Silicon in wheat crop under water limitation and seed tolerance to water stress during germination[1]

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ABSTRACT

Water stress during wheat seed germination and seedling establishment for affect the percentage and speed of germination. Silicon (Si) is related to plant tolerance to different types of stress, then the application of this nutrient in the mother plant during seed production could contribute to the improvement of the seed quality and, consequently, its tolerance to stress. The aim was to evaluate the effect of foliar application of silicon on the tolerance of wheat seeds, produced under different irrigation levels, to water stress during germination and initial seedling growth. Wheat seeds were produced under three irrigation depths (0, 50 and 100% of the total required irrigation) applied after the anthesis. The silicon was supplied in two doses by foliar application (0 and 5 mM of Si). The germination and seedling growth were performed under water stress induced by PEG 6000 at the -0.2; -0.4; -0.6 MPa osmotic potentials. In addition, in the control only distilled water was used. The reduction of the osmotic potential reduced seed germination, germination speed and seedling growth. Irrigation depths, as well as foliar application of Si, during seed production did not influence the physiological quality and tolerance of seeds to water stress during germination.

Keywords: *Triticum aestivum* L.; seeds quality; seeds vigor; abiotic stress.

INTRODUCTION

Water stress occurs in large extensions of cultivable areas (Nogueira *et al*., 2001; Girotto *et al*., 2012) and for many crops can affect the water relations of plants metabolism, leading to reduced plant production.

In Brazil, the water deficit in wheat crop (*Triticum aestivum* L.) occurs in a significant way in the northern regions of the Paraná state, towards the center of the country, which limits the cultivation of this cereal in certain regions of the Brazilian Cerrado (Monteiro, 2009; Andrade *et al*., 2015). In these regions, wheat crops under dry farming conditions may face water shortages. In addition to reducing the final yield, due to the occurrence of water deficit during the booting growth stage, this stress also contributes to failure on grain formation and inefficient filling of the grains. Also, it can affect the emergence and establishment of the crop, as the sowing stage occurs in the months of March and April (Monteiro, 2009), when in general there is a low rainfall occurrence.

Seed germination is a crucial stage in the establishment of crops and may be influenced by several abiotic stresses, including drought stress (Hubbard *et al*., 2012). For the germination process to take place, water availability is crucial for the activation of the chemical reactions involved in the metabolism and, therefore, generate the resumption of embryonic axis growth (Marcos-Filho, 2015). Thus, the reduction of the osmotic potential caused by water deficiency leads to a reduction in the percentage and speed of seed germination (Pereira & Lopes, 2011), as well as a significant reduction in seedling growth (Kashif, 2011).

Several authors reported the effect of water stress during seed germination on the germination rate and seedling growth in wheat, with reductions in the length

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and dry weight of shoot and root (Dhanda *et al*., 2004; Rauf *et al*., 2007; Yagmur & Kaydan, 2008), and in the final crop yield.

Silicon (Si) is a beneficial element for higher plants. Its effect is observed in situations of multiple stresses. When absorbed, it is accumulated in organs such as leaves and stems and forms a double layer structure composed of silica (SiO_2), which is deposited in the cuticle and acts in a structural way. It also influences the physiology and metabolism of plants, with increased antioxidant power (Tripathi *et al*., 2017; Bukhari *et al*., 2015). Its main effect under water deficit condition is the reduction of excessive water loss through the process of transpiration of plants (Rao *et al*., 2019).

This element plays an important role in plants, mainly of the Poaceae family, such as wheat, contributes to increase in growth and plant production, indirectly caused by upright leaves, decreased self-shading, and greater structural rigidity of the tissues (Sarto *et al*., 2014), besides generating some protection against abiotic (Khan *et al*., 2021) and biotic (Acevedo *et al*., 2021) stresses. Wheat plants that received foliar application of Si produced seeds with higher mass (Toledo *et al*., 2012). Although the effect on seed physiological quality has not been observed (Toledo *et al*., 2012; Segalin *et al*., 2013), it is expected that greater tolerance of the plants to the stresses contribute to the production of seeds with higher physiological quality under these conditions.

During the process of seed maturation, the environment is determinant for its physiological quality. Thus, abiotic stresses, such as water deficit, can reduce the quality of seed (Eskandari & Alizadeh-Amraie, 2017). On the other hand, some studies reported the increase of the tolerance of the seeds to certain stresses, conditioned by the effect of the environment where the maturation process occurred (Bilichak & Kovalchuk, 2016). According to Walter *et al*. (2011), in some grasses this effect can be observed, in such a way that plants that undergo cycles of water deficit present protection responses when again submitted to water shortage situations. Thus, seeds produced under conditions of water deficit could have a greater tolerance to this stress.

Thus, the objective of the present work was to evaluate the effect of foliar application of silicon on the tolerance of wheat seeds, produced under different irrigation depths, to water stress during germination and initial seedling growth.

MATERIAL AND METHODS

The research was carried out in the Laboratory of Seed Analysis of the Department of Plant Science of the Federal University of Viçosa (Portuguese acronym: UFV). Wheat seeds from cultivar BRS 264 were used. The seeds were produced under field conditions from April to August 2017, in Viçosa, MG. Acidity regulation and fertilization were performed based on the soil analysis, and the amount of acidity regulator and fertilizer as recommended by Ribeiro (1999). The climatic conditions during the production of the wheat seeds are presented in Figure 1.

During the seed production, the plants were submitted to different irrigation depths from the anthesis, with or without foliar application of Si. The experimental plots consisted of five rows of plants, spaced 0.20, ten meters in length. The useful area considered for application of the treatments comprised the total area occupied by plants in the three central lines minus 0.20 m at the ends. Irrigation treatments consisted of the application of three depths, 0, 50 and 100% of the total irrigation required for the crop (ITN), calculated by means of the ratio between the actual irrigation required for the crop (IRN) and the efficiency of the irrigation system. It was used a drip irrigation system.

IRN was calculated by means of the water balance of the system, with information about the entrance (irrigation + precipitation) and exit (crop evapotranspiration - ETc) of water. For the calculation of ETc, the following equation was used (Allen *et al*., 1998):

$$ETc = ETo \times Kc$$

In which: ETc - crop evapotranspiration in mm day^{-1};

ETo - reference evapotranspiration in mm day $^{-1}$;

Kc - coefficient of the crop, dimensionless.

Estimates of crop evapotranspiration were performed using the Penman-Monteith-FAO 56 method (Allen *et al*., 1998). The values of Kc, as obtained by Libardi & Costa (1997), used for each stage of the crop were: establishment, 0.29; tillering, 0.36; booting growth, 0.79; flowering, 1.11; grain formation, 1.16; and maturation, 0.45. The meteorological data used for the calculations were obtained through the Meteorological Station of Automatic Surface Observation of Viçosa-MG (*Estação Meteorológica de Observação de Superfície Automática de Viçosa* - MG), with access by the National Institute of Meteorology (*Instituto Nacional de Meteorologia* - INMET). The daily data of temperature (°C), relative humidity (%), dew point (°C), pressure (hPa), wind speed (m s^{-1}), radiation (kJm²) and rainfall (mm) were accessed.

The plants throughout the experiment were irrigated in a four-day shift. Up to the anthesis event, when 50% of the plants in field were with the anthers open, all plants were irrigated with 100% of ITN. From the anthesis, the plants were submitted to the different irrigation depths.

For the foliar application of silicon, the commercial product Supa Silica® (Potassium Silicate - K_2SiO_2), with

25.7% of SiO_2 and 12.23% K_2O was used. The silicon treatments consisted in the application of two doses, 0 mM and 5 mM of silicon, which were applied at the tillering stage, at 25 days after sowing (DAS). The application was performed with the aid of a system composed of a pressurized cylinder with CO_2 and a double bar of type TT 11002 that provided a constant pressure of 3 bars and a volume of syrup of 260 L ha^{-1}.

At 110 days after sowing (DAS), when the plants were already dry, the spikes were harvested manually, which were threshed with the help of an experimental thresher. The seeds were dried naturally in the shade in a laboratory environment, untill 12% moisture content. Each field plot was collected separately and constituted a laboratory repetition in the tests to evaluate the tolerance of the seeds to water deficit. The following evaluations were carried out:

Germination

Four replicates of 50 seeds were distributed on paper towel moistened with 2.5 times the dry paper weight with polyethylene glycol 6000 (PEG 6000) solutions equivalent to the osmotic potentials of -0.2; -0.4 and -0.6 MPa (Villela *et al*., 1991). The rolls were kept in germinator regulated at 20 ºC. In the control treatment, the paper towel was only moistened with distilled water. The evaluations consisted of daily counting of the number of normal seedlings until the values stabilized. With the data, the final germination percentage was calculated on the eighth day after sowing, and the first germination test count, on the fourth day after sowing (Brasil, 2009). The average germination time was determined according to Equation 1 and the germination rate according to Equation 2 (Bewley *et al*., 2013).

$$AGT = \Sigma(t_i \times n_i)/\Sigma n \qquad \text{(Eq. 1)}$$

$$GR = 1/AGT \qquad \text{(Eq. 2)}$$

In which:

AGT = Average germination time

GR = Germination rate

n_i = number of normal seedlings on day i;

t_i = is the number of days from the start of the test.

Seedling growth

The test was performed with four replicates of 10 seeds each, which were distributed in rolls of paper towel moistened with 2.5 times the weight of the dry paper, with solutions of PEG 6000, equivalent to the osmotic potentials of -0.2, -0.4 and -0.6 MPa, besides the control with distilled water. At the end of the test (8th day after sowing) the seedlings were submitted to shoot and root measurements, which were separated and measured with the aid of a graduated ruler. The results were expressed in cm $seedling^{-1}$ (Krzyzanowski *et al*., 2020).

Following length measurements, the seedlings had their shoots and roots separated and placed in an oven at 65 °C, where they remained for 72 hours. Subsequently, the dry material was weighed in an analytical balance (0.001g). The dry matter was expressed in mg $seedling^{-1}$ (Krzyzanowski *et al*., 2020).

Experimental design and statistical analysis

The experiment was conducted in a randomized complete block design, in subdivided plots scheme, with four replications. In the plots were allocated the three irrigation depths and the osmotic potentials in a 3 x 4 factorial scheme, that is, three depths and four potentials. In the subplots were allocated the treatments of foliar application of Si, at doses of 0 mM (control) and 5 mM of Si.

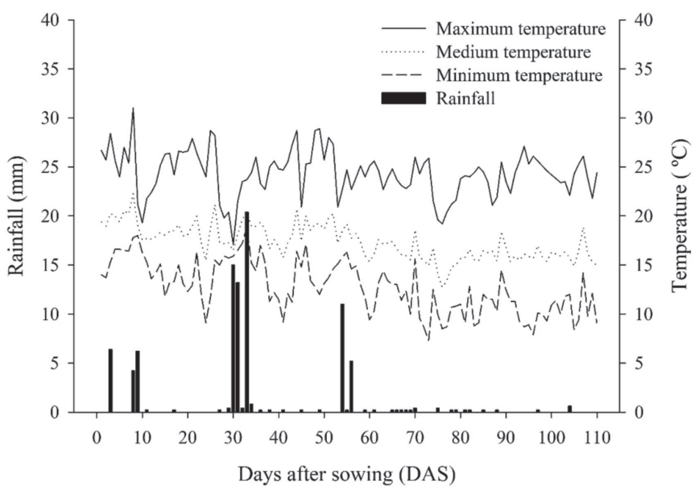

Figure 1: Rainfall distribution, maximum, average and minimum temperature during the wheat seed production period. Viçosa-MG, 2017.

Data were submitted to analysis of variance. The verification of the normality of the errors and of the homogeneity of variances was performed through the Shapiro-Wilk and Bartlett tests, respectively. The averages obtained for each irrigation depth were compared by the Tukey test ($p \leq 0.05$); the averages obtained for the osmotic potentials were submitted to regression analysis ($p \leq 0.05$); and the averages obtained for the Si doses were compared by the F test ($p \leq 0.05$). Statistical analysis was performed using statistical software R (R Core Team, 2021).

RESULTS AND DISCUSSION

Germination of wheat seeds produced under different irrigation depths, and foliar application of silicon at 0 and 5 mM of Si, was negatively influenced by the osmotic potential (Figure 2A). It was observed a reduction in seed germination percentage, which initially was of values above 90% in the control potential (0.0 MPa), to approximate values of 50% of germination in the most drastic potential, -0.6 MPa. This reduction shows the effect of water stress on germination of wheat seeds, which can be explained by its influence on the water absorption, which is compromised in low osmotic potentials and thus prevent the occurrence of events related to the germination process (Botelho & Perez, 2001). Despite the drastic reduction observed in seed germination at potential -0.6 MPa, it is considered that the seeds of cultivar BRS 264 used in this study present moderate tolerance to water stress during germination, since seed germination was still observed in the lowest potential, although this cultivar is classified in some works as sensitive to drought (Girotto *et al*., 2012).

No difference was observed in seed germination in the different osmotic potentials when comparing the different irrigation depths treatments and the doses of Si applied via foliar. Therefore, no beneficial effect of silicon was observed for increasing water stress tolerance during germination of wheat seeds (Figure 2A). However, Tavares *et al*. (2014) stated that the physiological quality of the seeds may not be directly related to silicon fertilization, but rather due to the improvement in plant development conditions, that is, plants that are more tolerant to both biotic and abiotic stresses produce seeds with higher physiological quality.

The germination speed was evaluated using the tests of first germination count, germination rate and average germination time (Figures 2B, 3A and B). A significant reduction of germination speed occurred, and null germination was observed for all treatments at the most extreme potential, -0.6 MPa, on the 4th day of the test.

The highest values for the germination rate were observed for the control treatment (0.0 MPa). As the osmotic potential decreased, reduction in the germination rate of the seeds was observed. Lower values of average germination time were observed for the control. An increase in the values was observed according to the reduction of the osmotic potential, that is, the germination of the seeds was slower. There was no significant effect of the different irrigation depths treatments and of the two silicon treatments for any of the variables related to germination speed.

In very negative osmotic potentials, responses such as reduction in germination and its delaying are observed (Brito *et al*., 2016), which can lead to complete inhibition, as verified for the potential of -0.6 MPa.

The water deficit usually influences the speed and percentage of seed germination (Bewley *et al*., 2013) and contributes to an increase in the time required for germination in this unfavorable condition. This may be

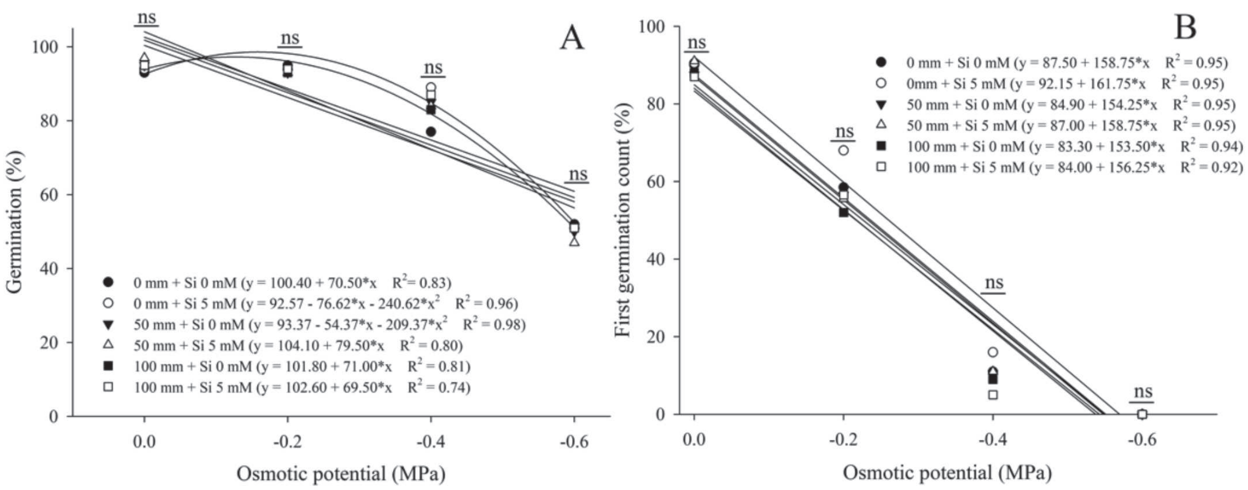

Figure 2: Germination (A) and first germination count (B) of wheat seeds from plants submitted to different irrigation depths from the anthesis, and two doses of silicon applied via foliar, as a function of the osmotic potential. * significant at 5% probability. ns – not significant by the F test. The horizontal bars compare the different treatments within each osmotic potential.

related to the time it takes for the seeds to develop adaptation mechanisms (Barroso, 2010). In reduced osmotic potentials, the process of seed imbibition occurs more slowly, and since the process of seed germination is dependent on water for the activation of the metabolic processes that lead to germination, water limitation reduces their speed germination and the increase in average germination time.

The reduction of the water potential in the germination substrate limits the water absorption by the seeds and causes a retardation of the metabolic processes that culminate with the germination (Botelho & Perez, 2001). Thus, both germination (Figure 2) and germination speed (Figures 2B, 3A and B) were reduced with water stress, but no effects of foliar application of Si or of the different irrigation depths imposed on plants during seed maturation were observed.

No difference was observed between the different irrigation depths, at 0 and 5 mM silicon doses, on the growth of seedlings under water deficit, when comparing the data of shoot length (Figure 4A) and root length (Figure 4B). On the other hand, there was an effect of the osmotic potential on the two variables, which showed reduction of the length with the reduction of the water potential.

The reduction of the osmotic potential, as well as affecting the percentage and the germination speed, as observed in this experiment, affects the growth of the seedlings due to the decrease in cell expansion (Taiz & Zeiger, 2017). However, the reduction of shoot length was more intense when compared to that observed for root length.

This smaller root length reduction can be attributed to a rapid osmotic adjustment to allow turgor pressure to be restored. Thus, the potential gradient is resumed, with consequent water absorption and root growth (Hsiao & Xu, 2000). In contrast, this osmotic adjustment in the leaves occurs slowly, which causes a decrease or stop in the increase of the cellular walls, causing less growth of the shoot. Therefore, the non-alteration of root growth under water deficiency conditions is a mechanism for adapting plants to this condition (Hsiao & Xu, 2000; Magalhães-Filho *et al*., 2008).

Analogous behavior was found for seedling shoot (Figure C) and root (Figure D) dry matter. For these variables, no difference was observed between the evaluated treatments, but there was a reduction in the dry mass of the shoot, starting from the potential -0.2 MPa, which was more drastic than the reduction observed for the dry mass of the root. This result may also be related to the plant's strategy of adapting to the water deficiency condition, maintaining or increasing the flow of reserves to the root system to maintain its growth in search of water. Steiner *et al*. (2017) stated that, during the establishment stage of the plant, the allocation of dry matter to the root system appears to be a protection mechanism for the crop to tolerate a condition of lack of water.

In general, irrigation depth treatments and foliar application of silicon during the production of wheat seeds did not influence germination and initial seedling growth, as well as seed tolerance to water stress in the germination stage and initial seedling development. However, an important strategy used to better stimulate defenses and adaptability to drought is the simulation of a recovery period, through reapplication of water (de Macêdo *et al*., 2019; Chen *et al*., 2016). Thus, a simulation of a recovery period could be an alternative to better assess the effect

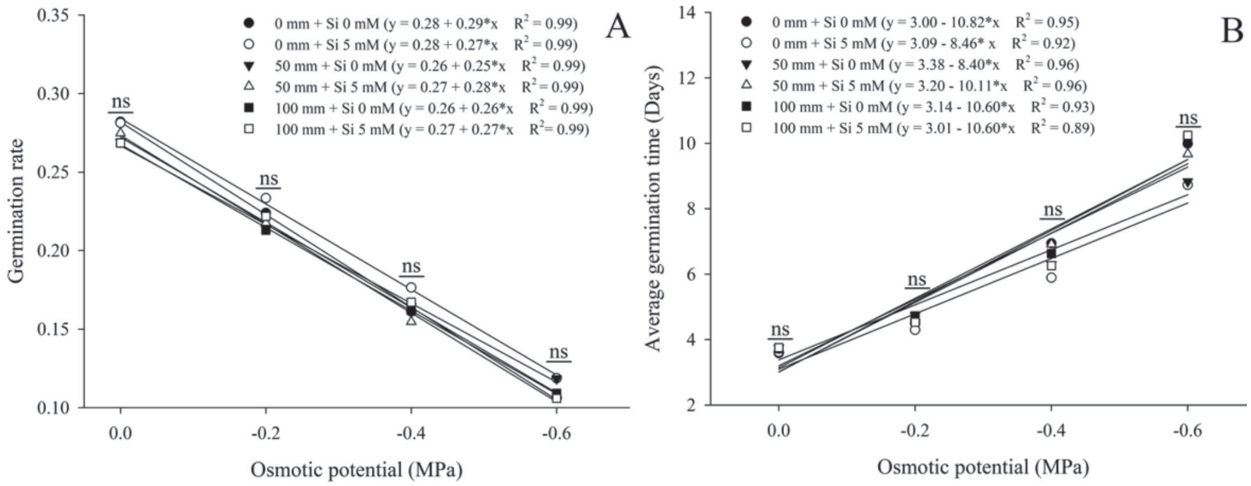

Figure 3: Germination rate (A) and average germination time (B) of wheat seeds from plants submitted to different irrigation depths from the anthesis, and two doses of silicon applied via foliar, as a function of the osmotic potential. * significant at 5% probability. ns – not significant by the F test. The horizontal bars compare the different treatments within each osmotic potential.

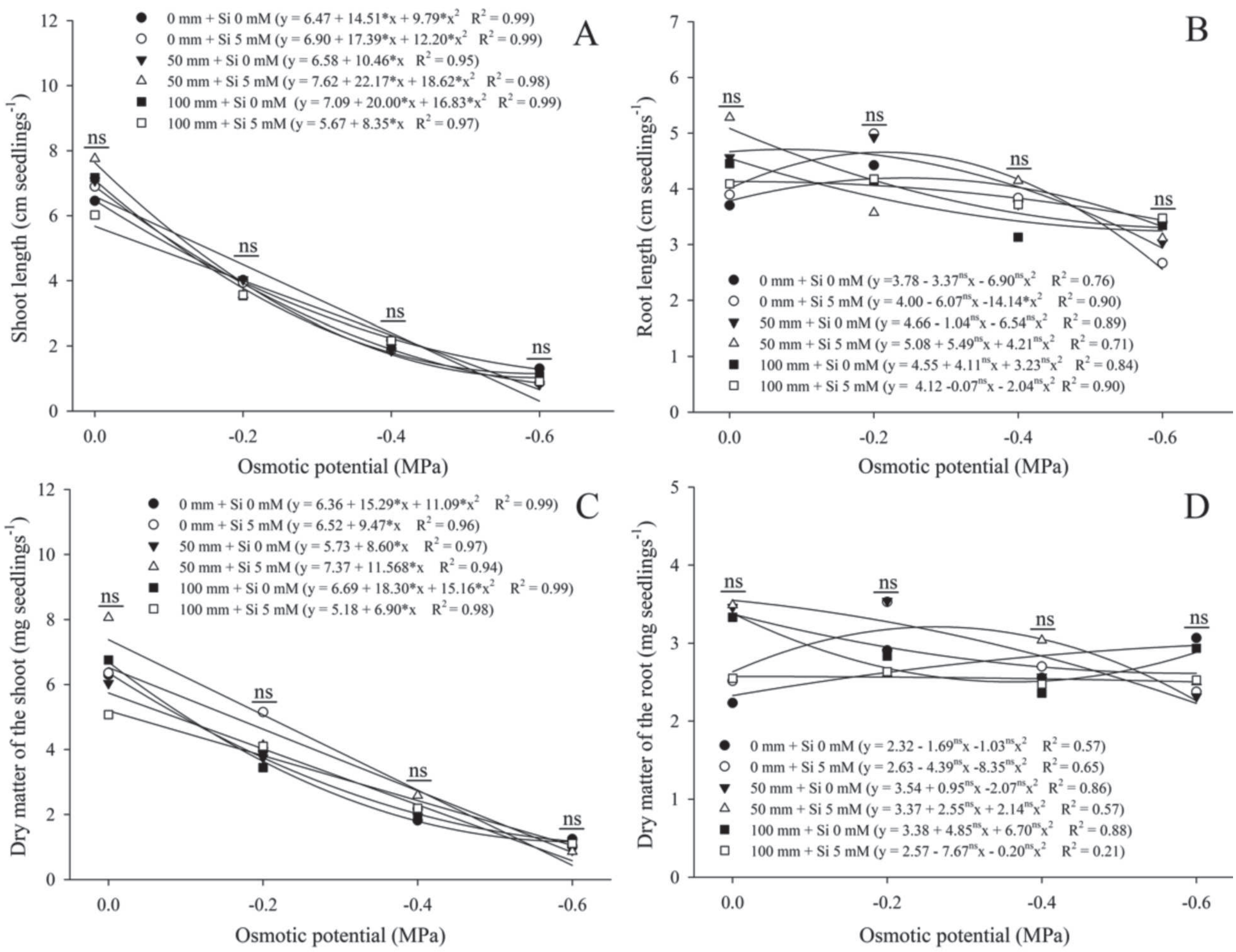

Figure 4: Shoot length (A), root length (B), dry matter of shoot (C) and root (D) of wheat seedlings from seeds produced in plants submitted to different irrigation depths from the anthesis, and two doses of silicon applied via foliar, as a function of the osmotic potential. * significant at 5% probability. ns – not significant by the F test. The horizontal bars compare the different treatments within each osmotic potential.

of tolerance on present study. On the other hand, seeds were sensitive to water stress simulated by PEG 6000 during germination, with reductions in germination and germination speed identified by germination tests, first germination count, germination rate and average germination time, as the potential osmotic reduced (Figures 2 and 3).

Seedling growth was also affected by water stress during germination of wheat seeds and seedling initial growth, which was more expressive in the shoot of the seedlings in relation to root growth (Figure 4). This was identified in the experiment by assessing the length of the shoot and the root and can be explained by the possible adaptation of the seedlings to the stress condition imposed.

CONCLUSIONS

The different irrigation depths in the seed maturation stage, as well as the foliar application of silicon in the wheat plants, did not influence the germination and initial seedling growth nor their tolerance to the condition of water stress during germination.

Wheat seeds of cultivar BRS 264 present moderate tolerance to water stress during germination, but there is a reduction in the number of normal seedlings and seedling growth with the increase of the stress level to the osmotic potential of -0.6 MPa.

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