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Full Length Research Paper

Effect of water and salt stress on the vigor and viability of seeds of pitaya genotypes using different osmotic potential gradients

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Lack of water and salinity are commonly encountered problems in many regions worldwide. For this reason, certain robust cactus species may represent promising crops. Because it is necessary to assess the ability of cactus species to survive and adapt under conditions of natural stress, the present study aimed to evaluate the effect of water and salt stress on the vigor and viability of seeds of pitaya genotypes using different osmotic potential gradients and different osmotically active agents. The experiment had a completely randomized design with a 3 × 6 × 4 factorial scheme corresponding to three pitaya genotypes (white, hybrid I, and hybrid II), six osmotic potential gradients (0.0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa), and four osmotically active agents (PEG 6000, KCl, NaCl, and $MgCl_2$), with four replicates. The following variables were analyzed: germination percentage, germination speed index (GSI), and mean germination time (MGT). Statistical analyses were performed for each pitaya genotype. The data pertaining to germination were fitted to a binomial model; the data pertaining to GSI and MGT were fitted to regression models. The germination, GSI, and MGT values for all three pitaya genotypes were optimal with the osmotically active agents KCl and NaCl, regardless of the osmotic potential gradient. At osmotic potential gradients lower than -0.2 MPa, the PEG 6000 polymer was detrimental to pitaya seed vigor and viability. The pitaya hybrid I seeds were more resistant to the adverse conditions, exhibiting higher rates of germination and GSI than those of the other genotypes. The osmotic effect negatively influenced the vigor and viability of seeds of the three pitaya genotypes to a greater extent than the salt effect.

Key words: Cactaceae, *Hylocereus undatus*, *Hylocereus costaricensis*, polyethylene glycol, vigor.

INTRODUCTION

With climate change occurs worldwide and water scarcity becoming increasingly pronounced in many areas, the species of family Cactaceae, which can be produced under conditions of limited water resources, have become promising for the future of mankind. The pitaya can adapt to different environmental conditions and has therefore been introduced into countries with different edaphoclimatic conditions; because of Its robustness, this, crop may, represent a cultivation option in unfavorable areas (Mizrahi et al., 2002; Tel-Zur et al., 2004).

The organoleptic characteristics and nutraceutical properties of the pitaya have made this crop attractive to the consumers; in addition, certain pitaya species are rich in antioxidants, vitamins, and fiber and are sources of vitamin A, phosphorus, calcium, potassium, and sodium (Crane and Balerdi, 2005; Wu et al., 2006; Esquivel et al., 2007).

Because the pitaya culture can be propagated by seeds, it becomes necessary to evaluate the germination process these in different conditions in order to know the possible factors influencing it; since the germination percentage is dependent on external and internal factors regarding the seed, for example, water, salinity, oxygen, temperature, light, the substrate, health, etc.

Water is one of the most important environmental factors influencing the seed germination process, because is the matrix where most of the biochemical and physiological processes occur; with reactivation of metabolism; participates in enzymatic reactions, in solubilization, and in the transport of metabolites, besides being a reagent in the hydrolytic digestion of proteins, carbohydrates and lipids of seed reserve tissues (Marcos Filho, 2005; Virgens et al., 2012).

Imbibition depends on the osmotic potential gradient (water tension) that exists between the seed and the external environment (Ávila et al., 2007). When the osmotic potential of the solution is lower than the potential within the embryonic cells, absorption of the water necessary for the seed to germinate becomes difficult, affecting the uniformity, speed, and percentage of seed germination, which, in turn, affects cell elongation, cell wall synthesis, and seedling formation (Marcos Filho, 2005; Machado Neto et al., 2006).

Thus, for the germination process to occur, it is essential that the moisture content, which is dependent on seed chemical composition and testa permeability, be minimal (critical point). For each species, there is a critical osmotic potential value below which germination does not occur (Carvalho and Nakagawa, 2012).

Salinity is an important environmental issue that can adversely affect crops worldwide, especially in arid and semi-arid regions. The sensitivity of species to salinity during the germination phase can affect the establishment of a crop and may affect the crop productivity. Salts act on the osmotic potential of the substrate, reducing the potential gradient between the substrate and the seed surface and thus restricting water uptake by the seed (Oliveira et al., 2011).

The reduced osmotic potential associated with salt toxicity not only prevents seeds from absorbing water but also affects seed germination, cell division and elongation, reserve mobilization, and the development of many species in different regions (Lima et al., 2005; Marcos Filho, 2005; Nogueira et al., 2005). It is known that high total salt concentrations in cells can inactivate enzymes and inhibit protein synthesis (Taiz and Zeiger, 2013). However, because adaptation to stressful conditions results in integrated events that occur at several levels, the mechanisms by which plants tolerate high levels of salinity remain unclear due to involve morphological, anatomical, cellular, biochemical, physiological, and molecular changes (Zhu, 2002; Abreu et al., 2008). These changes vary with plant species, the development stage of the plant, and the type, duration, and intensity of the stress (Larcher, 2000).

The ability of plants to tolerate water and salt stress has been extensively studied, with the goal of finding species that are more resistant to these conditions. In these studies, solutions with different osmotic potentials are used to moisten substrates (usually paper towels), and seeds are placed to germinate on these substrates in an attempt to simulate conditions of water and salt stress; the point of tolerance of different species to drought and salinity is then identified. Certain authors have observed that saline solutions have the strongest effect on seed germination, whereas other authors have observed that polyethylene glycol (PEG) solutions more strongly affect germination; some authors found that both solutions had equal effects on germination (Moraes and Menezes, 2003; Duan et al., 2004; Sosa et al., 2005; Zhang et al., 2010).

Water restriction is usually induced by adding osmotically active solutes such as PEG, potassium chloride (KCl), sodium chloride (NaCl), and magnesium chloride ($MgCl_2$). However, each osmotic agent has chemical differences that may affect seed germination differently, even in the presence of similar osmotic potentials.

PEG, which is not easily metabolized by living organisms, not absorbed by cells because of its high molecular weight (>4000), and is chemically inert, stable, and non-toxic to seeds, has been extensively used in seed germination studies (Mexal et al., 1975; Santos et al., 2008). Studies of the germination response of seeds subjected to conditions of artificial stress are important ecophysiological tools that can be used to understand the ability of species to survive and adapt to conditions of natural stress (such as drought and saline soils, which are commonly encountered in agricultural regions). These tools are also used to evaluate the sensitivity of these species and their ability to adapt aggressiveness and dominance strategies when subjected to adverse and/or new environments (Larcher, 2000; Rosa et al., 2005; Pereira et al., 2012).

In light of the diversity of the family Cactaceae, basic information on the optimal germination conditions of this plant group is lacking. Thus, the present study aimed to evaluate the effect of water and salt stress on the vigor and viability of seeds of pitaya genotypes using different osmotic potential gradients and different osmotically active agents.

MATERIALS AND METHODS

The study was conducted in May 2012 at the Seed Production and Technology Laboratory of Londrina State University (Universidade

Estadual de Londrina - UEL) located in Londrina, Paraná state, Brazil. The seeds were collected from ripe fruit from mother plants of three pitaya genotypes - *Hylocereus undatus* (white pitaya); *H. undatus* x *H. costaricensis* (pitaya hybrid I), and *H. costaricensis* x *H. undatus* (pitaya hybrid II) – grown at the experimental site of the UEL Department of Agronomy located at 23°23’ S and 51°11’ W at a mean altitude of 566 m. The approximately 10-year-old pitaya plants were grown on an area of soil classified as eutrophic, latosolic red nitosol (Embrapa, 2013). The plants were spaced 2.0 × 3.0 m apart and trellised with 2.5 m tall stands, with two plants per stand.

The pulp was manually extracted from the fruit with a spoon and placed in a beaker containing a solution of water (1 L) and sucrose (25 g L^{-1}); the mixture was left for 48 h at room temperature to promote the fermentation process and facilitate seed extraction. Subsequently, the solution was sieved under running water to eliminate the pulp residues and retain the seeds. The seeds were then placed on paper and shade-dried at room temperature for 48 h.

The following were used for the study: three pitaya genotypes (*Hylocereus undatus* (white pitaya); *H. undatus* x *H. costaricensis* (pitaya hybrid I), and *H. costaricensis* x *H. undatus* (pitaya hybrid II)), six osmotic potential gradients (0.0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa), and four osmotically active agents (PEG 6000 polymer and potassium chloride (KCl), sodium chloride (NaCl), and magnesium chloride ($MgCl_2$) salts) in a 3 x 6 x 4 factorial scheme. The experimental design was completely randomized with four replicates.

The osmotic potential gradients were obtained according to the methods described by Braccini et al. (1998). The physiological quality of the seeds was evaluated by conducting a germination test in which 50 seeds per replicate were arranged in crystal polystyrene boxes (Gerbox®) lined with blotting paper moistened with the respective solutions for each treatment (using two and a half times the dry weight of the substrate). The experiment was conducted in a germinator with a 24-hour photoperiod regulated with a fluorescent lamp and kept at a constant temperature of 25°C.

The seeds were evaluated daily for 23 days (that is, at which point the germination process was stabilized), and seeds were considered germinated when they exhibited root extension greater than or equal to 2 mm. The following variables were analyzed: germination percentage (G); germination speed index (GSI), calculated according to the method described by Maguire (1962); and mean germination time (MGT), in days, determined according to the method described by Lima et al. (2006).

The statistical analyses were performed using the software R (R DEVELOPMENT CORE TEAM, 2014). To test the germination potential of the three pitaya genotypes exposed to the different osmotic potential gradients and osmotically active agents, the data were fitted to three binomial models (one for each pitaya genotype), with the percentage of germinated seeds as the response variable. The variability of the observed responses was greater than the variability that the binomial model is able to accommodate, that is, the data exhibited over dispersion. To accommodate this extra variability, the variances of the binomial model parameters were multiplied by the heterogeneity factor (Mascarin et al., 2010). For the variables GSI and MGT, the data were fitted to regression models in which the explanatory variables were the osmotic potential gradients and the osmotically active agents. To test the fit of the models, semi-normal probability plots were constructed with simulation envelopes; the model provides a good fit if most of the points are within the envelope (Urbano et al., 2013).

RESULTS

Figure 1 shows the germination percentages of the white pitaya (A), pitaya hybrid I (B), and pitaya hybrid II (C) genotypes with the different osmotically active agents (KCl, $MgCl_2$, NaCl, and PEG) at each osmotic potential gradient (MPa).

Regardless of the osmotic potential gradient of the KCl and NaCl salts, the germination percentages of the white pitaya (Figure 1A) did not significantly differ (approximately 82% and 87%, respectively). For the $MgCl_2$ salt, lower osmotic potential gradients were associated with lower germination percentages (decreasing from 96% at 0.0 MPa to 42% at -1.0 MPa).

The PEG 6000 polymer was the osmotically active agent that most negatively affected white pitaya seed germination; reduced germination percentages were observed as the osmotic potential gradient decreased (from 97% at 0.0 MPa to 0% at -0.8 and -1.0 MPa). In other words, the white pitaya genotype did not germinate at osmotic potential gradients lower than -0.6 MPa.

For pitaya hybrids I and II (Figure 1B and C), the germination percentages (approximately 90% and 83%, respectively) did not significantly differ when different KCl, $MgCl_2$, and NaCl salt concentrations were used, that is, germination is statistically equal regardless of the osmotic potential gradient.

With PEG, the seed germination of both genotypes decreased as the osmotic potential gradient decreased. The germination of pitaya hybrid I was 97% at 0.0 MPa and 0% at -1.0 MPa; the germination of pitaya hybrid II decreased from 93% at 0.0 MPa to 0% at osmotic potential gradients lower than -0.6 MPa. Osmotic potential gradients lower than -0.2 MPa obtained with PEG were unfavorable for the germination of the seeds of all three genotypes.

It is noteworthy that for all of the osmotically active agents and for all osmotic potential gradients, the pitaya hybrid I achieved better seed germination than the other two genotypes, indicating that the pitaya hybrid I seeds are more resistant to the adverse conditions to which they were exposed.

The germination speed index (GSI) of all three pitaya genotypes (Figure 2A, B and C) did not significantly differ at the different osmotic potential gradients achieved with KCl and NaCl. However, for all three genotypes, the GSI decreased with decreasing osmotic potential gradient (MPa) for the osmotically active agents $MgCl_2$ and PEG.

PEG led to a more pronounced decrease in GSI for all of the pitaya genotypes: for the white pitaya, hybrid I, and hybrid II, the GSI decreased from 21.62, 22.30, and 17.91 at 0.0 MPa to less than 4.96 at -0.6, -0.8, and -0.6 MPa, respectively. The GSI was not calculated at potentials below -1.0 MPa for any of the genotypes, as no white pitaya or pitaya hybrid II seeds germinated at osmotic potentials lower than -0.6 MPa and no pitaya hybrid I seeds germinated at osmotic potentials lower than -0.8 MPa.

The GSI of pitaya hybrid I seeds (Figure 2) was higher than that of the other two genotypes under all conditions,

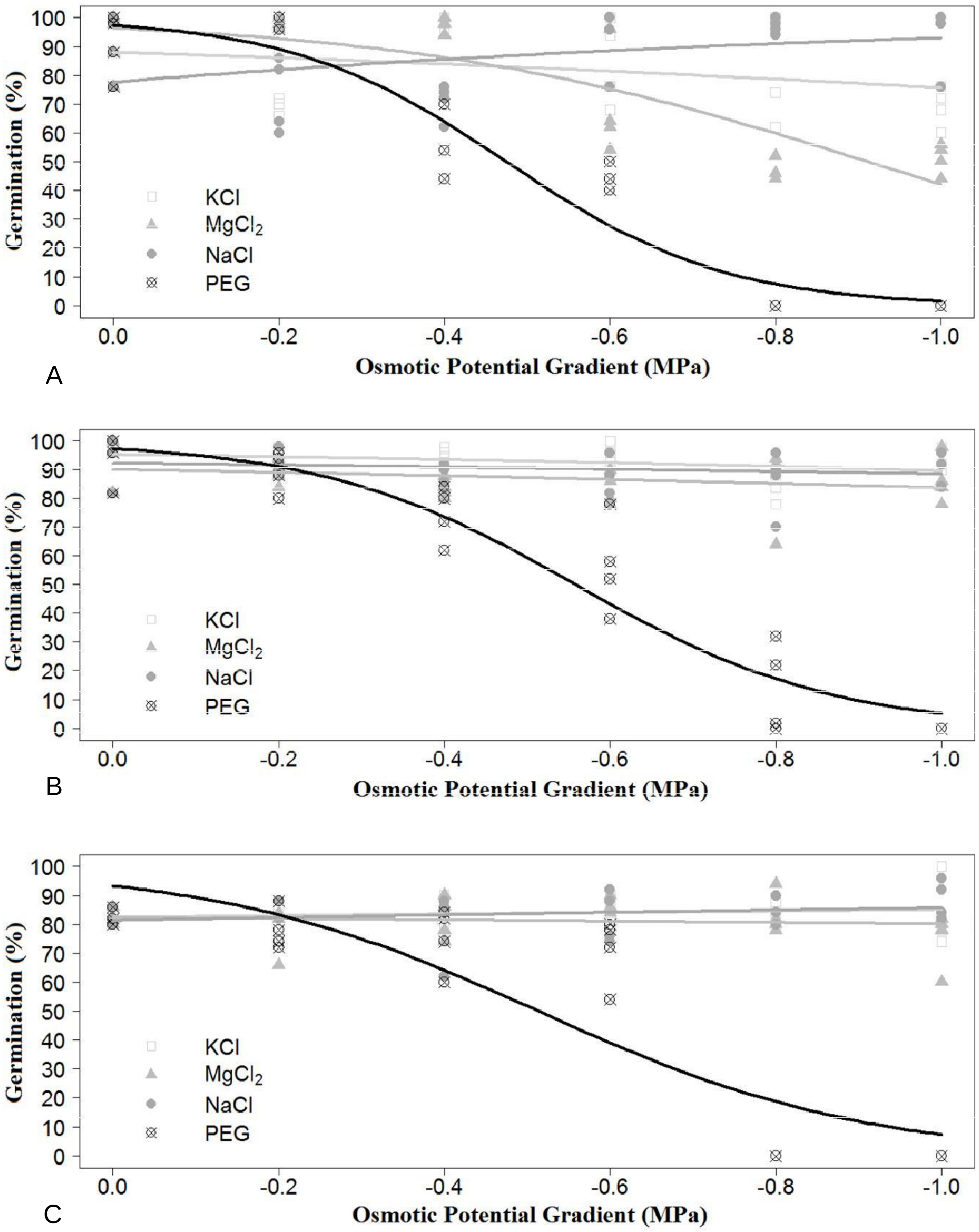

Figure 1. Germination (%) of white pitaya (A), pitaya hybrid I (B), and pitaya hybrid II (C) seeds exposed to different osmotically active agents (KCl, $MgCl_2$, NaCl, and PEG) and osmotic potential gradients (MPa) with fitted curves for the models.

indicating that this genotype develops to a greater extent under adverse conditions than the other genotypes.

For all three pitaya genotypes, the mean germination time (MGT - days) did not significantly differ by osmotic potential gradient (MPa) with the KCl and NaCl salts (Figure 3); the average MGT was 4.98 days for the white

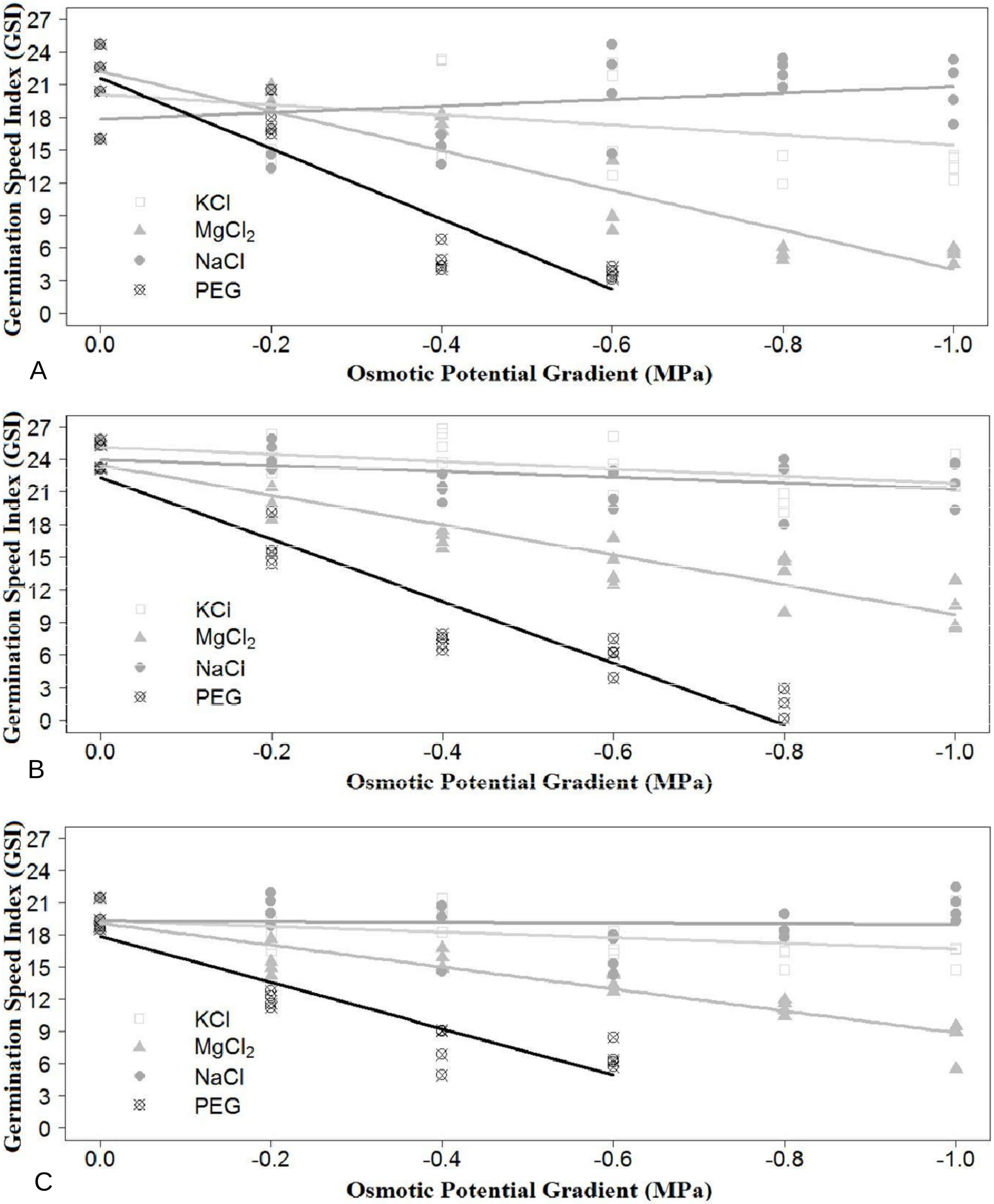

Figure 2. Germination speed index (GSI) of white pitaya (A), pitaya hybrid I (B), and pitaya hybrid II (C) seeds with different osmotically active agents (KCl, $MgCl_2$, NaCl, and PEG) and osmotic potential gradients (MPa) with fitted curves for the models.

pitaya (Figure 3A), 4.16 days for pitaya hybrid I (Figure 3B), and 4.83 days for pitaya hybrid II (Figure 3C).

For seeds exposed to $MgCl_2$, the MGT increased with decreasing osmotic potential gradient, indicating that osmotic potential gradients lower than - 0.4 MPa increase the duration of the germination process. In the field, such

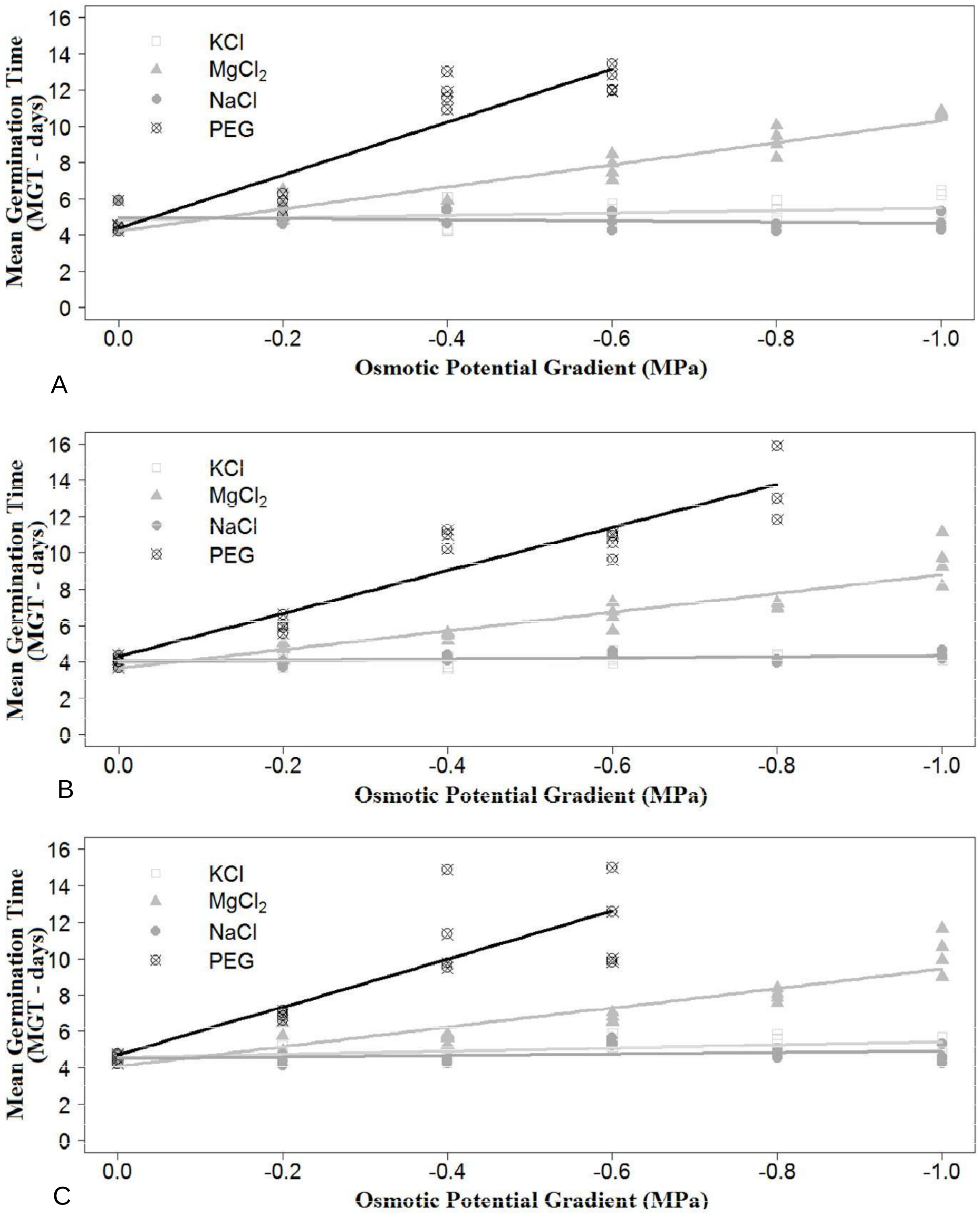

Figure 3. Mean germination time (MGT - days) of white pitaya (A), pitaya hybrid I (B), and pitaya hybrid II (C) seeds exposed to different osmotically active agents (KCl, MgCl2, NaCl, and PEG) and osmotic potential gradients (MPa) with fitted curves for the models.

an increased germination time would be detrimental to the crop, as the seeds would be exposed to unfavorable edaphoclimatic conditions for longer periods of time.

For the three pitaya genotypes, the MGT of seeds exposed to substrates moistened with PEG varied with the osmotic potential gradient; more specifically, lower gradients resulted in higher MGTs. The MGT was 4.39 days at 0.0 MPa and 13.17 days at -0.6 MPa for the white pitaya; the MGT increased from 4.30 and 4.69 days at 0.0 MPa to 13.77 and 12.64 days at -0.8 and -0.6 MPa for pitaya hybrids I and II, respectively. When the gradients were lower than -0.6 MPa for the white pitaya and pitaya hybrid II and lower than -0.8 MPa for pitaya hybrid I, no seeds germinated and it was impossible to calculate the MGT. It is noteworthy that PEG and osmotic potential gradients lower than -0.2 MPa were detrimental to the MGT for all of the pitaya genotypes.

DISCUSSION

According to Mizrahi et al. (1997), cacti in general are drought-tolerant and salt stress-susceptible. However, to confirm the tolerance and/or susceptibility of a species, its developmental stage should be considered. Strogonov (1964) studied plant physiological responses and concluded that the occurrence of salinity-related damage depends on the vegetative phase of the plant.

The three pitaya genotypes evaluated in this study were water stress-susceptible during the germination-emergence stage at osmotic potential gradients lower than -0.2 MPa obtained with the PEG 6000 polymer; the germination, GSI, and MGT were all lower at these gradients than at 0.0 MPa (distilled water). Similar results were obtained by Moraes and Menezes (2003) who found that PEG 6000 produces more stressful effects on the performance of *Glycine max* seeds than KCl, $MgCl_2$, and NaCl salts when the osmotic potential is reduced to - 0.8 MPa. This most likely occurs because the PEG solutions exhibit high viscosity, thus limiting the amount of O_2 available to the seeds and consequently reducing their germination potential (Yoon et al., 1997).

According to Moraes and Menezes (2003), osmotic potentials of -0.8 MPa induced by PEG 6000 and $MgCl_2$ prevent germination and reduce the vigor of *G. max* seeds. In the present study, the -0.6 MPa potential was able to prevent germination in the white pitaya seeds and pitaya hybrid II seeds and the -0.8 MPa potential produced the same effect in pitaya hybrid I. However, in solutions of $MgCl_2$, the germination process remained uninhibited for all of the genotypes evaluated until the potential reached a value of -1.0 MPa.

The results pertaining to the salt effect were inconsistent with those obtained by Mizrahi et al. (1997); in this study, none of the variables differed significantly when the seeds were exposed to substrates moistened with distilled water or with KCl and NaCl regardless of the osmotic potential gradient, indicating that all of the genotypes were salt-stress tolerant. Corroborating the results obtained in this study, Ungar (1978) reported that mannitol and PEG have a more pronounced inhibitory effect on several halophytes than inorganic ions, indicating that the seeds are affected by osmotic stress rather than by the toxicity of specific ions.

Salinity affects seed germination via osmotic effects (Bliss et al., 1986), ion toxicity (Hampson and Simpson, 1990), or both (Huang and Redmann, 1995). Zehra et al. (2013) support the claim that different salts produce both osmotic and ionic effects on seed germination and vigor; so only one salt is not applicable to field conditions, which is a mixture of different salts. For this reason, different osmotically active agents (KCl, $MgCl_2$, NaCl, and PEG) were used in this study to determine the specific toxicity of ions in different pitaya genotypes.

It is known that the osmotic and toxic effects of salts are exerted simultaneously on plants: toxicity directly affects plant physiological and metabolic processes, whereas the osmotic factor acts indirectly by reducing osmotic pressure and consequently limiting the absorption of water and nutrients (Hu and Schmidhalter, 2005). According to Zhang et al. (2010), ionic effects can be distinguished from osmotic effects by comparing the effects of saline solutions and iso -osmotic solutions with those of an inert osmotic agent such as PEG (which cannot penetrate the cell wall). Germination inhibition in PEG-treated seeds is attributed to osmotic effects only, and any difference in the germination of salt-treated seeds and PEG-treated seeds is usually attributed to ionic effects (Dodd and Donovan, 1999).

Thus, in this study, the osmotic effect was more detrimental to seed vigor and viability in the three pitaya genotypes than the toxic effect of the salts; indeed, reduced germination, GSI, and MGT occurred in the seeds exposed to the PEG solution (which is not a salt), suggesting that the osmotic effect was responsible.

According to Gulzar and Khan (2001), the salinity threshold for a significant decrease in germination varies among species. Moraes and Menezes (2003) suggest that the negative effects caused by salt stress may be related to the type of salt used. According to Sosa et al. (2005), the germination of *Prosopis strombulifera* seeds was not only affected by salt concentrations (or osmotic potential) but also by the nature of the ions in the saline solutions and their interactions. These authors found that the osmotic agent KCl inhibited germination to a greater extent than NaCl; indeed, at an osmotic potential of -0.8 MPa, the germination percentage was inhibited by a KCl solution but not by a NaCl solution. High intracellular concentrations of Na^+ and Cl^- can inhibit cell division and expansion, slowing seed germination and even leading to seed death (Neumann, 1997; Zhang et al., 2010).

For Duan et al. (2004), the germination of *C. glaucum* seeds decreased with increasing salinity, and germination was inhibited to a greater extent by $MgCl_2$ than by NaCl. Zehra et al. (2013) found that $MgCl_2$ had more pronounced toxic effects on *Phragmites karka* seeds than KCl, which, in turn, was more toxic than NaCl.

In the present study, $MgCl_2$ was the only salt that reduced the GSI and increased the MGT for all three pitaya genotypes and delayed germination in the white pitaya. At osmotic potential gradients lower than -0.4 Mpa, the toxic effect of this salt was intensified.

Ungar (1978) reported that the toxic effects of specific ions have less influence on seed germination than the osmotic potential. This phenomenon was observed in the present study, as PEG (i.e., the osmotic effect) reduced germination, the GSI, and the MGT more than the saline solutions (ion toxicity).

Corroborating the results obtained in the present study for the pitaya genotypes, Zhang et al. (2010) assessed the germination and GSI of *Hordeum vulgare* seeds exposed to PEG or NaCl and found that both variables were higher in the saline solution and that germination occurred faster and at lower osmotic potentials in seeds exposed to NaCl. Thus, these authors suggested that seeds incubated in NaCl were less negatively affected by osmotic potential or better able to adapt to decreasing osmotic potentials than seeds incubated in PEG. According to these authors, *H. vulgare* seeds absorb sodium, which results in an additional osmotic potential, higher water absorption, and faster germination, even in environments with lower osmotic potential.

In contrast, for Sosa et al. (2005), the germination of *P. strombulifera* seeds was greater in treatments with PEG than in all of the salt treatments at osmotic potential gradients less than or equal to -0.8 MPa. Additionally, Katembe et al. (1998) demonstrated that high NaCl concentrations were more inhibitory to water absorption, germination, and seedling root length in *Atriplex prostrata* than PEG. In opposition to, Duan et al. (2004) found that similar concentrations of NaCl and PEG have similar effects on *C. glaucum* germination.

Because sodium chloride is commonly encountered in soils (Khan and Gul, 2006) and causes salinization, it has been extensively used in studies of germination. Thus, it is not surprising that plants have developed mechanisms to regulate the accumulation of sodium chloride (Munns and Tester, 2008). However, other chloride-, sulfate- , and carbonate-based salts and their interactions can also affect seed germination (Khan, 2002).

Zehra et al. (2013) found that the germination of *P. karka* seeds decreases with increasing salinity and attributed this decrease to ion toxicity and variable osmotic stress due to composition of salts; more specifically, the K^+ cation was usually the most toxic, followed by Mg^{2+} and Na^+. However, the salts did not affect seed viability, which suggests that the seeds went into dormancy. Shaikh et al. (2007) found that the germination of *Urochondra setulosa* seeds was inhibited by increasing concentrations of salts (NaCl, Na_2SO_4, $MgSO_4$, and KCl).

Luders and McMahon (2006) found that the pitaya does not tolerate saline environments. However, Bárcenas-Abogado et al. (2002), in a comparative study of different *Hylocereus*, found that the number of shoots and the root dry matter yield did not decrease with increasing salinity. These findings confirm the results of the present study that found that the salt effect was not detrimental to the plants at the development stage. In contrast, Cavalcante et al. (2008) found that salinity resulted in reduced plant height, stem diameter, root length, number of additional stems, and dry root and shoot weight in *H. undatus;* these authors also found that the stem tissues collapsed under conditions of high salinity and concluded that pitaya roots are as sensitive to salt effects as the shoots.

Information on pitaya salt tolerance remains limited. However, the fact that this species displays cross fertilization and can consequently experience high genetic variability may explain the different saline classifications recorded in the scientific literature (Cavalcante et al., 2008). The pitaya hybrid I seeds were more resistant to the adverse conditions of water restriction and salinity, as they exhibited higher germination and GSI values than the other genotypes. This result may be explained by heterosis. Coimbra et al. (2006) also concluded that achieving heterosis in hybrid rice cultivars is one of the most important technical applications of genetics in agriculture and can produce more vigorous cultivars with higher production.

For species such as the pitaya (*H. undatus*), the critical level of saline solution has not yet been determined. Thus, studies involving salt tolerance are valuable, especially in regions with arid and semi-arid climates where these conditions are a major environmental problem (Cavalcante et al., 2008).

Conclusion

The germination, germination speed index, and mean germination time were more optimal for all three genotypes in the presence of the osmotically active agents KCl and NaCl, regardless of the osmotic potential gradient.

At osmotic potential gradients lower than -0.2 MPa, the PEG 6000 polymer was detrimental to pitaya seed vigor and viability.

The pitaya hybrid I seeds exhibited higher germination percentages and GSI values than the other genotypes and were thus more resistant to the adverse conditions to which they were exposed.

The osmotic effect negatively influences the vigor and viability of the three pitaya genotypes to a greater extent than the salt effect.

Conflict of Interest

The authors have not declared any conflict of interest.

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