-) at toxic levels (Hossain *et al*., 2015; Rady *et al*., 2018), which results in nutritional problems (Ceccarini *et al*., 2019; Shahzad *et al*., 2019; Talhouni *et al*., 2019).

Plants' response to salt stress depend on several factors, varying among species/cultivars (Fu *et al*., 2018; Öner and Kirli, 2018), organs, and growth stages (Kalhor *et al*., 2018; Tanveer *et al*., 2018). For several plant species, the mechanisms of salt tolerance are widely reported (Kodikara *et al*., 2018). However, for seed germination and seedling growth, there are several gaps because these stages are the most sensitive to salinity (Oliveira and Steiner, 2017; Aloui *et al*., 2017; Chichanoski *et al*., 2019; Leal *et al*., 2019; Farooq *et al*., 2020).

Reduced seedling emergence causes an uneven establishment of the stand that results in crop yield reduction. In this context, several studies show that pre-treatment of seeds with different products is a simple and economical approach to improve plant tolerance to salt stress (Andrade and Laurentin, 2015; Mekawy *et al.*, 2018; Samsampour *et al.*, 2018; Silva *et al.*, 2018; Silva *et al.*, 2019; Arif *et al.*, 2019; Wu *et al.*, 2019; Feghhenabi *et al.*, 2020).

The techniques used in seed pre-treatment include physiological priming (Lopes *et al.*, 2019), which consists in controlled hydration of the seeds to a certain level, enabling the occurrence of the initial stages of germination (Lopez del Egido *et al.*, 2018; Ebert and Wu, 2019). Osmopriming is one of the main techniques for seed priming (Garruña-Hernández *et al.*, 2014).

It refers to the immersion of seeds in saline solutions (Zhang *et al.*, 2018) such as $Ca(NO_3)_2$ in *Citrullus lanatus* in Brazil (Barbosa *et al.*, 2016); NaCl in *Capsicum annuum* L. in India (Yadav *et al.*, 2011) and *Solanum lycopersicum* L. in Bangladesh (Rashed *et al.*, 2016); KNO_3 in *Triticum aestivum* L. in Brazil (Steiner *et al.*, 2018); KCl in *Phaseolus vulgaris* L. in Brazil (Oliveira *et al.*, 2019); and K_2SiO_3 in *Triticum aestivum* L. in Iran (Feghhenabi *et al.*, 2020).

The time duration of seed immersion in saline solutions is important; therefore, the objective of this study was to evaluate the germination and initial growth of pepper under different times of seed imbibition in saline solutions prepared with NaCl. It is expected the results of the study will bring knowledge about the best growing conditions concerning the identification of salt tolerance of pepper and exposure time to salts. The findings may be relevant for the places where the use of brackish water is necessary since the economic and agricultural potential and uses of the pepper plant are already known.

MATERIAL AND METHODS

Experiment location and seeds material. The present study was conducted at the Laboratory of Soil, Water and Plant Tissue of the Federal Institute of Education, Science, and Technology of Ceará, Iguatu, Ceará, Brazil. The tests were conducted under laboratory conditions, at a temperature of 25°C, 8-hour photoperiod, and 60% relative humidity. The seeds of pepper varieties used were: pepper 'De Cheiro – Lupita' (*Capsicum chinense* Jacq.) (guaranteed germination percentage of 93% - Feltrin® Sementes, Farroupilha, Brazil) and pepper 'Malagueta – De Cayenne' (*Capsicum frutescens* L.) (guaranteed germination percentage of 95% - Isla Sementes Ltda., Porto Alegre, Brazil). These varieties were the only ones found on the local market.

Experimental design and treatments. The experimental design used was completely randomized, with three replicates, each one with five seeds. The treatments were arranged in a 2 × 4 × 5 factorial scheme: two varieties of pepper (*C. chinense* and *C. frutescens*), four levels of electrical conductivity of solutions - EC (1.5, 3.0, 4.5, and 6.0dS m^{-1}), and five times of seed soaking in saline solutions (2, 4, 6, 8, and 10h), totaling 120 experimental units (Table 1). The salinity level of 1.5dS m^{-1} was adopted as a control based on the results of a previous study (Sá *et al.*, 2019), where the species *C. chinense* was considered as tolerant in its initial development up to EC of 1.55dS m^{-1}.

Table 1. Description of treatments used.

Pepper species	Solutions EC (dS m^{-1})	Times of seed soaking (hours)				
C. chinense[1]	1.5 (control)*	2[1]	4	6	8	10
	3.0	2	4	6	8	10
	4.5	2	4	6	8	10
	6.0	2	4	6	8	10
C. frutescens	1.5 (control)	2	4	6	8	10
	3.0	2	4	6	8	10
	4.5	2	4	6	8	10
	6.0	2	4	6	8	10

* Example of an experimental unit consisting of five seeds.

Preparation of solutions and germination test. The solutions were prepared in distilled water by adding sodium chloride (NaCl). After the dissolution of salt for each treatment, the EC values were measured at 25^{0}C with the aid of a benchtop conductivity meter.

A germination test was performed in disposable cups with a capacity of 250mL. Due to the reduced availability of seeds, only five seeds of each pepper variety were placed in each cup containing 25 mL of respective saline solution without aeration, covered with plastic film, and stored under controlled conditions (20-25^{0}C temperature and 60% relative humidity). The seeds remained under these conditions until the end of the established time. Afterward, the seeds were removed from the solution and immediately washed with distilled water.

Subsequently, the seeds were placed on germination paper moistened with distilled water and put in a germination chamber with a temperature regulated at 20-25^{0}C and 60% relative humidity.

Variables evaluated. After installing the assay on germination paper, the number of germinated seedlings on the fourth and twelfth day were computed. Seeds with radicle emission of 2mm were considered as germinated according to Demir *et al*. (2008) and Eskandari and Alizadeh-Amraie, 2014).

The observations of radicle emission were performed daily until the twelfth day after sowing on germination paper. The number of germinated seedlings per plot was computed, and subsequently, the germination percentage was calculated.

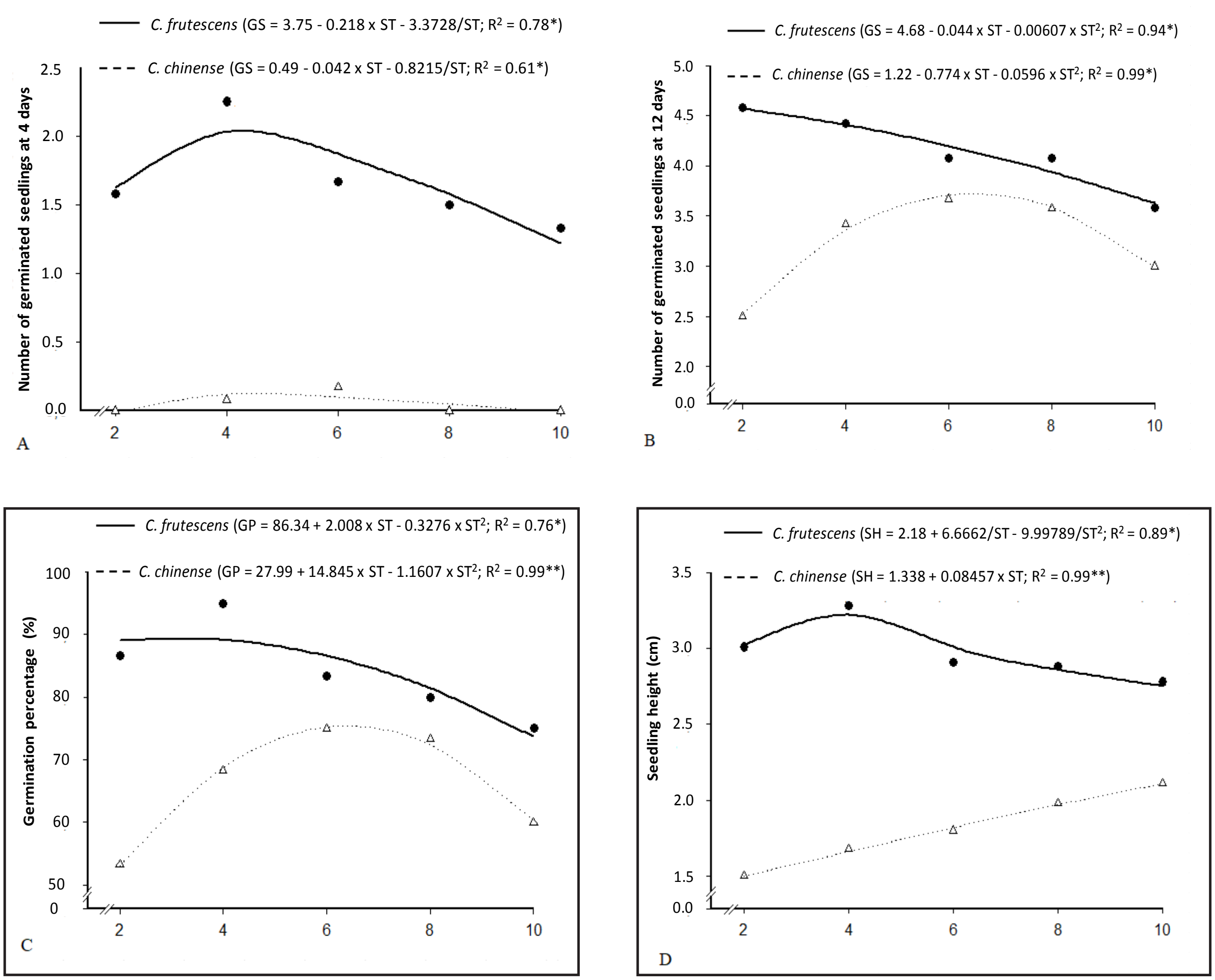
At the end of the assay (twelve days after sowing), seedling height (hypocotyl + primary root) and root length were determined using a millimeter ruler according to the norms established in the *Rules for Seed Testing* (Ministério da Agricultura Pecuária e Abastecimento, 2009) and adopted by other authors (Marinho *et al*., 2019; Nunes *et al*., 2019).

Statistical analysis. The data were subjected to analysis of variance by F test and regression analysis, adjusting different types of mathematical models according to the response of the variable.

RESULTS AND DISCUSSION

There were significant effects of the isolated factors on the number of seedlings germinated on the fourth and twelfth day, germination percentage, seedling height, and root length. As for the interactions, there were significant effects between pepper varieties and soaking times for all evaluated variables and between varieties and levels of electrical conductivity (EC) of the solutions, except for root length. Therefore, follow-up tests were conducted for the respective variables.

In general, for the interactions between varieties and soaking times (Figure 1) or with solutions salinity (Figure 2), higher rates of germination and initial growth were observed in *C. frutescens* pepper seedlings compared to the *C. chinense* pepper. These results corroborate the study conducted by Mavi (2018), who reported a higher percentage of germination for *C. frutescens* in comparison to the *C. chinense* under a controlled environment (inside a growing chamber at 23 ± 2°C) in Antakya, Turkey.



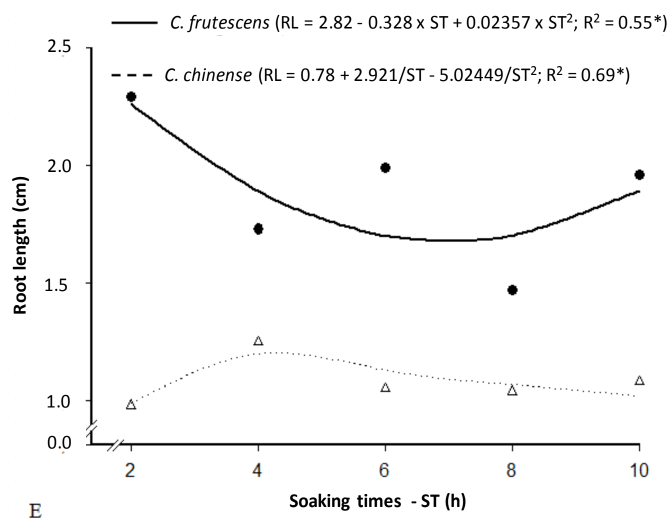


Figure 1. Follow-up analyses of the interaction between pepper varieties and soaking times in the solutions for the number of germinated seedlings - GS at 4 days (A) and 12 days (B), germination percentage - GP (C), seedlings height - SH (D), and root length - RL (E). *: significant at the P < 0.05 level by t-test; **: significant at the P < 0.01 level by t-test.

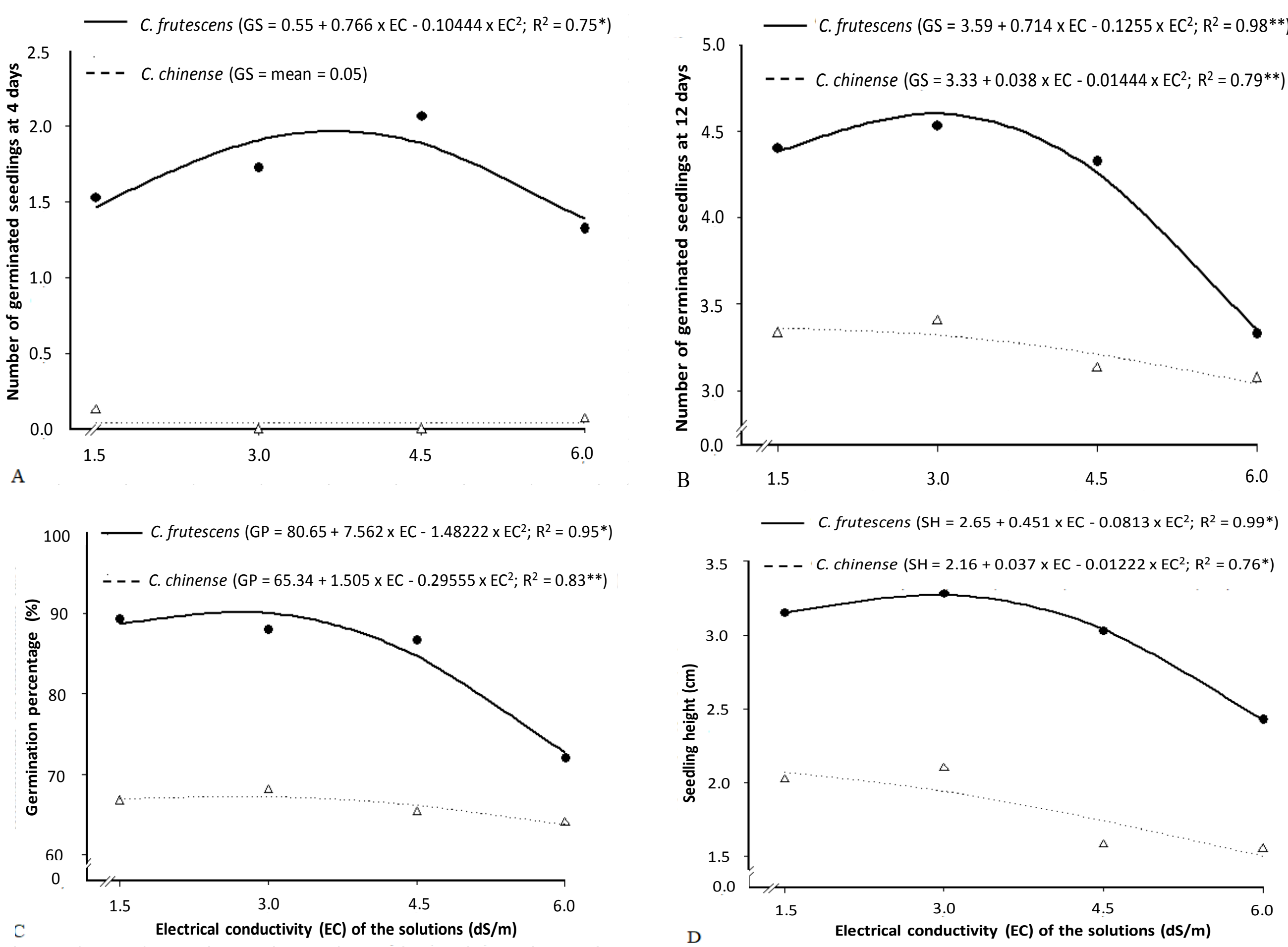


Figure 2. Follow-up analyses of the interaction between pepper varieties and electrical conductivity of the solutions for the number of germinated seedlings - GS at 4 days (A) and 12 days (B), germination percentage - GP (C), and seedlings height - SH (D). *: significant at the P < 0.05 level by t-test; **: significant at the P < 0.01 level by t-test.

In the follow-up analysis between pepper varieties and seed soaking times in saline solutions, *C. frutescens* pepper in the first count (on the fourth day) had the highest number of germinated seedlings (2.03) when its seeds were soaked in the solution for up to 4.3h. The means obtained with soaking times of 2, 6, and 8h were similar (1.6-1.9 seedling) (Figure 1A). In the second count on the twelfth day (Figure 1B), the number of germinated seedlings was lower for times greater than 2h (mean of 4.57 seedlings). However, from the soaking time of 8h, the number was less than 4 seedlings, and there was a reduction of approximately 21% under the soaking time of 10h in comparison to that of 2h. For *C. chinense* pepper, there was no germination in the first count with soaking times of 2 and 10 h, reaching means with times of up to 5h, of the order of 0.12 seedlings (Figure 1A). In the count on the twelfth day, seedling germination increased with soaking time, reaching a mean maximum of 3.73 seedlings with a time of 6.5h (Figure 1B). It is worth pointing out that germination was higher (3.0 seedlings) under the longest time (10h) of seed immersion in saline solution compared to the time of 2h (2.53 seedlings).

The results show that the *C. chinense* pepper species required a longer time for germination when the seeds were pre-treated in saline solutions. In general, this species is characterized by dormancy problems, with slow and uneven germination according to Monteiro *et al*. (2008). Andrade and Laurentin (2015) recorded low germination of *C. chinense* seeds, which did not exceed 80% in 14 days with soaking times of 2, 5, and 10min in solutions with different concentrations of KNO_3. These results agreed with those from Sá *et al*. (2019) who studied *C. chinense* seeds germinated under salinity stress for 30 days (in a protected environment - greenhouse, Campina Grande, Brazil). Seeds germination did not exceed 80% under salt concentrations ranging from 1.4 to 3.0dS m^{-1}. The results of the present study show that presoaking of pepper seeds in saline solutions is necessary to improve their germination capacity. Therefore, they are of great relevance in conditions where brackish waters are employed in the process of seedling production.

Regarding germination percentage, *C. frutescens* pepper, the means were approximately 90% with seed times of up to 4h. Under a soaking time of 10h, the percentage of germination was approximately 74%. The highest germination percentage (75.46%) for *C. chinense* pepper was observed with a soaking time of 6.4h, while the lowest germination was obtained with the time of 2h (53.04%) (Figure 1C). The values of germination percentages found in the present study are in line with the results of other studies for the same pepper species. For example, Adebisi *et al*. (2015) in Nigeria recorded germination percentage of 90.48 and 88.75% for C. *frutescens* and C. *chinense*, respectively, primed in different solutions (KCl and KH_2PO_4) for 12h (under laboratory conditions), at 8 days after germination. Batista *et al*. (2015) in Brazil reported germination of 88% when seeds were pre-treated with KNO_3 for 20h, at 14 days after sowing (under a controlled environment - growing chamber at 25°C). At 15 days after sowing (under a controlled environment at 30°C in Nigeria), Eremrena and Mensah (2016) recorded a germination percentage of 80% in *C. frutescens* L when the seeds were pre-treated with KNO_3.

For seedling height, the opposite behavior was observed between the pepper varieties as a function of the soaking times. For *C. frutescens* pepper, the highest value of height (3.29cm) was observed with a soaking time of 3.2h, with a subsequent reduction, reaching approximately 16% with the soaking time of 10h (Figure 1D). For *C. chinense* pepper, there was an increase in seedling height of approximately 31% with the time of 10h when compared to the time of 2h (2.18cm).

Different behaviors between pepper varieties were also recorded for root length (Figure 1E), when the highest means of 2.26 and 1.20cm for *C. frutescens* and *C. chinense* peppers were obtained with soaking times of 2 and 4h respectively.

Adequate establishment of the time for the soaking of seeds in salt solutions is extremely important to obtain success in the initial stand of seedlings, thus improving the establishment of the crop in the field. This has been done in studies for different species, such as *Nicotiana tabacum* (Caldeira *et al*., 2014; Lopes *et al*., 2019), pepper (Garruña-Hernández *et al*., 2014; Andrade and Laurentin, 2015; Ermis *et al*., 2016; Alcalá-Rico *et al*., 2019), *Dianthus barbatus* L. (González-Amaya *et al*., 2018), and canola (Shirazi *et al*., 2019).

According to the interaction between varieties and EC levels of the solutions, for the first and second count, the highest number of germinated seedlings for *C. frutescens* pepper decreased with increasing salinity. For example, while on the fourth day the maximum number of germination (1.95 seedlings) was estimated with an EC value of 3.67dS m^{-1} (Figure 2A), on the twelfth day the maximum number of 4.61 seedlings was estimated for the EC value of 2.84dS m^{-1} (Figure 2B). However, with the highest EC (6.0dS m^{-1}), there was a greater reduction in the number of seedlings on the fourth day, approximately 29% in comparison to EC of 3.67dS m^{-1}, while on the twelfth day, it was approximately 9% less in comparison to EC of 2.84dS m^{-1}.

For *C. chinense* pepper in the first count on the fourth day, there was no satisfactory adjustment of any type of mathematical model to the data, with a mean value of 0.05 seedlings (Figure 2A). In the second count on the twelfth day (Figure 2B), the highest number of seedlings (3.35) was obtained under the lowest salinity (1.5dS m^{-1}), while under the highest salinity (6.0dS m^{-1}) the reduction was approximately 9%.

Regarding germination percentage, the maximum estimated value was 90.29% at solution EC of 2.55dS m^{-1} for *C. frutescens* pepper (Figure 2C). Nevertheless, under the EC levels of 1.5 and 3.0dS m^{-1}, the values were around 90%, and under EC of 4.5dS m^{-1}, the values were approximately 85%. For *C. chinense* pepper, the percentage of germination did not exceed 70%; however, the behavior was similar to that observed for *C. frutescens* pepper, when maximum germination percentage (67.26%) was observed at EC of 2.55dS m^{-1}, and for the other EC levels mentioned above, the germination percentages were between 66-67%.

In the evaluation of seedling height, the maximum height of 2.19cm was observed at lower EC (1.5dS m^{-1}) and 2.08cm at higher EC (6.0dS m^{-1}) (Figure 2D). For the same variable, salt stress was more pronounced in *C. frutescens* pepper, reaching a reduction of approximately 26% at EC of 6.0dS m^{-1} when compared to the EC level of 2.77dS m^{-1} (3.28cm).

In the present study, the responses of the variables of initial growth of *C. frutescens* and *C. chinense* pepper species differed under solution salinity levels (Figure 2). The yield of crops depends on the rate and percentage of germination, the emergence of seedlings, and also on their uniformity (Balouchi *et al*., 2015), so seedling germination is a sensitive and crucial stage that plays an important role in the production process (Hozayn *et al*., 2019).

Different responses between plant species are reported in the literature regarding pre-treatment in solutions to attenuate salt stress (Moreno *et al*., 2017). Soaking increased the germination of *Physalis angulata* L. (Souza *et al*., 2016) and chia (Stefanello *et al*., 2019) seeds, and consequently their salt tolerance. Fredj *et al*. (2013), when evaluating different soaking times (12, 24, and 36h) of coriander seeds in solutions prepared with different NaCl concentrations (0, 2, 4, 6, and 8g L^{-1}), reported germination percentage above 90% up to the concentration of 4g L^{-1}, especially when the seeds were soaked for 12h. In the study conducted by Dalchiavon *et al*. (2016), the germination of *Stageolus vulgaris* seeds was on average equal to 99%, regardless of NaCl concentrations (0, 1.309, 1.964, 2.620, 3.273, and 3.928g L^{-1}).

According to Oliveira *et al*. (2019), melon seeds can be pre-treated with KNO_3 to improve germination and growth rate of seedlings under mild salt stress conditions; however, for severe stress conditions, the use of hydropriming should be preferred because it resulted in more vigorous seedlings. Jiang *et al*. (2020) with *Isatis indigotica*, found that salt resulted in a distinct decrease in the germination and seedling performance, indicating an unfavorable role of salt in the growth of this species.

According to Oliveira and Silva (2019), knowing how the different plant species react to salt stress is important because only in this way it is possible to seek mitigating strategies of salinity control in arid and semiarid regions, which are constantly more affected by the processes of soil salinization. While some species survive in soils with high salt concentrations, others are unable to establish in soils with small amounts of salts.

According to the various results presented, each species responds differently to the pre-treatment of seeds in saline solutions, which is reinforced by José *et al*. (2016), who reported that effective priming is highly dependent on both the osmotic potential of the solution and the duration of the treatment.

CONCLUSIONS

The data show that the responses of the evaluated variables of the pepper varieties differed with the times of soaking in saline solutions and their levels of electrical conductivity. *Capsicum frutescens* pepper was more tolerant to different times of soaking in saline solutions prepared with NaCl in relation to *Capsicum chinense*. For this reason, depending on the pepper species, pre-treatment is recommended in a salt solution with salinity levels compatible with those that will be employed in field conditions (in saline soils and/or irrigation with brackish waters). Thus, in addition to ensuring uniformity in the seedling standard, it is possible to avoid eventual losses in the pepper yield when cultivated under salinity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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