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Physiological quality of chickpea seeds submitted to hydric and saline stress

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Abstract

The use of high physiological quality seeds reduces problems in the seedlings' development, allowing the proper stand establishment under different environments, especially in stressful conditions. This paper aimed to assess the effect of the hydric and saline stress on chickpea seeds' physiological quality and growth as they are submitted to different osmotic potentials induced by polyethylene glycol 6000 (PEG) and sodium chloride (NaCl). The experiment was performed in a completely randomized experimental design in a 2x5 factorial design (Two osmotic solutions, PEG and NaCl, and five osmotic potentials (-0.2, -0.4, -0.6, and -0.8 MPa) with four repetitions of 200 seeds each. In the beginning, the water content of the seeds was established. As the treatment's effects, the seedlings' physiological quality and initial growth were assessed (germination, first germination counting, abnormal seedlings, germination velocity index, average germination time, epicotyl and primary root length, and seedlings dry mass). Osmotic potentials under -0.2 reduce the budburst and the chickpea seedlings' growth, independently from the stressing agent. NaCl's osmotic stress induces more severe effects than PEG 6000 on the physiological seedlings' quality.

Keywords: *Cicer arietinum* L., sodium chloride, polyethylene glycol, osmotic potential

Introduction

Chickpea (*Cicer arietinum* L.) is the second world's most consumed legume. It is widespread worldwide, with an approximate 11 million hectares cultivated area and a total production of 11.6 million tons (Icrisat, 2017). The grains have high nutritional value and can be used in human feeding, animal supplementation, medicine, and industry (Nascimento et al., 2016).

Abiotic factors may limit this plant's culture development. Among the factors that most affect the seeds' physiological quality and plants' growth, the water and salt stress stand out, as water is crucial for the seeds' budding process (Bewley et al., 2013). Seeds' tissues rehydration occurs from the absorption of water and subsequent increase of breathing and all remaining metabolic activities that culminate with the provision of the necessary energy and nutrients to recover the embryonic axis growth (Carvalho & Nakagawa, 2012).

If seeds are submitted to the soil's low osmotic potential, the seeds may not germinate as drought occurs. A similar effect may be observed in salts' presence, affecting the hydric potential of the soil. Salts reduce the gradient of the potential between the soil and the seeds' surface, reducing the water uptake and invalidating the events' sequence related to the seeds budding process (Silva et al., 2019), hampering the plant's growth.

The budding and seedlings growth phases have been studied enough in the laboratory, under the effects of these abiotic stresses. These studied used chemical compounds, such as the polyethylene glycol 6000 (PEG) and sodium chloride (NaCl), to simulate water and saline stress conditions. Studies performed on different cultures, such as *Salvia hispanica* (Stefanello et al., 2020), *Pisum sativum* (Pereira et al., 2020), *Crambe abyssinica* Hochst (Silva et al., 2019), *Cucumis sativus* L. (Albuquerque et al., 2016) and *Zea mays* L. (Silva et al., 2016) used aqueous

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solutions with different osmotic potentials to regulate the budding substrate humidity, simulating soil water and saline stressful conditions, producing a complete set of high scientific values results.

Considering the economic relevance of the chickpea culture, few studies in the literature described the effects of water and saline stress on the seeds' physiological quality and growth of the seedlings justifying the present study's performance.

This paper aimed to assess the effect of the hydric and saline stress on the physiological quality of chickpea seeds and the seedlings' growth submitted to different osmotic potentials induced by polyethylene glycol 6000 (PEG) and sodium chloride (NaCl).

Material and Methods

The experiment was performed in the Seeds Analysis Teaching Laboratory (LDAS: *Laboratório Didático de Análise de Sementes*), belonging to the post-graduation program in Seeds Technology, Pelotas Federal University (FAEM/UFPel). This experiment used BRS Aleppo cultivar chickpea seeds.

The polyethylene glycol 6000 (PEG) solutions to simulate the water stress were prepared according to Villela et al. (1991). The sodium chloride (NaCl) solutions to simulate the salt stress were prepared according the Van`t Hoff equation (Salisbury & Ross, 1992), where Ψos = osmotic potential (MPa), C = concentration (mol L -1), i = isotonic coefficient, R = general gas constant (0.0082 MPa mol -1 K -1), and T = temperature (K).

PEG and NaCl solutions were prepared with the following osmotic potentials: 0.0, -0.2, -0,4, -0.6, and -0.8 MPa. Zero potential represented the control without any stress. The zero potential was produced by using distilled water.

The initial seeds water content was assessed previously by the oven method at 105 °C for 24 hours (Brasil, 2009).

Germination test was performed with four repetitions, with 200 seeds each. The seeds were seeded on two germitest paper leaves, moistened with each salt's solution volume, equivalent to 2.5 times the weight of the dry substrate at the different osmotic potentials and, after that, covered with one more leaf. After that, the papers were enrolled in rolls and preserved in a Mangelsdorf model germinator at a constant 20°C temperature. At the fifth and eight days after seeding, the number of normal seedlings (Figure 1A) was assessed according to the criteria established by the "*Regras para Análise de Sementes*" (Brasil, 2009). The abnormal seedlings were also computed (Figure 1B).

Figure 1. Chickpea seedlings classified as normals (A) and abnormals (B) according to the criteria established by the seeds analysis rules.

The data of normal seedlings percentage on the fifth day after the germination test setting were used to test the first germination counting (Brasil, 2009).

Germination velocity index was performed together with the germination test, calculated by the sum of the amount of germinated seeds every day, divided by the number of days passed between the seeding and germination, according to the formula of Maguire (1962).

The average germination time was calculated from the number of daily germinated seeds until the eighth day after seeding. The result was expressed as days after seeding.

The epicotyl and primary root length were considered exclusively in the normal seedlings, using a graduated ruler. The results were expressed as centimeters per seedlings^{-1}.

Normal seedlings derived from the germination tests were weighed in a 0.0001 g precision scale to obtain the fresh mass weight.

The experiment was performed in a completely randomized experimental design in a 2x5 factorial design (two osmotic solutions, PEG 6000 and NaCl, and five osmotic potentials: -0.2, -0.4, -0.6, and -0.8 MPa).

Data were submitted to the Analysis of Variance at 5% probability and subsequent regression analysis. As significant, the solutions' effects were studied by the F test at 5% significance, as long as the osmotic potentials' effects were studied by the regression analysis, choosing the best models to present them according to their biological behavior, the significance of the model's coefficients, and the coefficient of determination value (R^2).

Results and Discussion

The initial water content determination displayed 12.1% humidity. This result is crucial to the analyses, as the harmonization of the water content is fundamental to standardize the analyses and obtain consistent results, providing higher confidence in the tests' results (Tunes et al., 2011).

The analysis results pointed out that the interaction between the factors of osmotic solutions x osmotic potentials influenced the germination, first germination counting, abnormal seedlings, epicotyl, and primary root length. The isolated factor osmotic potentials displayed a significant effect for the variables mean germination time, germination velocity index, and seedlings fresh mass.

The results of the germination percentage and vigor (first germination counting) of the chickpea seeds displayed linear behavior (Figure 2A and B). The control treatment (0MPa) displayed the highest germination and vigor percentages. On the other side, the chickpea seeds' germination and vigor were progressively reduced with the osmotic potential reductions, both for the PEG and NaCl moistened substrates (Figura 2 A e B), pointing out the sensitivity of the chickpea seeds to these stresses. In exchange, an increase of abnormal seedlings occurred on both osmotic agents used (Figure 2C).

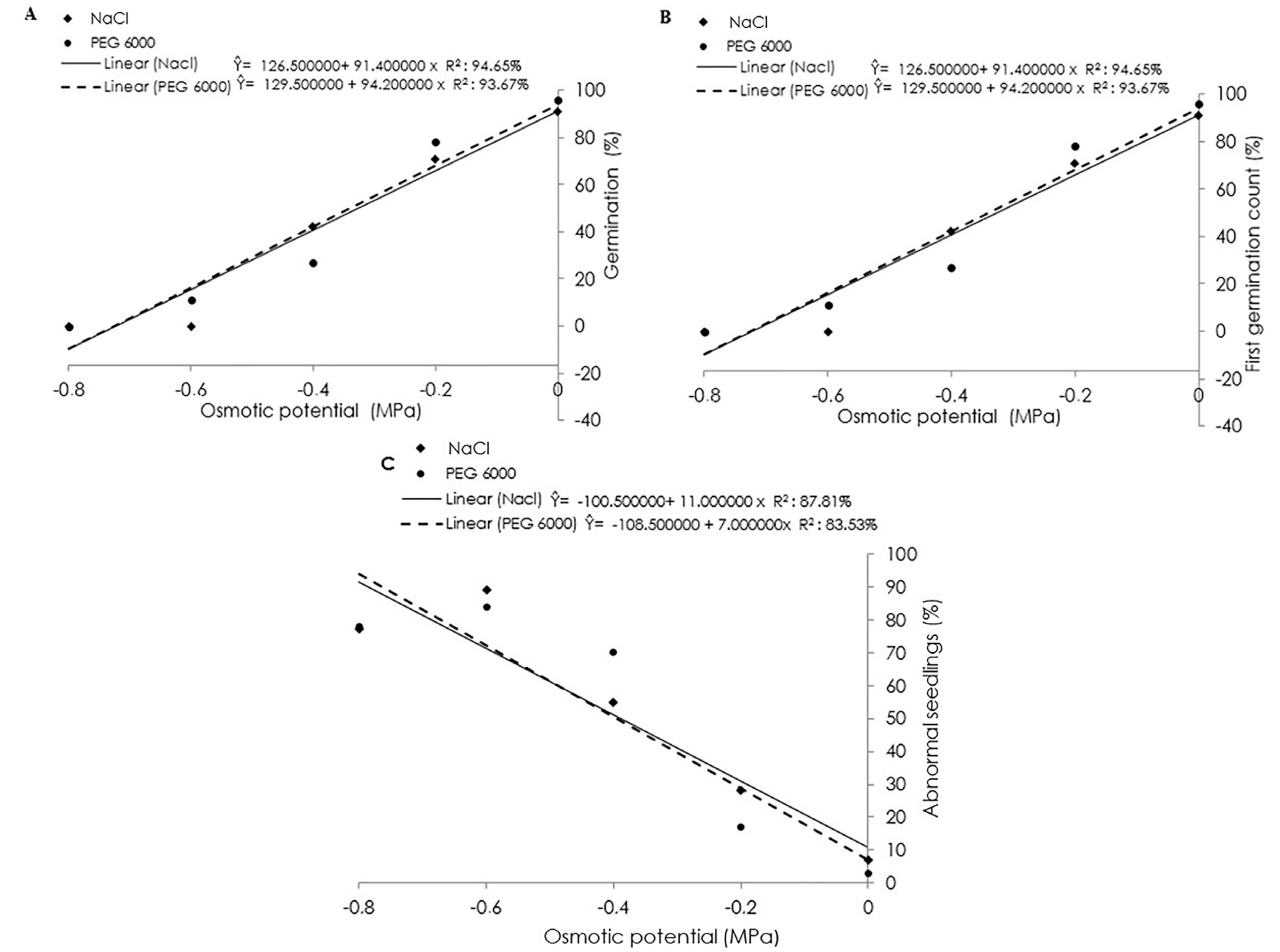

Figure 2. Germination (A), first germination counting (B), and abnormal seedlings (C) of chickpea submitted to different osmotic potentials induced by the use of PEG 6000 or NaCl.

The modification of the cellular membranes system selectivity and permeability can produce higher leaching of the reserves and increase abnormal plants' number (Aumonde et al., 2012). This collaborates with reducing germination and vigor, measured by the number of normal seedlings.

The germination percentage and vigor reduction of the seeds as the osmotic potential decreases might be associated with the extension of the soaking time, as, according to the triphasic standard proposed by Bewley & Black (1994), The intense water uptake and main root growth occur during this phase. Besides this, a higher salts concentration inside the cells can deactivate enzymes, inhibit the protein synthesis and prevent the seeds germination (Taiz & Zeiger, 2013).

Other researchers claimed similar results to those of this research, as they discovered that the water and saline stresses reduce the germination percentage and the vigor of several cultures, such as *Crambe abyssinica* Hochst (Silva et al., 2019), *Pisum sativum (Pereira et al., 2020), Phaseolus vulgaris* L. (Cangussú et al., 2020) *and Vigna unguiculata* (L) (Gomes Filho *et al., 2019)*. A reduction of viability, vigor, and quality of the cowpea seeds (*Vigna unguiculata*) was also described by Ferreira et al. (2017) as the osmotic potentials were below -0.6 MPa.

The chickpea seeds' physiological quality was more affected by the saline stress than by the water stress, with a higher germination potential and vigor at the osmotic potentials -0.2 and -0.6 MPainduced by PEG than the same potential as induced by NaCl (Figure 2A). At the -0.8 MPa osmotic potential, there was no germination in any used osmotic solutions. The germination absence was probably due to the ions' toxicity and water lack to begin the seeds' metabolic processes.

At the osmotic potential of -0.2 and -0.4 MPa, the abnormal seedlings percentage was higher in the samples submitted to saline stress than water stress (Figure 2A).

Figures 3A and B highlight how the mean germination time and germination velocity index of the chickpea seeds fitted a linear decreasing regression equation. Seeds vigor reductions occurred as the reduction of the solution's osmotic potential (i.e., higher salts amount and lower water availability).

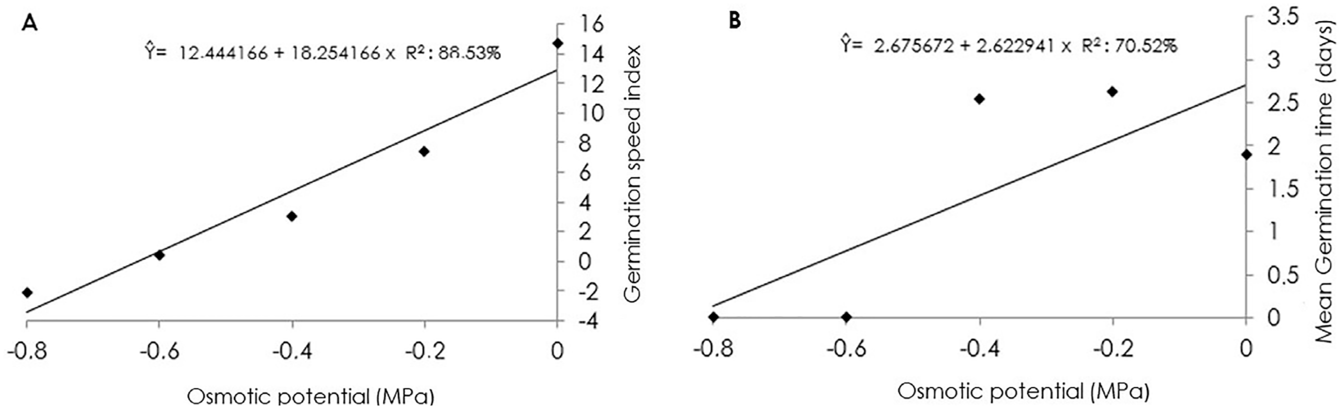

Figure 3. Germination velocity index (A) and mean germination time (B) of chickpea seeds under different osmotic potentials.

The osmotic stress affects the amid synthesis reactions and energy production process (adenosine triphosphate- ATP) through the seeds' respiration (Bewley et al., 2013), reducing the germination velocity index and, therefore, a delay in the germination time. The most common plants' responses to the osmotic potential reduction are an initial germination delay and a germination rate reduction (Soares et al., 2015; Steiner et al., 2017). The salts excess causes phytotoxicity, cells dehydration (Taiz & Zeiger, 2013) and decreases the metabolic activity and the synthesis of new tissues performed by the seeds due to reduced water availability (Marcos Filho, 2015), causing a lower germination velocity index and, in the most severe cases, the germination capacity loss.

Figures 4A and B display the behavior of the variables epicotyl and primary root length of chickpea seedlings in NaCl and PEG solutions according to their osmotic potentials. Seeds submitted to PEG and NaCl solutions displayed longer epicotyl in the control treatment (0.0 MPa) and -0.2 MPa osmotic potential (Figure 4A). The used osmotic agents caused significantly different effects in the -0.4 MPa osmotic potential, reaching 1.41 and 1.77 cm in the PEG and NaCl solutions, respectively.

These results agree with Taiz & Zeiger (2013), as the author claimed that water stress, besides affecting soaking, seeds' germination velocity and percentage, causes the reduction of the seedlings' growth by reducing the cellular expansion.

The reduction of the PEG and NaCl solutions' osmotic potential reduced the chickpea seedlings' primary roots' length (Figure 4B). The results also displayed that the longest roots occurred in the control treatment (0 MPa) and in the -0.2 and -0.4 MPa potentials using PEG solution (Figure 4B).

Pereira et al. (2020) also observed roots' length reduction in pea seedlings as a response to salinity increase. According to Maia et al. (2010), the root's growth inhibition is the primary response in plants submitted to osmotic stress. In a general form, salt excess in the root zone is detrimental to the plants' growth

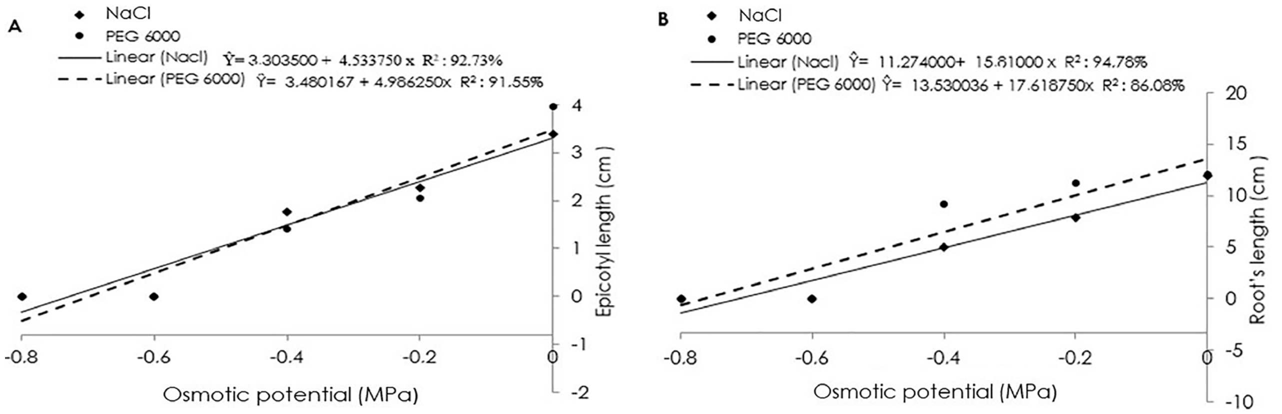

Figure 4. Epicotyl (A) and primary root (B) length of chickpea seedlings submitted to different osmotic potentials induced by PEG 6000 or NaCl.

Figure 5 describes the fresh mass behavior according to the varying osmotic potentials. The results fitted a linear regression equation, with a reduction of fresh mass accumulation proportional to the osmotic potential decrease.

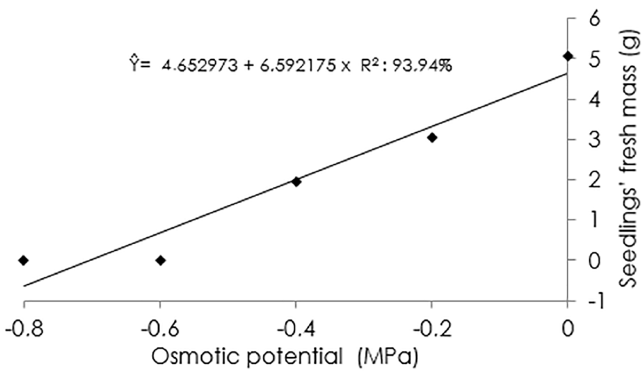

Figure 5. Fresh mass of chickpea seedlings under different osmotic potentials.

The salts' effects affect the seedlings' development: growth rate and biomass production are reasonable criteria to assess the stress and the plant's capacity to overcome the osmotic stress (Gomes et al., 2015).

Conclusions

Saline and water stress undermine the chickpea seeds' germination and vigor.

Sodium chloride (NaCl) caused more severe effects than polyethylene glycol 6000 (PEG) on the seedlings' growth.

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