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Bell pepper germination and seedling's parameters under different osmotic potentials

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Abstract

Bell pepper (*Capsicum annuum* L.) is the third most cultivated Solanaceae species in Brazil and is highly sensitive to osmotic stresses. The increasing soil salinity is a frequent problem that hinders the plant absorption of water and nutrients by the roots, limiting crop production. Therefore, this study's objectives were to evaluate the germination, biometrical and physiological responses of bell pepper cultivars after NaCl treatments to simulate osmotic stress. Five cultivars (Yolo wonder, SF134 yellow, Marta, All big red, Cascadura) were submitted to four osmotic potentials (0, -0.3, -0.6, -0.9 MPa) in a factorial scheme of 5×4, with four repetitions in completely randomized design. The results indicated that the bell pepper germination is highly affected by the presence of NaCl. The bell pepper germination bears NaCl concentrations equal to or higher than -0.3 MPa osmotic potential. Significant reductions in germination, germination speed index, seedling length, and dry mass as the salinity increases - osmotic potential decreases. The yellow bell pepper 'SF134' presented similar germination down to -0.6 MPa and can be cultivated in regions with salinity problems. The green bell pepper 'Cascadura' presented great germination, germination speed index and dry mass, and superior seedling length.

Keywords: *Capsicum annuum*, germination rate, NaCl, osmotic stress, seedling

Introduction

Bell pepper (*Capsicum annum* L.) fruits are a good source of carotenoids, vitamin C, antioxidants, and phenolic compounds, such as capsaicin, that give their pungent and chili flavor (Rawat et al., 2018). The antioxidant properties are directly related to the vegetables' functional or nutraceutical properties (Cisternas-Jamet et al., 2020), which increases the interest in plant species-rich in these compounds.

The bell pepper is the third most cultivated species of the Solanaceae plant family in Brasil, especially in the southeastern region (Santos et al., 2020). The importance of this horticultural crop highlights the growing demand for high-quality seeds. The success of the germination proces initially depends solely on the water movement in the seed tissues (Pereira et al., 2014). However, Duarte & Souza (2016) reported that studies are still needed about the *C. annuum* cultivation to uncover missing information about seedling production under stressful conditions.

The high seed quality is a stepping stone for higher productivity, which can increase the yield by up to 40% (Verma & Mehta, 2019). The seed metabolic activity is mainly affected by the water availability and temperature, which start the process of germination, stimulate the emergence of the radicle and regulate the energy and nutrients present in seeds (Silva et al., 2011; Bareke 2018; Carrera-Castaño et al., 2020).

The increase in soil salinity is a recurrent problem affecting crops production in modern agriculture. This condition is worse in regions with low precipitation, irrigation with low-quality water, successive application of fertilizers and amendments with high salt content (Silva et al., 2020). The soil salinity effects on plants are usually related to the dynamics of water and nutrient absorption by roots and the toxic levels of some elements (Maciel et al., 2012; Oliveira et al., 2018; Sá et al., 2019).

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Water restrictions and elevated salt concentration increase the concentration of plant solutes, interfere with the soil water potential, restrict the absorption of water by the seed, change the soil pH, facilitate the entrance of toxic ions, and accelerate the degenerative reactions causing abnormal seedling development and survival (Pereira et al., 2014, Soares et al., 2015).

Despite fertigation is widely used in pepper cultivation, this technique needs attention as it can provide salt accumulation in the plant root zone, which promotes oxidative stress and negatively affect plant growth (Kaya et al., 2018; Chauhdary et al., 2019).

Bell pepper is considered a salt-sensitive plant species (Silva et al., 2020); however, the productivity and the quality parameters of the bell pepper fruit could be improved by certain levels of deficit in irrigation, which highlights the importance of optimal irrigation management (Abdelkhalik et al., 2019).

Therefore, this study's objective was to evaluate the seed germination, biometrical and physiological responses of commercial bell pepper cultivars under different salt concentrations to simulate water stress during the germination process.

Material and Methods

The study was conducted at the LAGEN (Laboratory of Seed Analysis and Genetic Resources) of the Universidade Federal de Uberlândia (UFU), Campus Monte Carmelo, Minas Gerais state, Brazil.

Bell pepper seeds of five commercial cultivars were evaluated under four salt stresses (5×4 factorial scheme) with four repetitions, totaling 80 parcels. Each parcel was represented by one transparent plastic box (seedbox or germbox type) (11×11×3.5 cm), treated with sodium chloride (NaCl) solutions: 0, -0.3, -0.6 or -0.9 Mpa (Table 1).

Table 1. Characterization of the treatments to evaluate bell pepper germination and seedling's parameters in different osmotic potentials.

Bell pepper genotype	Osmotic potential (Mpa)
Green bell pepper 'Yolo wonder'	0, -0.3, -0.6, -0.9
Yellow bell pepper 'SF134'	0, -0.3, -0.6, -0.9
Green bell pepper 'Marta'	0, -0.3, -0.6, -0.9
Red bell pepper 'All big'	0, -0.3, -0.6, -0.9
Green bell pepper 'Cascadura'	0, -0.3, -0.6, -0.9

The bell pepper seedling evaluation and determination of the tolerance to salt stress were performed by the germination test, germination speed index (GSI), seedling length, and dry mass evaluation. Reductions in the seed germination, compared to the control (0 MPa), served as an index of the tolerance to salinity (Pereira et al., 2014).

The germination test was conducted following the recommendations of the RAS (*Seed Analysis Rules*) (Brasil, 2009). RAS suggests four replications of 100 seeds for each treatment, and each repetition consisted of 25 seeds in each germbox (seeds were spaced by 1 cm from each other), totaling four germbox per treatment. In each germbox, two sheets of blotter paper were placed, which were previously autoclaved for 20 minutes at 121 °C and 1 Kgf cm^{-2}. The blotter papers were soaked with the respective NaCl solutions (Table 1).

The concentrations were calculated by the Van't Hoff formula, considering the ambient temperature of 25 °C: $Yos = -R \times T \times C$, where, Yos: osmotic potential (atm); R: constant line of perfect gases (0.082 atm L mol^{-1} K^{-1}); T: temperature (K); C: concentration (mol L^{-1}).

After inserting the bell paper seeds and soaking the blotter papers, the germboxes were closed and sealed with plastic film. The germboxes were sorted in a completely randomized design. All germboxes were kept in a BOD incubator (D type), regulated to maintain a constant temperature at 25 °C and 12 hours of photoperiod for 14 days before the evaluations previously described.

The germination was evaluated daily, starting 24 hours after the experiment installation. The seeds considered germinated were those that initiated the emission of primary root and hypocotyl. The seedling length measurements - using a millimeter ruler - were performed only in seedlings considered normal. The samples were placed in paper bags and dried in a force air-circulation oven (402/D Model) at 105 °C for 24 hours before weight to assess the seedling dry mass using an analytical balance (0.001 g precision).

The germination speed index was calculated based on the daily observation of the number of emerged plants from the beginning of the emergency/germination until stabilization (Maguire 1962; Nakagawa 1999): GSI = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn), where: G1, G2, G3, ..., Gn = number of seedlings computed at first, second, third and last count, respectively; N1, N2, N3, ..., Nn = number of days from sowing to first, second, third and last count.

Extreme values (*outliers*) in the results were identified using boxplot graphs of residuals (Chambers et al., 1983) generated by the software SPSS® Statistics. The outliers identified were calculated as a lost parcel to replace the extreme values. The resulting data were submitted to the ANOVA presumption of normality of residues (p>0.01). If no data transformation was needed, the data was then submitted to the ANOVA (p<0.01). The averages were distinguished by Tukey's test (p<0.05). The results of seedling length and dry mass were also used to compose significant ($p<0.05$; $R^2>0.7$) regression curves.

Results and Discussion

The results observed for bell pepper germination (Table 2) indicated a significant interaction effect between the bell pepper genotypes and the osmotic potentials (NaCl concentration in solution). Maciel et al. (2012) observed that the increasing salt concentration in solution reduced the water absorption by broccoli seeds from organic or conventional cropping systems. Other studies also observed similar results for turnip (*Raphanus raphanistrum*) (Pereira et al., 2014), and soybean (*Glycine max*) (Soares et al., 2015) seeds when submitted to osmotic potentials.

Irrigation is common in bell pepper production, even during the rainy season, due to poor rainfall distribution. Fertigation has also been widely used, but salinization is a risk mainly caused by salt fertilizers, which negatively affects seed germination (Maciel et al., 2012). Dhotre et al. (2018) concluded that the combination of irrigation replenishment at 60% of ETc and fertigation with 75% recommended fertilizer dose was optimal to obtain higher bell pepper yields and better fruit quality.

Table 2. Bell pepper seed germination (%) of different genotypes in different osmotic potentials.

Bell pepper genotypes	Osmotic potentials (MPa)			
	0	-0.3	-0.6	-0.9
	------------------------- *Germination (%)* -------------------------			
Green bell pepper 'Yolo wonder'	94.0 Aa*	86.0 ABab	71.0 Ab	25.3 Bc
Yellow bell pepper 'SF134'	82.0 Aa	76.0 Ba	74.7 Aa	27.0 Bb
Green bell pepper 'Marta'	96.0 Aa	96.0 Aa	65.3 ABb	49.0 Ab
Red bell pepper 'All big'	82.0 Aa	81.0 ABa	50.0 Bb	42.0 ABb
Green bell pepper 'Cascadura'	96.0 Aa	96.0 Aa	46.7 Bb	49.3 Ab
C.V. (%)	13.67			

*Averages followed by the same uppercase and lowercase letter do not differ the bell pepper genotypes or the osmotic potentials, respectively, by Tukey's test (p<0.05). C.V. (%): coefficient of variation.

According to Sheldon et al. (2017), the seeds of a salt-sensitive plant at higher salinities has the NaCl toxicity as the dominant factor, which consequently reduces the seed water use capacity due to the low osmotic potential of the salty soil solution. This situation imposes more energy spending by the seed to keep its metabolism stable, consequently influencing its germination potential (Pereira et al., 2014).

Throughout the development of seedlings and plants, the dehydration of cell membranes also can reduce the permeability to CO_2 influx, compromising its assimilation (Cavalcante et al., 2019) and may cause a physiological limitation in the environment under saline stress (Giuffrida et al., 2014).

The germination (%) for all bell pepper genotypes at -0.3 MPa osmotic potential did not differ from the germination at 0 MPa (Table 2), indicating that these genotypes can germinate up to specific stressful conditions. The bell pepper genotype 'SF134' (yellow) presented similar germination down to -0.6 MPa osmotic potential, varying from 82.7 to 74.7%, being a bell pepper genotype indicated for regions with salinity problems. The germination of the green bell pepper 'Marta' was always prominent, even at -0.9 MPa (49.0%).

The germination speed index (GSI) of the bell pepper genotypes was similar to each other below -0.3 MPa osmotic potential (Table 3), showing that greater salt concentrations will affect equally any of the bell pepper genotypes.

Table 3. Germination speed index of different bell pepper genotypes in different osmotic potentials.

Bell pepper genotypes	Osmotic potentials (MPa)			
	0	-0.3	-0.6	-0.9
	----------------- *Germination Speed Index* -----------------			
Green bell pepper 'Yolo wonder'	5.12 ABa*	3.43 Bb	2.14 Ac	0.71 Ad
Yellow bell pepper 'SF134'	4.01 BCa	3.15 Bab	2.17 Ab	0.73 Ac
Green bell pepper 'Marta'	5.95 Aa	4.83 Aa	2.58 Ab	1.49 Ab
Red bell pepper 'All big'	3.75 Ca	2.90 Ba	1.62 Ab	1.14 Ab
Green bell pepper 'Cascadura'	5.98 Aa	3.93 ABb	1.55 Ac	1.04 Ac
C.V. (%)	21.59			

*Averages followed by the same uppercase and lowercase letter do not differ the bell pepper genotypes or the osmotic potentials, respectively, by Tukey's test (p<0.05). C.V. (%): coefficient of variation.

At -0.3 MPa osmotic potential, the NaCl influence was enough to only decrease the GSI for the green bell pepper 'Yolo wonder' and 'Cascadura'. These dissimilar responses among bell pepper genotypes were also observed when no NaCl was added to the solution (0 MPa), indicating the existence of natural genetic variations among the genotypes, just as Silva et al. (2011) observed for carrots, Abbas et al. (2014) for okras and Tehseen et al. (2016) for bell peppers.

No interaction was detected (p>0.01) between the factors (bell pepper genotypes and osmotic potential) for the biometric variables seedling length (mm) and seedling dry mass (mg); thus, the factors were studied separately. The seedling length and dry mass responses of the bell pepper genotypes are presented in Figure 1.

Only the seedling length of the green bell pepper 'Cascadura' (17.59 mm) differed from other genotypes (11.58 mm average), being about 51% larger. Seedling length is a genotype-linked characteristic and indicates that great initial seedling lengths will be observed for this genotype grown in a great diversity of soil salinities. However, the dry biomass of the 'Cascadura' genotype was similar to other genotypes (903 mg average), except for the red bell pepper 'All big', which was about 47% heavier. The seedling dry mass is highly influenced by the amount of initial seed reserves; thus, bigger seeds generate more vigorous seedlings (Soares et al., 2015; Steiner et al., 2019).

The regression analyses of the bell pepper seedling length and dry mass in different osmotic potentials are explored in Figure 2. The bell pepper seedling length was linearly affected (R^2 = 96.85%) by the osmotic potential caused by increasing NaCl concentration. The linear polynomial model indicates that each 0.1 MPa reduction in the osmotic potential causes a 1.783 mm decrease in the bell pepper seedling length. The bell pepper seedling dry mass adjusted to a quadratic polynomial model (R^2 = 99.68%) indicates that osmotic potentials below -0.26 MPa sharply decrease seedling dry mass (Figure 2). This inflection point (-0.26 MPa) in seedling dry mass accumulation indicates the ideal soil solution osmotic potential for bell pepper development.

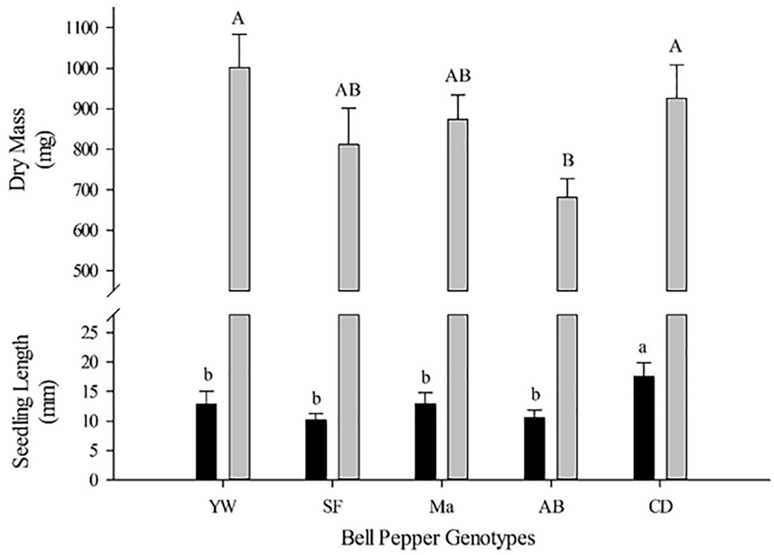

Figure 1. Effect of osmotic potential caused by NaCl concentration in bell pepper genotypes (YW: green bell pepper 'Yolo wonder'; SF: yellow bell pepper 'SF134'; Ma: green bell pepper 'Marta'; AB: red bell pepper 'All big'; CD: green bell pepper 'Cascadura'). Bars of each variable with the same letter do not differ bell pepper genotypes by Tukey's test (p<0.05). Coefficient of Variation = 26.04% (dry mass), 27.87% (seedling length).

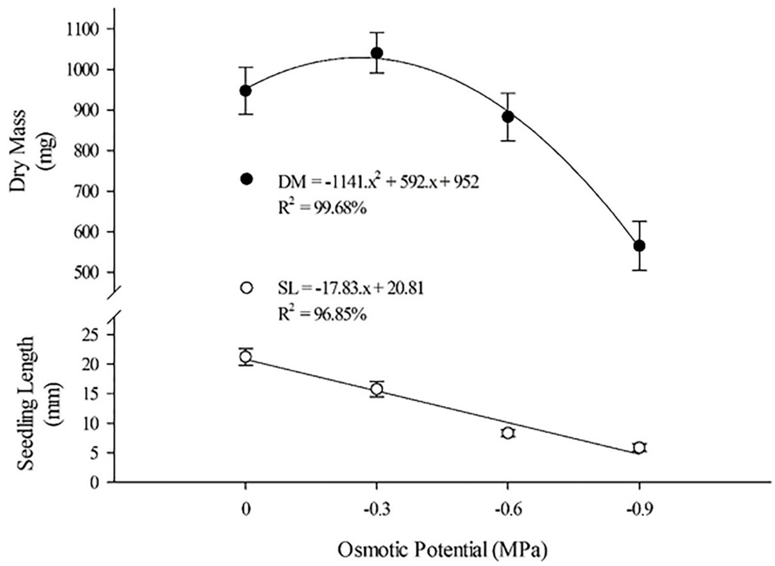

Figure 2. Effect of osmotic potential caused by NaCl concentration in bell pepper seedling length and dry mass. Coefficient of Variation = 26.04% (dry mass), 27.87% (seedling length).

Silva et al. (2011) observed that osmotic potentials down to -0.3 MPa were not negatively affecting the carrot seedling development. For soybean, the hypocotyl and root length were significantly affected in potentials as low as -0.2 MPa (Soares et al., 2015). The results of the present study indicated that as the osmotic potential progressive reductions in germination, germination speed index, seedling length, and dry mass occurred. Abbas et al. (2014) also found a strong negative correlation between seedling shoot and root length, seedling fresh and dry weight with salinity, in a test with okra cultivars.

According to Soltani & Zeinali (2006), the salinity stress negatively affected the wheat seed reserve mobilization to the seedling development. This observation corroborates the bell pepper seedling length and dry mass reduction with increasing NaCl concentration.

Seed evaluations from various species were negatively affected as the NaCl concentration of the germination medium increases, presenting low germination and reduced rate of plant growth (Lopes &Macedo, 2008; Maciel et al., 2012); thus, the response to salinity stress differ depending on the stress intensity, plant species, and genotypes (Yamashita & Guimaraes, 2010).

Laffray et al. (2018) related salt stress tolerance to morphological changes and osmotic adjustment. The authors observed that oak seedlings could cope with moderate salt stress but emphasized that root responses are insufficient to maintain adequate water uptake to aerial parts.

Therefore, the present study is important for knowing the limits of saline stress supported by bell pepper seeds, indicating a possibility to, increase productivity and reduce economic losses arising from the right choice of a genotype and proper fertigation.

The growers must consider the salinity response function and seasonal productivity alongside an appropriate irrigation regime. The knowledge can help farmers' decisions and avoid negative results reflections on productivity as a reduction in the number of marketable fruits and an increase in the number of small fruits and fruits without commercial quality in plants fertigated with nutrient solutions of higher salinity (Silva et al., 2020).

Conclusions

The bell pepper seed germination bear NaCl concentrations equal or higher than -0.3 MPa.

The bell pepper germination, germination speed index, seedling length, and dry mass decrease as the NaCl concentration increases and the osmotic potential decreases.

Yellow bell pepper 'SF134' presented similar germination down to -0.6 MPa osmotic potential, a genotype indicated for regions with salinity problems.

The green bell pepper 'Cascadura' presented great germination, germination speed index and dry mass, and superior seedling length.

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