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Seed physiological quality and seedling growth of pea under water and salt stress

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ABSTRACT: The aim of this study was to evaluate seed physiological quality and initial pea (*Pisum sativum*) seedling development at different water and salt potentials. Polyethylene glycol 6000, sodium chloride and potassium chloride solutions with different osmotic potentials (0; –0.2; –0.4; –0.6; –0.8; –1.0, and –1.2 MPa) were used, and the experiment had a 3 × 7 factorial arrangement. The physiological quality and initial seedling growth characteristics (first germination count, final germination count, strong normal seedlings, epicotyl and taproot lengths, epicotyl dry matter, and root dry matter) were evaluated in a completely randomized experimental design, with four repetitions. Decreases in the observed variables showed that the simulated stress conditions negatively affected physiological performance, germination and initial seedling growth. The water and salt stresses induced by the sodium chloride and potassium chloride solutions were greater than the stress induced by polyethylene glycol 6000.

Key words: *Pisum sativum*, vegetable, polyethylene glycol, osmotic potential

Qualidade fisiológica de sementes e crescimento de plântulas de ervilha sob estresse hídrico e salino

RESUMO: Objetivou-se avaliar a qualidade fisiológica das sementes e o crescimento inicial de plântulas de ervilha sob efeito de diferentes potenciais hídricos e salinos. Foram utilizadas soluções de polietilenoglicol 6000, cloreto de sódio e cloreto de potássio com diferentes potenciais osmóticos (0; –0.2; –0.4; –0.6; –0.8; –1.0 and –1.2 MPa), sendo o experimento conduzido em esquema fatorial 3 x 7. Nos efeitos dos tratamentos, avaliou-se a qualidade fisiológica e o crescimento inicial das plântulas (primeira contagem e contagem final de germinação, plântulas normais fortes, comprimentos do epicótilo e da raiz primária, massa seca do epicótilo e massa seca das raízes), em delineamento experimental inteiramente ao acaso, com quatro repetições. As simulações das condições de estresse prejudicaram o desempenho fisiológico e permitiram a constatação dos efeitos do estresse na germinação e no crescimento inicial das plântulas pelo decréscimo das variáveis observadas. Os estresses hídrico e salino induzidos pelas soluções cloreto de sódio e cloreto de potássio foram mais drásticos do que o induzido por polietilenoglicol 6000.

Palavras-chave: *Pisum sativum*, hortaliça, polietilenoglicol, potencial osmótico

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Introduction

Water stress has negative effects on the survival and initial growth of plants (Machado et al., 2017). Furthermore, water is the most important factor in the germination process because it is the medium in which most of the biochemical and physiological processes occur that result in the growth of the taproot (Silva et al., 2016).

Water availability that is below the limit tolerated by the cell can lead to an increase in solute concentration, changes in the pH of the intracellular solution, an acceleration in degenerative reactions, denaturation of proteins, loss of membrane integrity, and increases in the number of damaged and abnormal seedlings (Pereira et al., 2014).

Laboratory studies often use aqueous solutions with different osmotic potentials to regulate moisture in the germination substrate. The aim is to simulate water and salt stress conditions in the soil (Taiz & Zeiger, 2013).

Several chemical compounds, including polyethylene glycol (PEG) and sodium chloride (NaCl), have been used to simulate water and salt stress conditions (Pereira et al., 2012). High salt concentrations and water deficiency may prevent water uptake and inhibit seed germination due to a reduction in the external osmotic potential or the toxic effects caused by the uptake of ions, such as Na^+ and Cl^- (Silva et al., 2016). Therefore, the aim of this study was to evaluate the physiological quality of pea seeds and the initial growth of pea seedlings under different water and salt potentials.

Material and Methods

The study was conducted at the Seed Analysis Laboratory of the Plant Science Department of the Fundação Gammon de Ensino em Paraguaçu Paulista, SP, Brazil. Pea seeds (*Pisum sativum)* var Aragorn were used in this study.

The experiment had a completely randomized design in a 3 × 7 factorial arrangement. There were three osmotic conditioner (PEG 6000, KCl and NaCl) and seven water potential: 0, −0.2, −0.4, −0.6, −0.8, −1.0 and −1.2 MPa, with four repetitions. The control contained distilled water instead of the osmotic conditioners. The seeds were germinated in solutions adjusted to the different osmotic potentials, which simulated various water and salt stress situations. Then, the initial performance of the seedlings under these conditions was evaluated.

The PEG 6000 solutions were prepared according to the specifications in the table cited by Villela et al. (1991). The KCl and NaCl salt solutions were prepared using deionized water, and the salt concentrations were obtained from the equation used by Van't Hoff: $\Psi os = -RTC$, where Ψos = osmotic potential (atm); R = the general constant for perfect gases (8.32 J mol^{-1} K^{-1}); T = temperature (K) = 273 + T (°C); and C = concentration (mol L^{-1}) (Salisbury & Ross, 1991). The NaCl concentrations were corrected according to the calibration curve established by Braccini et al. (1996), namely, $\Psi os = 0.194699 + 0.750394C$; $R^2 = 0.9999$; where Ψos = osmotic potential (bar); and C = concentration (g L^{-1}).

A total of 50 seeds per treatment were distributed on germinating paper and then the paper was rolled up. The paper toweling was moistened with the PEG 6000, KCl, or NaCl solutions to 2.5 times the weight of the dry paper. There were no further solution applications during the experiment. The control treatment was moistened with distilled and deionized water.

The rolls were kept in plastic bags throughout the test period to avoid water evaporation and to ensure that the desired osmotic potential was maintained. The rolls containing the seeds were placed in a biological oxygen demand (B.O.D.) germination chamber and maintained at 20 °C with a photoperiod of 12 h light. The evaluations were made at 5 and 8 days after the beginning of the test, and comparisons were made with normal seedlings, i.e., those that had grown a normal taproot and shoot. The results were expressed as a percentage. The first germination count recorded the percentage of normal seedlings on the fifth day after beginning the test. The final germination count was made on the eighth day after sowing. The seedlings were also evaluated to see if they were strong normal seedlings, following the criteria established by the Rules for Seed Analysis (Brazil, 2009).

After the germination test, seedling development was analyzed. The epicotyl and taproot length was evaluated by allowing 20 pea seeds to germinate on germitest paper in a substrate pre-moistened at the same potentials used in the germination test. The seeds were arranged in two rows containing 10 seeds that were uniformly spaced to allow the free development of the seedlings. Each treatment contained four repetitions. The rolls were placed in plastic bags and grown in the germinator under the same conditions as the germination test. On the fifth day, the epicotyl and taproot lengths of the normal seedlings were measured with a ruler.

After the seedlings had been measured, they were cut up, and the shoots and roots were left to dry for 72 h in a forced air laboratory oven regulated to 65 °C. After drying, the materials were cooled in a desiccator and weighed on an analytical balance with an accuracy of 0.001 g. The root to shoot ratio was calculated based on the dry matter values (g) obtained.

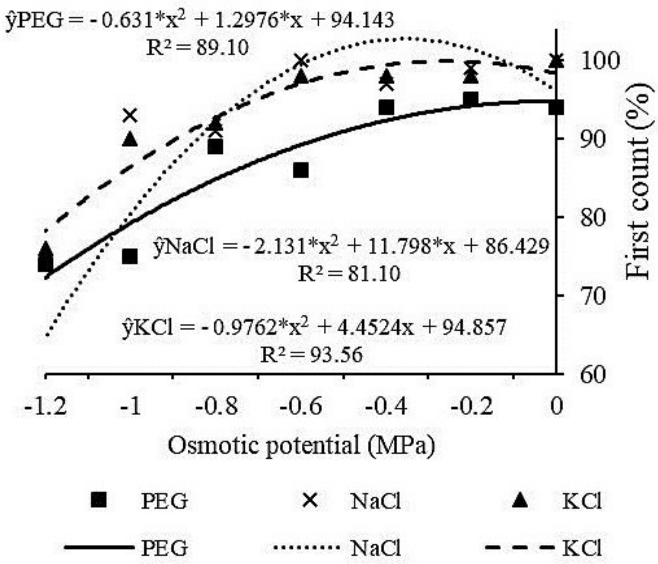
The data were subjected to analysis of variance using the F test, and the mean values were grouped by the Scott-Knott test at $p \leq 0.05$. The osmotic potential data were analyzed by polynomial regression at $p \leq 0.05$. All statistical analyses were undertaken using SISVAR statistical software (Ferreira, 2011).

Results and Discussion

The physiological quality of the pea seeds was affected by the water and salt potentials. The control (0 MPa) treatment produced the highest viability and vigor percentages. The mean percentages after the first germination count (FCG), the final germination (GERM) count, and for the strong normal seedlings (SNS) are shown in Table 1. The first count results showed that seed vigor declined when PEG was used and the potential was −0.6 MPa. However, when NaCl and KCl were used, seed vigor only began to decline at -0.8 MPa (Figure 1). There was no decline in seed vigor recorded in the −0.2 and −0.4 MPa treatments.

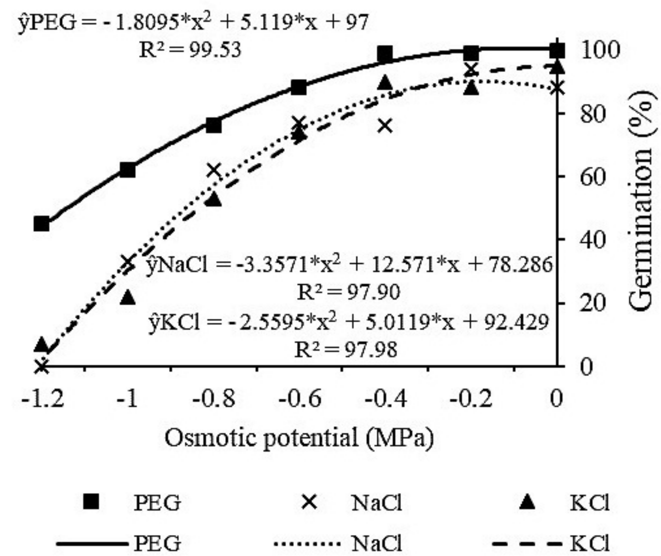
Pea seed germination began to significantly decline when the potentials was −0.6 MPa for PEG, −0.4 MPa for NaCl and −0.8 MPa for KCl (Figure 2). There was a sharp decline in the percentage of strong normal seedlings as water and saline stress increased (Table 1 and Figure 3).

Similar results have also been reported by other researchers, who found that an osmotic potential of –0.9 MPa after PEG, NaCl or mannitol treatment led to a reduction in maize and soybean seed germination (Abreu et al., 2014; Soares et al., 2015). A decline in cowpea (*Vigna unguiculata*) seed viability, vigor and quality was also reported by Ferreira et al. (2017) when the osmotic potentials were below –0.6 MPa.



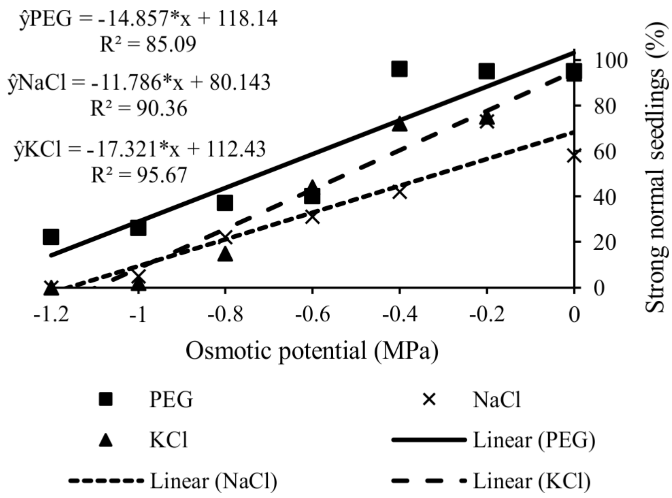
* Significant at p ≤ 0.05 by F test

Figure 1. First germination count results for pea seeds subjected to water and salt stress at different osmotic potentials



* Significant at p ≤ 0.05 by F test

Figure 2. Final germination counts of pea seeds subjected to water and salt stress at different osmotic potentials



* Significant at p ≤ 0.05 by F test

Figure 3. Strong normal pea seedlings from seeds under water and salt stress at different osmotic potentials

The reduction in germination percentage when the osmotic potential becomes more negative could be due to the increase in the length of time corresponding to phase III of the imbibition process because, according to the triphasic pattern proposed by Bewley & Black (1994), intense water uptake and taproot growth by the seed occurs in this phase. Another factor that may also explain this reduction in germination is the high molecular weight of PEG, which is not absorbed due to its high viscosity. This, together with the correspondingly low O_2 diffusion rate, can compromise the availability of oxygen to the seeds during the germination process (Braccini et al., 1996).

The seeds that were subject to water stress by PEG germinated when the osmotic potential was –0.4 MPa or above. Lower potentials reduced germination. Similar results were reported by Pereira et al. (2014) in a study on *Raphanus raphanistrum* and *Senna obtusifolia* seeds subjected to water stress (induced by PEG and NaCl). In their study, there was no germination when the seeds were subjected to an osmotic potential of –0.8 MPa, regardless of the osmotic agent used. In this study, NaCl led to the greatest decline in pea seed vigor (Figure 1). Both NaCl and KCl led to greater declines in germination than PEG when the osmotic potential was –0.6 MPa (Figure 2).

Table 1. First germination count (FGC), final germination count (GERM), and strong normal seedling percentages (SNS) for pea seeds subjected to water and salt stress at different osmotic potentials

ψo (MPa)	FGC			GERM (%)			SNS		
	PEG	NaCl	KCl	PEG	NaCl	KCl	PEG	NaCl	KCl
0	94 a	100 a	100 a	100 a	88 a	95 a	95 a	58 b	94 a
-0.2	95 a	99 a	98 a	99 a	94 a	88 a	95 a	73 b	75 b
-0.4	94 a	97 a	98 a	99 a	76 b	90 a	96 a	42 c	72 b
-0.6	86 b	100 a	98 a	88 a	77 b	74 b	40 a	31 b	44 a
-0.8	89 a	91 a	92 a	76 a	62 b	53 b	37 a	22 b	15 c
-1.0	75 b	93 a	90 a	62 a	33 b	22 c	26 a	5 b	2 b
-1.2	74 a	57 b	76 a	45 a	0 b	7 b	22 a	0 b	0 b
CV (%)		5.01			5.39			6.45	

*Mean values followed by the same lowercase letter in the row, are not significantly different according to the Scott-Knott test at p ≤ 0.05
PEG: Polyethylene glycol 6000

Mortele et al. (2008) attribute this reduction in germination to the amount of water taken up by seeds in the salt medium. A high concentration of salts is a stress factor for plants because water is osmotically retained in the salt solution. Therefore, the increase in salt concentration makes water less available to plants (Lopes et al., 2014). Species can adapt to salinity, but when they are sown in salt substrates, there is first a decrease in water uptake, which consequently reduces the speed of their physiological and biochemical processes. Excessive salt levels lead to cytotoxicity and cell dehydration (Taiz & Zeiger, 2013), and reduces metabolic activity and the synthesis of new seed tissues due to the reduction in water availability (Marcos-Filho, 2015). Low water availability results in a lower speed of germination and, in more serious cases, germination capacity losses.

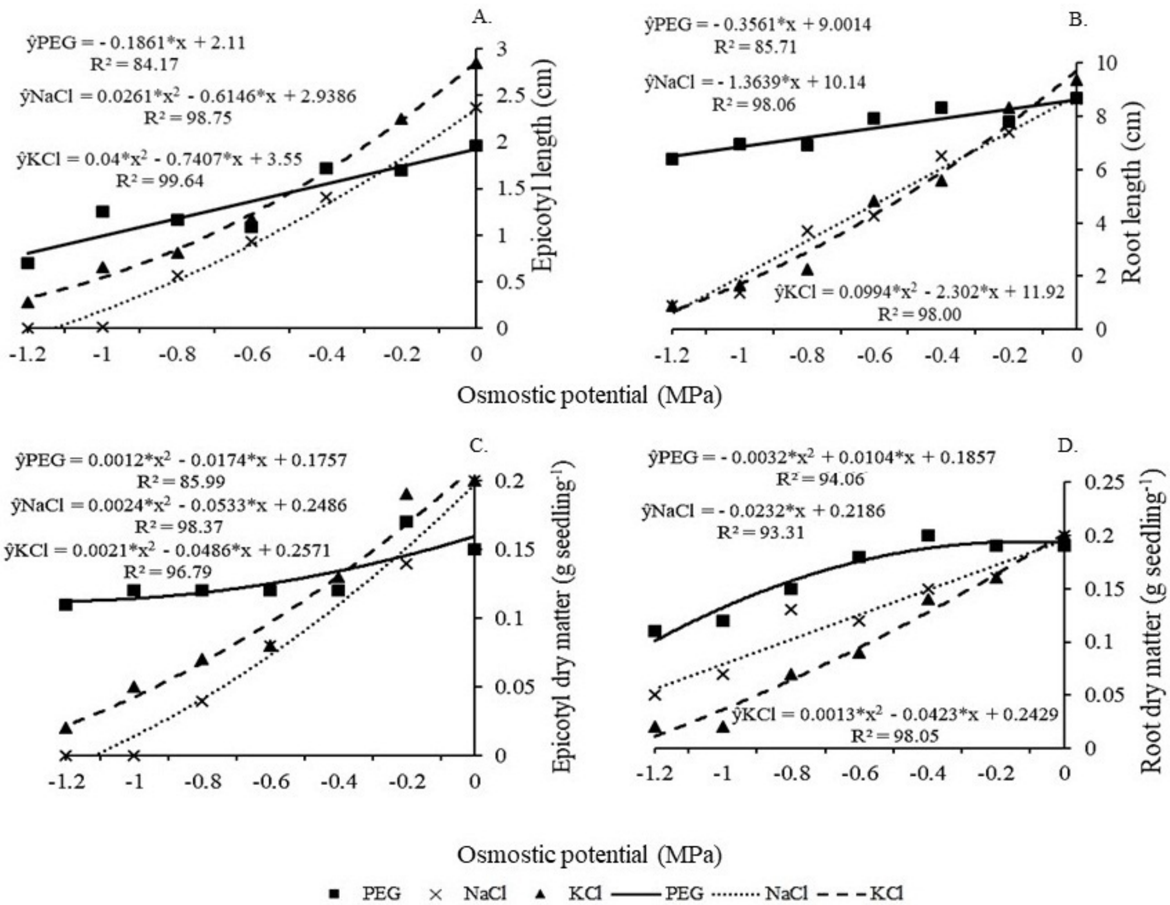
Lopes et al. (2014) suggested that the germination inhibition brought by salinity is due both to the osmotic effect, i.e., the “physiological drought” produced, and to the toxic effect, resulting from the concentration of ions in the protoplasm. Therefore, salt stress can lead to water stress, i.e., the germination of seeds that were only subjected to water stress was greater than the germination of seeds that were subjected to salt stress (Table 1 and Figure 2). This occurs due to ion toxicity and the lack of water to begin seed metabolic processes.

The regression curves plotted in Figure 3 show that there was a reduction in the number of normal seedlings as water restriction and salinity increased. When the osmotic potential was ≤ –0.2 MPa, the percentage of strong normal seedlings that were subjected to water stress was greater than the percentage of seedlings that were subjected to salt stress by KCl and NaCl (Table 1). Furthermore, when the osmotic potential was ≤ –0.6 MPa, there was no significant statistical difference between KCl and NaCl with regards to the number of strong normal seedlings produced (Figure 3).

Similar results to those found in this study were reported by Moterle et al. (2008) and Abreu et al. (2014), who evaluated the effects of water stress and salt stress on maize seeds and popcorn seeds, respectively. They recorded a reduction in the percentage of strong normal seedlings when the osmotic potential was ≤ –0.3 MPa. Similar results were also reported when the physiological and biochemical aspects of soybean cultivars were evaluated (Soares et al., 2015).

The seedlings subjected to the different osmotic potentials showed statistically significant differences in epicotyl and root lengths compared to the control, except for PEG where the osmotic potential reached –0.6 MPa before any differences in root length were recorded. Reductions in epicotyl and root length occurred when the osmotic potential reached ≤ -0.2 MPa for the NaCl and KCl conditioners (Figures 4A and B).

Similar results were also obtained by Taiz & Zeiger (2013), who reported that water and salt stress affected imbibition, and seed germination speed and percentage. They also reported that the stresses reduced seedling growth. Medeiros et al. (2015) suggested that the reduction in seedling length was due to changes in cell turgidity caused by a reduction in protein



* Significant at $p \leq 0.05$ by F test

Figure 4. Epicotyl length (A), root length (B), epicotyl dry matter (C), and root dry matter (D) of the seedlings from pea seeds subjected to water and salt stress at different osmotic potentials

synthesis under water stress conditions. Furthermore, Taiz & Zeiger (2013) reported that the first measurable effect of water stress is reduced growth, which is caused by a reduction in cell expansion.

The pea seedling root length results showed that decreases caused by PEG only occurred when the osmotic potential was ≤ –0.6 MPa. The results also showed that the longest roots occurred in the control treatment (0 MPa) (Figure 4B). This difference is probably because vigorous seeds give rise to seedlings with a higher growth rate due to their greater capacity for transforming supplies from storage tissues reserves and their incorporation by the embryonic axis (Nascimento et al., 2017).

The results showed that excess salt significantly reduced root growth because it reduced the osmotic potential (Figure 4B). In general, excess salt in the plant root zone has harmful effects on growth. The hypothesis that best explains this is that excess salinity reduces growth and development and may even cause plant death because of the greater osmotic effect outside the root and the resulting restriction in water flow from the soil into plants, which plants need if they are to survive and grow under salt stress conditions (Gomes et al., 2015).

The dry matter values of the seedling epicotyl and roots were affected by the reduction in water and the increase in salt concentration (Figures 4C and D). Both decreased the soil osmotic potentials, which confirmed the results reported by Nascimento et al. (2017). The epicotyl dry matter of the seedlings declined when the osmotic potential reached ≤ –0.4 MPa, regardless of the salt conditioner used. However, this decrease only occurred when the osmotic potential was ≤ –0.8 MPa for PEG. There were no significant differences among the potentials above –0.8 MPa for PEG or –0.4 MPa for the salt conditioners.

Seedling development processes are sensitive to the effect of salts, which means that growth rate and biomass production are good criteria for evaluating the degree of stress and the capacity of a plant to overcome salt stress (Gomes et al., 2015). Nascimento et al. (2017) reported that the dry matter values for *Phaseolus lunatus* seedling shoots and roots (biomass) were also affected by a reduction in moisture content.

These reductions in dry biomass and seedling growth can be explained by the reduction in seed metabolism due to the lower availability of water, which is needed for the digestion of reserves and the translocation of metabolized products (Bewley & Black, 1994). The large accumulation of Na^+ and Cl^- in plant tissues during seedling exposure to salt stress is one of the main effects of salt stress on plant metabolism because the ionic component of salinity can cause irreparable damage to cell structures. This can compromise metabolic efficiency and even lead to cell death (Silva et al., 2016).

Conclusions

1. Simulation of water and salt stress in the laboratory by creating different osmotic potentials using conditioners has confirmed the effects of salt and water stress on pea seed physiological performance and on seedling development because the values for the variables evaluated decreased as the osmotic potential decreased.

2. Water stress and salt stress induced by the NaCl and KCl solutions were more harmful than the stress induced by PEG 6000 for the specie studied.

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