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Original Article

Does silicon attenuate PEG 6000-induced water deficit in germination and growth initial the seedlings corn

O silício atenua o déficit hídrico induzido pelo PEG 6000 na germinação e crescimento inicial das mudas de milho

L. I. S. Sousa[a], A. E. A. Brito[a], L. C. Souza[a*], K. B. S. Teixeira[a], V. R. Nascimento[a], G. D. P. Albuquerque[a], C. F. Oliveira Neto[a], R. S. Okumura[b], G. A. S. Nogueira[a], J. M. N. Freitas[a] and G. G. T. N. Monteiro[a]

[a]Universidade Federal Rural da Amazônia – UFRA, Instituto de Ciências Agrárias, Grupo de Estudos da Biodiversidade em Plantas Superiores, Laboratório de Fisiologia Vegetal, Belém, PA, Brasil

[b]Universidade Federal Rural da Amazônia, Instituto de Ciências Agrárias, Parauapebas, PA, Brasil

Abstract

Water stress limits the initial growth and development of maize mass and grain, as well as the physiological process for absorbing the amount of mineral elements. The objective was to evaluate the effect of silicon on germination and growth of corn seedlings submitted to water deficit. The experiment was carried out in the laboratory and the experimental design was completely randomized (factorial 3 × 4), with three concentrations of calcium silicate (0.0; 1.0 and 2.0 mM) and 4 solutions of PEG-6000 to simulate different osmotic potentials (0, 0; -0.3; -0.6; -0.9 MPa). Germination percentage, germination speed index (GSI), mean germination time (MGT), percentage of non-germinated and abnormal germinated, length and dry matter of shoot, root and total seedlings were evaluated. Water deficiency reduced the parameters TG, GSI and MGT. The water deficit reduce the MSPA, MSR and MST with more than 80% reduction in mass from seedlings without deficiency to seedlings with deficiency. For CPA, CR and CT there was a reduction of at least 87%, 70% and 77%, respectively, among seeds without deficiency compared to seeds submitted to deficiency. The use of silicon in corn seeds did not attenuate the stress caused by water deficit simulated by PEG-6000.

Keywords: Esther deficit, silicate, vigor, *Zea mays*.

Resumo

O estresse hídrico limita o crescimento inicial e o desenvolvimento da massa e do grão de milho, assim como no processo fisiológico para a absorção da quantidade de elementos minerais. Objetivou-se avaliar o efeito do silício na germinação e no crescimento de plântulas de milho submetidas a deficiência hídrica. O experimento foi desenvolvido em laboratório e o delineamento experimental foi inteiramente casualizado (DIC) (fatorial 3 × 4), sendo três concentrações de Silicato de cálcio (0,0; 1,0 e 2,0 mM) e 4 soluções de PEG-6000 a simular diferentes potenciais osmóticos (0,0; -0,3; -0,6; -0,9 MPa). Foram avaliados a porcentagem de germinação, o índice de velocidade de germinação (IVG), o tempo médio de germinação (TMG), a porcentagem de não germinadas e de germinadas anormais, o comprimento e matéria seca da parte aérea, raiz e total das plântulas. A deficiência hídrica diminuiu os parâmetros TG, IVG e TMG. O déficit hídrico reduz a MSPA, MSR e MST com mais de 80% de redução da massa das plântulas sem deficiência para as plântulas com deficiência. Para CPA, CR e CT houve redução de, no mínimo, 87%, 70% e 77%, respectivamente entre as sementes sem deficiência em comparação as sementes submetidas a deficiência. A utilização de silício em sementes de milho não atenuou o estresse causado pela deficiência hídrica simulada por PEG-6000.

Palavras-chave: déficit hídrico, silicato, vigor, *Zea mays*.

1. Introduction

Corn (*Zea mays* L.) is a crop that has economic relevance because it is one of the most produced grains on the planet (USDA, 2016). Water stress limits the initial growth and development of corn mass and grain, as well as the physiological process for absorbing the amount of mineral elements (Quintas, 2022). It stands out for being one of the crops of great interest to the Brazilian economy and is the second crop that most produces grains in Brazil, having a significant share in exports, in addition to meeting the consumption of people, animals and bioenergy (Simão, 2016).

*e-mail: lumasouza30@hotmail.com
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Global climate change will reduce the productivity of the most valuable crops and induce negative impact on ecological fitness causing significant losses in major crops (Ferguson, 2019). Drought is one of these consequences and is considered a primary abiotic constraint, which affects agricultural production around the world, such as corn. Therefore, the most vulnerable stages to water restriction and saline stress are germination and seedling growth (Waterworth et al., 2015). Seed germination is negatively affected by water deficit, causing significant decreases in crop yield (Guo et al., 2017). In seeds, polyethylene glycol 6000 (PEG-6000) is one of the most used solutes to induce water deficit. It is a solute that is not absorbed by the seeds, but affects the absorption of water by generating negative osmotic potentials, consequently it is harmful to the germination process (Nonogaki et al., 2010).

In this context, the adoption of strategies such as the use of more tolerant genotypes, application of growth regulators, use of osmoprotectants and the induction of tolerance through techniques called priming have been used to overcome or minimize the damage caused by these abiotic factors during these stages (Li and Liu, 2016). In addition to these techniques, a promising alternative that can be used to mitigate the negative effect of water deficit is the use of silicon.

Many works address how silicon attenuated water deficit in growth parameters, biomass production, enzyme activity of the antioxidant system and parameters of gas exchange, chlorophyll a fluorescence in sorghum cultures (Ahmed et al., 2011), corn (Sattar et al., 2016), wheat (Ahmad et al., 2007) and rice (Chen et al., 2011). Hasanuzzaman et al. (2018), studying the effect of silicon on the germination of Brassica napus seeds under water deficit, found that the exogenous application of Si (1 mM SiO_2) significantly induced tolerance to short-term drought exposure (10 and 20% PEG).

Among the alternatives for reducing water deficit, silicon (Si), despite not being considered an essential element for plant growth and development, has been associated with several beneficial effects, among which stand out tolerance to water deficit, stress saline and lower plant transpiration (Lima et al., 2011). Its importance for plants is directly related to plant-environment interactions, as it provides better conditions to tolerate the adversities of the environment, generating significant results in productivity and improvement in product quality (Zuffo and Aguilera, 2020). However, the literature still lacks research elucidating the mechanisms of action of Si in plants under water stress in the early stages (Pang et al., 2019).

Given this context, research aimed at finding ways to mitigate the negative effect of water deficiency is essential. Thus, the objective of this work is to study whether silicon attenuates the water deficit in the germination and initial growth of maize.

2. Material and Methods

The research was carried out at the Federal Rural University of the Amazon (UFRA – Campus Belém), at the Laboratory of Biodiversity Studies in Higher Plants (EBPS), with geographic coordinates 27'21" S and 30'16" W, whose average altitude is 10 meters. The experiment was carried out in October 2019 with a duration of seven days, as established by the RAS for corn (Brasil, 2009). The temperature in the germination room was controlled to 25 °C, relative humidity of 85% and a photoperiod of 8 hours of light and 16 hours in the dark during the seven days of conducting the germination test. Corn seeds (*Zea mays* L.) of the cultivar K9960 obtained from KWSeds were used.

Before soaking the seeds, the gerboxes were disinfected with a 70% alcohol solution and the germitest paper sheets were sterilized in an oven at 105 °C for 1 hour. PEG-6000 solutions were prepared according to Villela et al. (1991), in which the seeds were soaked in a solution for 24 hours. The following day, the calcium silicate solutions were prepared and added to properly identified trays. Each tray contained a calcium silicate solution, in which the germitest paper sheets were moistened with the solution in a proportion of 2.5 times the weight of the dry substrate. The seeds were placed between the two sheets of germitest paper (Brasil, 2009) for 7 days. For each repetition, two sheets of germitest paper were used, forming rolls. These rolls were wrapped in plastic bags and secured with elastic bands, in order to avoid evaporation of the solution and any type of contamination (Brasil, 2009). The bags containing the paper rolls were placed vertically inside plastic cups, allowing geotropism to occur naturally, which would later facilitate the evaluation of the seedlings (Coelho et al., 2014). Asepsis of all plastic materials with 70% alcohol was previously performed in order to inhibit possible proliferation of fungi (Tomazi et al., 2019).

The experiment was installed in a completely randomized design (DIC) in a 3 × 4 factorial scheme with 4 replications, consisting of three concentrations of calcium silicate (0.0, 1.0 and 2.0 mM of Ca_2SiO_4), according to the method used. by Gao et al. (2005) and 4 PEG-6000 solutions simulating different osmotic potentials (0.0; -0.3; -0.6; -0.9 MPa). These PEG-6000 solutions were prepared according to Villela et al. (1991). Fifty seeds per roll of paper were used for each treatment, as recommended by Brasil (2009), totaling 2,400 seeds.

To evaluate the germination parameters, the following variables were considered: percentage of abnormal seedlings and percentage of non-germinated seeds according to the recommended by the Rules of Seed Analysis (Brasil, 2009), the germination speed index (IVG) according to the formula proposed by Maguire (1962), in which the IVG= (G1/N1)+ (G2/N2)+ ... + (Gn/Nn), where IVG corresponds to the emergence speed index, the G to the number of normal seedlings computed in the counts, the N to the number of days of sowing since the 1st, 2nd... 7th evaluation. The mean germination time (ATM) was calculated according to the formula proposed by Labouriau (1983): TM = (Σniti) / Σni, with the TM corresponding to the mean germination time, the ni to the number of seeds germinated per day and ti to incubation time in days.

To evaluate the initial growth, the length of the shoot and the root were evaluated with the aid of a millimeter ruler, and the total length was also calculated from the sum of the length of the shoot and the root (Coelho et al., 2014), shoot dry mass and root dry mass, in which the plant material was packed in kraft paper bags and taken to an oven with forced air circulation at 65 °C for 72 hours.

Subsequently, the material was weighed and the results expressed in grams. The total dry mass was also calculated from the sum of the dry mass of the shoot and the root.

The results were compared by means of analysis of variance using the F test and the analysis of means comparison using the Tukey test ($p<0.05$). To obtain the statistical analysis, the A grostat *software* was used (Barbosa and Maldonado Junior, 2015).

3. Results and Discussion

Table 1 shows that there was a significant effect of the interaction between silicon and PEG-6000 only for the variable length of the shoot. It is also observed that there was a significant effect of the osmotic potentials simulated by PEG-6000 for all the variables analyzed, demonstrating that the water deficiency induced by PEG-6000 affects the germination and the initial growth of the corn.

The 2 mM silicon dosage was superior to the others at the same osmotic potential 0 (MP). Regardless of the silicon dosage applied, lower osmotic potentials influence shoot length (Figure 1). This result indicates that plants that suffer water deficiency induced by PEG-6000 are negatively affected, demonstrating sensitivity to water deficiency, since there was a reduction of approximately 17% in the CPA of seedlings without water deficiency (potential of 0 MPa), for the seedlings that were submitted to the lowest concentration of PEG-6000 (-0.3 MPa). In this case, Si at these doses did not attenuate the water deficiency. Possibly the concentrations of calcium silicate used were not efficient because this silicate source presents a slow release of Si and needs to be used in larger quantities compared to other silicate sources to meet the demand of plants (Queiroz et al., 2018).

For the root length (CR) and total length (TC) there was a significant difference ($p<0.001$) for the different osmotic potentials, in which it was observed that the greatest results were in the seedlings submitted to the potential of 0 MPa (without water deficit) (Figure 2A-2B). While the seedlings submitted to the potential of -0.9 MPa presented the smallest length of both the root (CR) and the total length (TC), reducing 99, 05% and 98.87% respectively, when compared to plants that did not suffer water deficit (0 MPa PEG-6000). The roots are the first organs of the plant to be affected by water deficiency, and for this reason the length of the root is reduced, since there is a decrease in turgor in the cells, making it difficult for the plant to grow. According to Padilha et al. (2016), corn plants subjected to water deficit showed a reduction in growth caused by the decrease in cell turgor that is associated with cell enlargement.

Figure 3A-3C shows how much water deficiency affected the dry mass of the seedling. For the variables analyzed, a decrease in seedling mass was observed when submitted to an osmotic potential of 0 MPa (without deficiency) when compared to seedlings with deficiency (-0.3; -0.6 and -0.9 MPa). As the osmotic potential became more negative, the production of MSR, MSPA and MST decreased. This result indicates that there may have been a decrease in turgor affecting its growth, therefore the leaves may have their expansion reduced and the growth of the roots is compromised, consequently reducing the mass of the shoot and root.

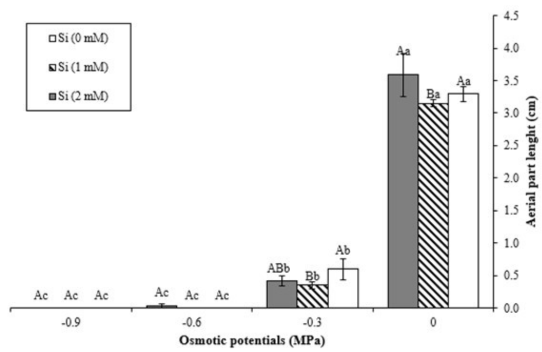

Figure 1. Shoot length in corn seedlings as a function of silicon and PEG-6000 doses. The same capital letters do not differ from each other within the same osmotic potential (0MPa; -0.3 MPa; -0.6 MPa; -0.9MPa). Equal lowercase letters do not differ from each other within the same silicon concentration (0 mM; 1 Mm; 2 mM).

Table 1. Analysis of variance (ANOVA) of germination and growth variables subjected to Si doses and osmotic potentials in maize.

	Causes of variation				
	Si	**PEG-6000**	**Si x PEG**	**Mean**	**CV (%)**
RDM	0.002^{ns}	2.571*	0.002^{ns}	5.157	7.1
SDM	0.002^{ns}	1.979*	0.001^{ns}	4.455	22.7
TDM	0.000^{ns}	9.343*	0.003^{ns}	9.612	9.3
RL	0.077^{ns}	75.058*	0.071^{ns}	4.454	18.2
SL	0.078*	31.041*	0.063*	0.953	12.1
TL	0.316^{ns}	122.210*	0.114^{ns}	5.277	20.4
G	0.333^{ns}	23674.083*	0.333^{ns}	22.208	12.4
NG	2.729^{ns}	1224.300*	3.585^{ns}	35.208	20.8
AS	40.583^{ns}	18261.889*	50.806^{ns}	42.583	18.8
MGT	0.001^{ns}	51.400*	0.001^{ns}	1.035	3.3
GSI	0.003^{ns}	351.619*	0.003^{ns}	2.707	11.6

*Significant ($p < 0.05$). ^{NS}Not significant. CV (%) – coefficient of variation; RDM – root dry mass; SDM – shoot dry mass; TDM – total dry mass; RL – root length; SL – shoot length; TL – total length; G – germination; NG – not germinated; AS – abnormal seedlings; MGT – mean germination time; GSI – germination speed index; Si – silicon; PEG - polyethylene glycol 6000.

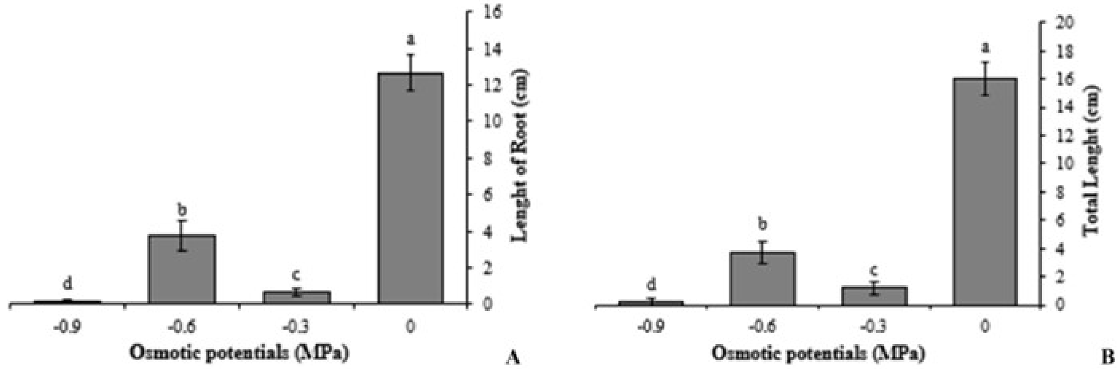

Figure 2. Root length (A) and total length (B) in corn seedlings as a function of osmotic potentials (PEG-6000). Equal lowercase letters do not differ from each other within the same osmotic potential.

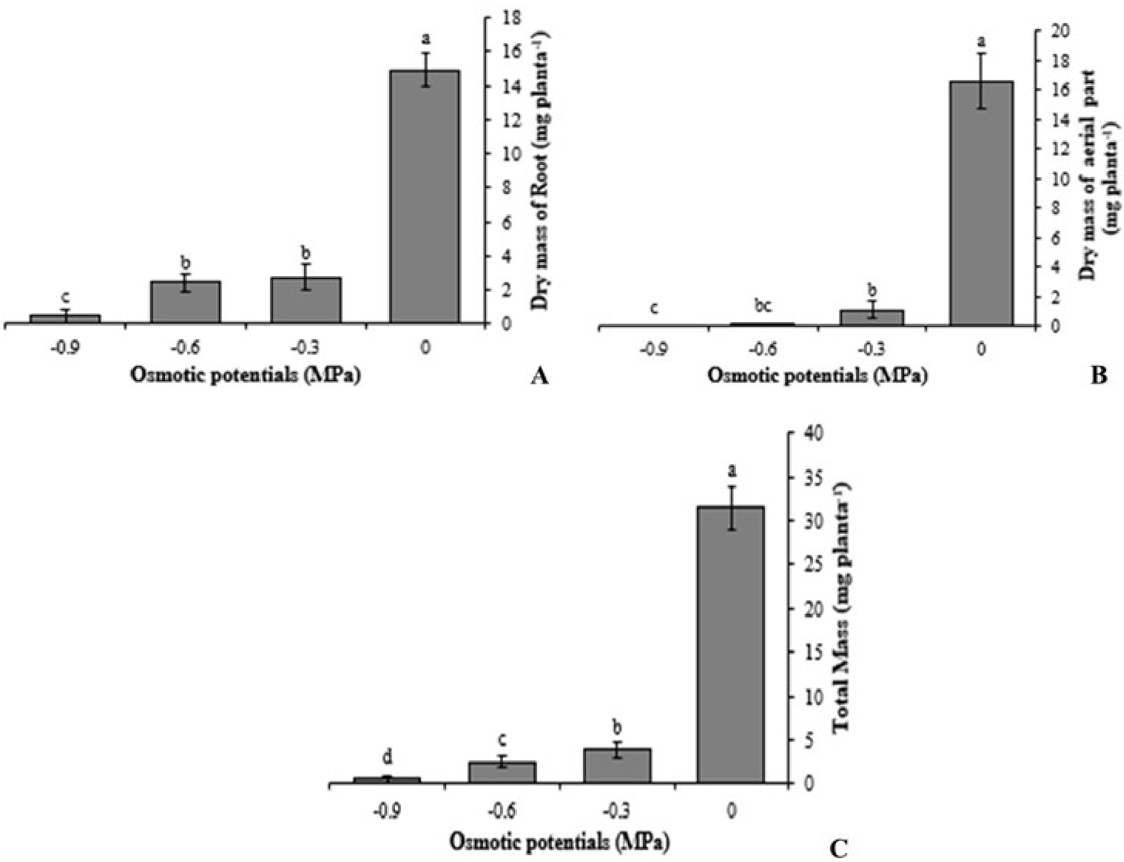

Figure 3. Root dry mass (A), shoot dry mass (B) and total dry mass (C) in corn seedlings as a function of osmotic potentials (PEG-6000). Equal lowercase letters do not differ from each other within the sameosmotic potential.

Therefore, the low water content in the cells of stressed seedlings possibly reduced or paralyzed cell elongation and consequently the expansion of plant tissue (Colman et al., 2014; Santos et al., 2012). Oliveira et al. (2017) report that water deficiency during the germination process reduces the action of hydrolytic and digestive enzymes important for the degradation of seed energy reserves (carbohydrates, proteins and lipids).

In their study on phytomass production in maize plants Xavier et al. (2014) found that the water deficit condition decreases the turgor pressure and also the flow within the conducting vessels. The reduction in turgidity and in the flow of solutes and water in the cases of xylem and phloem vessels may have limited cell expansion and seedling development, which favored a decrease in leaf area, root and shoot length and biomass.

For the variables germination test (TG), germination speed index (IVG) and average germination time (TMG) normal seeds were considered and thus it was observed that normal corn seeds did not germinate at osmotic potentials of -0 .3, -0.6 and -0.9 MPa (Figure 4A-4C). The germination of corn seeds reached values close to 90%, in seeds without PEG-6000 (0 MPa). The application of PEG 6000 negatively affected the germination of seeds submitted to -0.3; -0.6 and -0.9 MPa, with a percentage of 100% of non-germinated seeds (Figure 4A). This result indicates that the water deficit simulated by the PEG-6000 was severe to the point of not having germinated the corn, possibly the PEG-6000 impaired the water absorption of the medium and culminated in damages regarding the normal germination. In addition, PEG-6000 may have reduced the germination of abnormal seeds because the solution has a high viscosity, hindering the diffusion of oxygen, which impairs the availability of oxygen for the respiratory process during germination. The negative effect of PEG-6000 on seed germination was also observed by Avcı et al. (2017) in seven sorghum cultivars. The results showed that the GSI was higher in the seeds submitted to the potential of 0 MPa, while the seeds submitted to the other potentials presented null GSI (Figure 4B), because at potentials above MPa the corn seeds were not able to germinate. This result indicates that corn seeds are highly sensitive to water deficit induced by these osmotic potentials. Similar results for the GSI were also found by Vibhuti et al. (2015) working with three rice varieties subjected to water deficit.

Regarding the TMG, the results showed that seeds without PEG-6000 (0 MPa) took approximately 4 days to germinate, and seeds submitted to potentials of -0.3; -0.6 and -0.9 MPa did not germinate during the experiment (Figure 4C). This result was possibly due to the fact that the seed needs a longer time to reach an adequate water level to germinate under these conditions of this experiment, because in a way the GSI tends to reduce and the TMG to increase, which ends up prolonging the germination process.

For the percentage of abnormal seedlings, a significant difference was found between the seeds in the different osmotic potentials (Figure 5A), with a reduction of 73.89% of abnormal seedlings in the potential of 0 MPa compared to the potential of -0.3 MPa (Figure 4A). This result demonstrates that there is a higher percentage of abnormal seedlings when submitted to the potential of -0.3 MPa compared to the other potentials, demonstrating that the reduction of water for the seeds negatively affected the normal growth of corn seedlings. Lucchese et al. (2018) in their study on germination and seedling growth of *Toona ciliata* found that water limitation during the germination process favored the development of seedlings with some abnormality, such as: small and weak main and seminal roots, small or absent shoots, of poorly developed, no extended plume.

It is observed that corn seeds submitted to the potential of -0.9 MPa presented 72% of non-germinated seeds (Figure 5B).

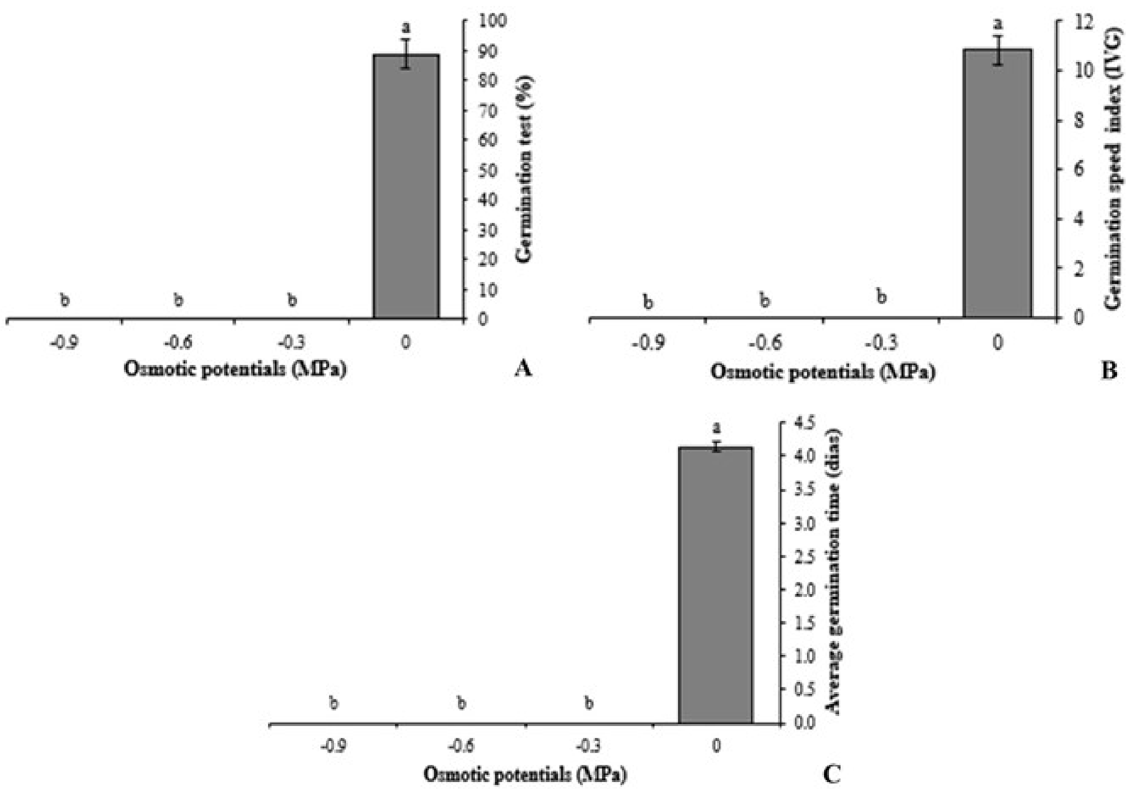

Figure 4. Germination test (A), germination speed index (B) and mean germination time (C) in corn seeds as a function of osmotic potentials (PEG-6000). Equal lowercase letters do not differ from each other within the same osmotic potential.

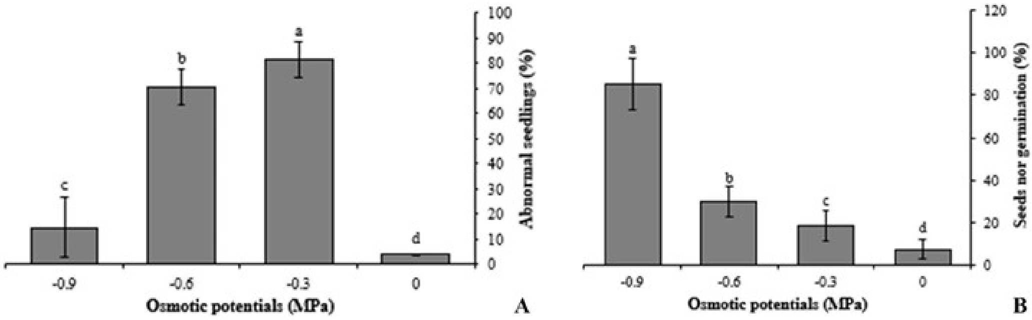

Figure 5. Percentage of abnormal seedlings (A) and non-germinated (B) corn seeds as a function of osmotic potentials (PEG-6000). Equal lowercase letters do not differ from each other within the same osmotic potential.

The results obtained for the percentage of non-germinated seeds showed a significant difference between the seeds submitted to the increasing osmotic potentials simulated by the PEG-6000. Only 7.33% of the seeds submitted to the potential of 0 MPa of PEG-6000 did not germinate, while the corn seeds submitted to the potential of -0.9 MPa showed 85% of non-germinated seeds (Figure 5B). This result indicates that the water deficit induced by PEG-6000 at powers of -0.9 MPa severely affected seed germination, due to lack of water, since water is essential for the reactivation of seed metabolism to occur.

4. Conclusion

The use of silicon in corn seeds did not attenuate the stress caused by water deficit simulated by PEG-6000.

The use of PEG-6000 as a water deficit simulator was harmful in terms of germination parameters and in terms of growth parameters and dry mass production.

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