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Silicon and boron mitigate the effects of water deficit on sunflower

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ABSTRACT: The objective of this study was to evaluate the effect of Ca and Mg silicate and B on the gas exchange, leaf water potential and chlorophyll fluorescence parameters in the sunflower variety Embrapa 122 -V2000 under water stress conditions. The trial was conducted in Red Yellow Latosol with very clayey texture, with contrasting levels of Si and B and subjected to water deficit. The experimental design was randomized blocks in a 2^4 factorial arrangement, with five replicates. Treatments consisted of the combination of two acidity corrective agents (calcium magnesium silicate and dolomitic limestone), two levels of base saturation (30 and 70%), two levels of B (0.18 mg dm^{-3} - value available in the soil and 1.20 mg dm^{-3}) and two water regimes (with and without water stress from the beginning of flowering). It can be concluded that the supply of Si and B can reduce the damage to sunflower caused by water stress.

Key words: *Helianthus annuus*, chlorophyll fluorescence, gas exchange, leaf water potential

Silício e boro atenuam os efeitos do déficit hídrico sobre o girassol

RESUMO: Objetivou-se avaliar o efeito do silicato de Ca e Mg e do B sobre as trocas gasosas, potencial hídrico foliar e parâmetros da fluorescência da clorofila na variedade de girassol Embrapa 122-V2000, em condições de deficiência hídrica. O ensaio foi realizado em Latossolo Vermelho-Amarelo de textura muito argilosa, com níveis contrastantes de Si e B, e submetido à deficiência hídrica. O delineamento experimental foi em blocos casualizados, num arranjo fatorial 2^4, com cinco repetições. Os tratamentos foram constituídos da combinação de dois materiais corretivos de acidez (silicato de cálcio e magnésio e calcário dolomítico), dois níveis de saturação por bases (30 e 70%), dois níveis de B (0,18 mg dm^{-3} valor disponível no solo e 1,20 mg dm^{-3}) e dois regimes hídricos (sem e com deficiência hídrica a partir do início do florescimento). O suprimento de Si e B podem reduzir os danos causados ao girassol pela deficiência hídrica.

Palavras-chave: *Helianthus annuus*, fluorescência da clorofila, trocas gasosas, potencial hídrico foliar

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Introduction

Despite the favorable scenario for the sunflower crop in Brazil, the occurrence of climatic adversities is still a factor of risk and a failure for the crop. This is due to the main period of its cultivation (between seasons), when plants are more prone to water stress (Castro & Leite, 2018). Thus, water deficit has been pointed as one of the main factors causing the reduction of yield and quality of sunflower grains/seeds (Castro & Leite, 2018). In addition to the effects on gas exchanges, water deficit can influence the transport of nutrients in the soil, their absorption and mineral metabolism in the plant.

One of the options for higher tolerance of sunflower to water deficit periods would be fertilization with silicon (Si). The beneficial effects of this element in agriculture have been observed when plants are cultivated under stressful conditions, including water deficit (Zhu & Gong, 2014). Si accumulated in organs of transpiration promotes the formation of a double layer of silica, which reduces transpiration and consequently increases water use efficiency by plants (Moreira et al., 2010).

Among the nutrients of great importance for the crop, there is also boron (B), which has as the main mechanism of transport in the soil the mass flow, which is influenced by the water deficit (Ramos et al., 2013).

After flowering starts until the complete filling of achenes, the sunflower plant becomes more sensitive to water deficit and its yield decreases in variable magnitudes according to the intensity and duration of the water deficit (Castro et al., 2006). Studies related to the supply of B and Si as a strategy to reduce transpiration are scarce. These elements can attenuate the effects of water deficit and allow sunflower yield to be less harmed by the adverse condition of lack of water in the soil.

Thus, this study aimed to evaluate the effect of Si and B on the gas exchanges, leaf water potential and on the chlorophyll a fluorescence variables of the sunflower cv. Embrapa 122-V2000, cultivated in a very clayey Red Yellow Latosol and subjected to water deficit after the beginning of flowering.

Material and Methods

The experiment was carried out in a greenhouse of the Plant Science Department of the Federal University of Viçosa, in Viçosa, MG, Brazil (20° 45' S, 42° 52' W and altitude of 651 m), in 30 dm^3 polyethylene pots with no holes at the bottom.

The experimental design was randomized blocks, in a 2^4 factorial arrangement with five replicates. Treatments consisted of the combination of two acidity corrective materials (calcium and magnesium silicate and dolomitic limestone), two levels of base saturation (30 and 70%), two levels of B (0.18 mg dm^{-3} - value available in the soil and 1.20 mg dm^{-3}) and two water regimes (without and with water deficit from the beginning of flowering).

The soil used was a dystrophic Red Yellow Latosol, collected from the 30-70 cm layer, near the municipality of Viçosa, MG. The chemical and granulometric characteristics of this soil were determined prior to the experiment (Table 1). To determine the relationship between soil water retention and tension, three undisturbed soil samples were collected at the site. These samples were saturated and subjected to the matric potentials of -10, -30, -60, -100, -500 and -1500 kPa in Richards' pressure plate apparatus. Then, they were dried in the oven at 105 °C, for 48 h, to determine the water content related to the applied tension (Table 1).

Table 1. Chemical and physical characteristics and moisture content of the soil (kg kg^{-1}) related to the matric potentials (kPa) of the Red Yellow Latosol and the chemical composition of the Ca and Mg silicate (Agro-silicon) and limestone used in sunflower cultivation

Soil chemical characteristics (1)								
pH $CaCl_2$	Ca	Mg	Al	H + Al	t	T	P	K
	($cmol_c$ dm^{-3})						(mg dm^{-3})	
4.2	0.30	0.10	0.80	6.50	1.21	6.94	1.90	3.91
Cu	Zn	Fe	Mn	B	V	m	OM	Prem
(mg dm^{-3})					(%)		(dag kg^{-1})	(mg L^{-1})
0.18	0.30	99.00	2.50	0.18	6.00	64.52	0.01	0.89

Soil granulometry (2)			
Clay	Silt	Fine sand	Coarse sand
(dag kg^{-1})			
75	3	13	9

Potentials – Moisture contents (kPa)					
-10	-30	-60	-100	-500	-1500
(kg kg^{-1})					
0.410	0.286	0.273	0.261	0.254	0.238

Correctives					
	NP	RE	TRNP	CaO	MgO
	(%)			(dag kg^{-1})	
Silicate	85.0	68.0	57.8	37.9	9.3
Limestone	102.5	97.0	99.4	39.8	13.1

(1) Determinations: pH with $CaCl_2$; soil - solution 1:2.5; Ca, Mg and Al, extracted with 1 mol L^{-1} KCl (EMBRAPA, 1997); 0.5 mol L^{-1} $Ca(OAc)^2$ pH 7.0 - H + Al; P, K, Cu, Zn, Fe, Mn, extracted with Mehlich-1; B - Hot water; V - Base saturation index; m - Aluminum saturation index; OM - Organic matter; Prem - Remaining P. (2) Ruiz (2005); NP - Neutralizing Power; RE - Reactivity and TRNP - Total relative neutralizing power

The values of the soil water retention curve were used to estimate the parameters α, n and m and the values of θr and θs to fit the data with the model proposed by Genuchten (1980), using the computer program Soil Water Retention Curve (SWRC). The coefficients estimated by the SWRC were used to fit the data with the model of Genuchten (1980):

$$\theta = \frac{\theta r + \left(\theta s - \theta r\right)}{\left[1 + \left(\alpha \Psi\right) n\right] m} \tag{1}$$

where:

θ - soil moisture, kg kg^{-1};
θr - residual soil moisture, at tension of 1.5 MPa;
θs - saturated soil moisture, kg kg^{-1};
Ψ - soil water potential, kPa; and,

α, m, and n - empirical equation parameters obtained by fitting the model. It was established that the moisture content at the potential of -60 kPa, fitted by the model proposed by Genuchten (1980), corresponded to the condition of water deficit.

The need for liming was calculated by the base saturation method, to increase it to 30 and 70%, with the correctives Ca and Mg silicate and dolomitic limestone (Table 1). The corrected soil was incubated in a greenhouse, for 80 days, with moisture equivalent to 80% of field capacity.

After the incubation period and one day before sowing, K deficiency in the soil was corrected using KCl, and P deficiency was corrected using monoammonium phosphate (MAP). Phosphorus was applied in 10% of the total volume, in the upper part of each pot. Nitrogen, in the form of urea and ammonium sulfate, and the micronutrients were split into four applications: 12, 19, 26 and 33 days after emergence of seedlings. The doses of nutrients, except B, were applied according to Novais et al. (1991). Boric acid (A.R.) was used as source of B.

In each pot, 12 seeds were planted at 2 cm depth. One week after emergence of seedlings, thinning was performed to leave the three most vigorous plants. The plants were trained to grow upright during cultivation. For disease control, two applications of tebuconazole (0.75 L ha^{-1}) were carried out in a preventive manner.

The soil was maintained at field capacity, estimated at a matric potential of -10 kPa from sowing to the end of the R3 stage (second phase of elongation of the flower bud, located at a distance greater than 2.0 cm above the last leaf), which occurred at 52 DAS (days after sowing). The treatments with the matric potentials of -10 and -60 kPa were established from the 52th DAS - within the interval from the final phase of the stage R3 to R4 (beginning of flowering, characterized by showing the first visible ligulate flowers, with yellow color) and maintained until the R8 stage, corresponding to the second phase of achene development, visually exhibiting the back of the capitulum in a dark yellow color and the bracts still green (Castiglioni et al., 1997). Soil water potential in the soil was monitored using the digital mini-tensiometer model T5 (UMS-MUC), and the moisture content in the pot was adjusted by adding water.

Gas exchange variables were evaluated at 2 (55 DAS), 9 (62 DAS), 16 (69 DAS), 21 (74 DAS) and 30 (83 DAS) days after water deficit application (DAWDA). One day after each period mentioned, chlorophyll fluorescence variables were evaluated. The reading of leaf water potential was taken only at 22 DAWDA.

Gas exchanges were determined in the third or fourth fully expanded leaf, below the capitulum. Photosynthetic rate (A), stomatal conductance (g_s), leaf transpiration rate (E), water use efficiency (WUE_i), leaf temperature (LT) and intracellular CO_2 concentration (C_i) were determined using a portable infrared gas analyzer (IRGA, Li-Cor, Li-6400 model), in five plants of each treatment. These variables were analyzed in plants exposed to solar radiation, at air temperature of 25 °C, photosynthetically active radiation of 1,200 μmol m^{-2} s^{-1}, CO_2 concentration and relative humidity of the natural air between 8 h and 10 h 30 min.

Chlorophyll fluorescence efficiency was evaluated using a portable fluorometer (Multi-Mode Chlorophyll Fluorometer OS5P) between 8 and 11 h, in the same leaves in which gas exchange evaluations were carried out. To determine the variables of the dark-adapted state, namely, initial fluorescence (F_o), maximum fluorescence (F_m) and F_v/F_m ratio (maximum quantum yield), the leaves were prepared using leaf clips for a period of 30 min, to simulate a dark condition.

After the parameters were determined in the dark-adapted state, the leaves were subjected to a saturating light pulse, with intensity of 1,400 μmol photons m^{-2} s^{-1} and duration of 15 s, to determine the parameters of the light-adapted state, which permit to obtain the relations: initial fluorescence (F_0'), maximum fluorescence (F_m'), Y (effective photochemical quantum yield of PSII in light-adapted state), ETR (electron transport rate in photosystem II), Q_P (photochemical quenching coefficient), Q_N (non-photochemical quenching coefficient) and NPQ (non-photochemical dissipation).

Leaf water potential (Ψw) was determined at the R7 stage, i.e., at 22 DAWDA, between 3 and 5 a.m., by means of a pressure chamber (Scholander bomb - Model 600 Spec), in fully expanded leaves, in five replicates of each treatment.

The results were subjected to analysis of variance and treatment means were compared by Tukey test at 0.05 probability level. The values for least significant difference (LSD) were represented in Figures 3 and 4.

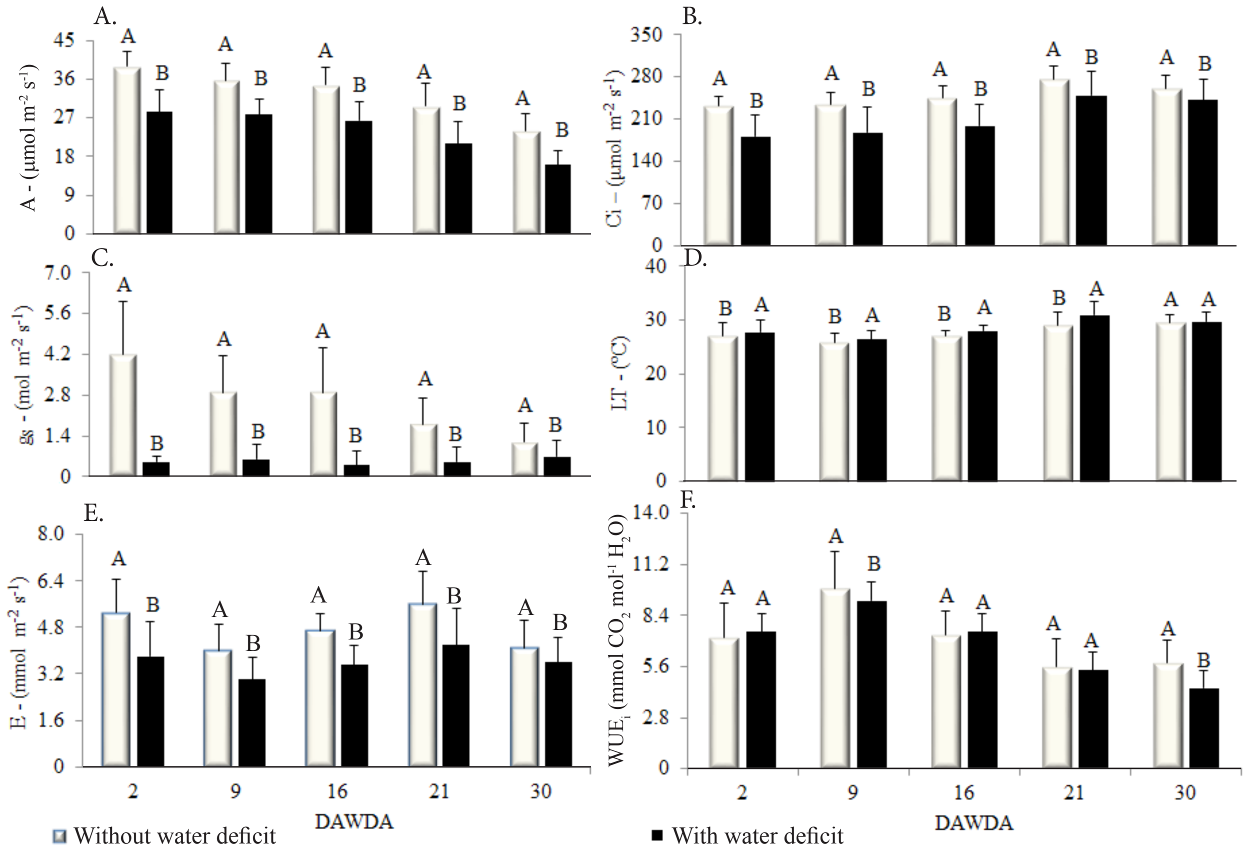
RESULTS AND DISCUSSION

Two days after induction of water deficit, there was a reduction in A, g_s, E and C_i and an increase in LT (Figure 1). In relation to the periods of gas exchange evaluations, it can be noted that the mean of A decreased along all periods, both in plants grown with irrigation and in those under water deficit conditions. Highest value of A was observed on the second DAWDA in plants under irrigation (39 μmol m^{-2} s^{-1}) and water deficit conditions (28.4 μmol m^{-2} s^{-1}). In this period of evaluation, A was higher than in the other stages of the crop. Silva et al. (2013) observed higher net assimilation rate at 52 DAS (32 μmol m^{-2} s^{-1}), and it decreased along the phenological stages of the crop. Highest rates in this period are related to the period of intense accumulation of photo assimilates in seed reserves.

Plants under water deficit rapidly reduced g_s, on the second day after water deficit (Figure 1), maintaining it between 0.4 and 0.7 mol m^{-2} s^{-1} during the steps of evaluation. This indicates that stomatal closure affected the process of carbon assimilation due to water deficit. Carneiro (2011) questioned what the main component of gas exchanges influencing sunflower development and production under water deficit conditions would be. For some researchers, stomatal limitation is the main cause of reduction in carbon assimilation; others attributed the reduction in photosynthetic rate to non-stomatal limitation (Pankovic et al., 1999; Steduto et al., 2000), suggesting that such behavior depends on the species and on each cultivar.

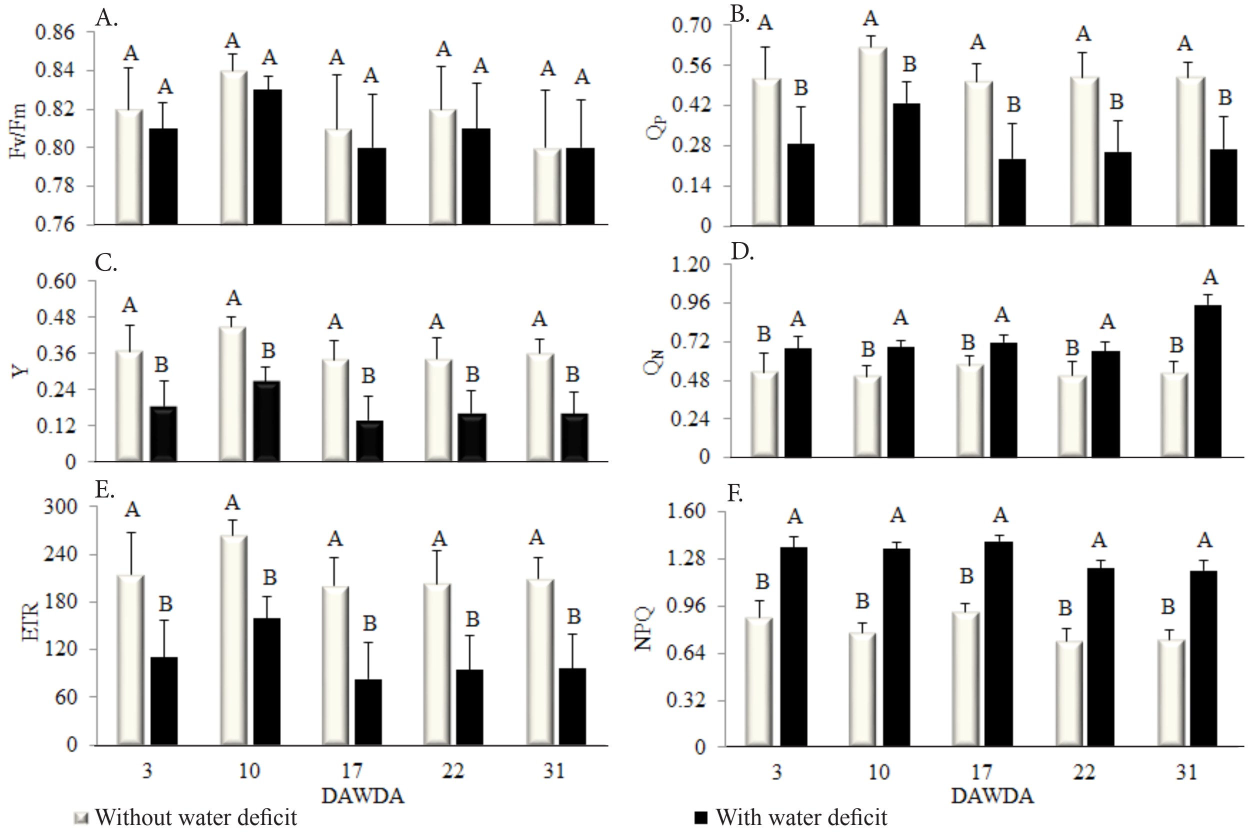
Stomatal limitation in plants subjected to stress indicates the restriction of water loss by transpiration and lower Ci. As CO_2 enters with greater difficulty, due to the stomatal limitation imposed to the diffusion process, there is a reduction in the photosynthetic rate of the plant resulting from lack of substrate. Regulation of stomatal opening is a mechanism used by many species to restrict water loss and overcome drought periods (Nascimento et al., 2011). The results of this experiment indicate a conservative behavior of sunflower plants to minimize water deficit (Figure 1).

Despite the stomatal limitation of photosynthesis, the maximum photochemical efficiency of PSII (F_V/F_m) was not affected by the water deficit, i.e., no significant difference was observed between control plants and those subjected to water deficit (Figure 2). Similar responses were also found by



Same letters between two water regimes of each period do not differ statistically by Tukey test at 0.05 probability level. Bars of error represent the standard deviation of the mean of five replicates

Figure 1. Effects of water regime on photosynthetic rate - A (A), intracellular CO_2 concentration - C_i (B), stomatal conductance - g_s (C), leaf temperature - LT (D), leaf transpiration rate - E (E) and water use efficiency - WUE_i (F) until 30 days after water deficit application (DAWDA)



Same uppercase letters between the two water regimes do not differ statistically by Tukey test t 0.05 probability level. Bars of errors represent the standard deviation of the mean of five replicates

Figure 2. Effects of water regime on maximum quantum yield - F_v/F_m (A), photochemical quenching coefficient - Q_P (B), effective quantum yield of PSII in light-adapted state - Y (C), non-photochemical quenching coefficient - Q_N (D), electron transport rate in PSII - ETR (E) and non-photochemical dissipation - NPQ (F) in sunflower until 31 days after water deficit application (DAWDA)

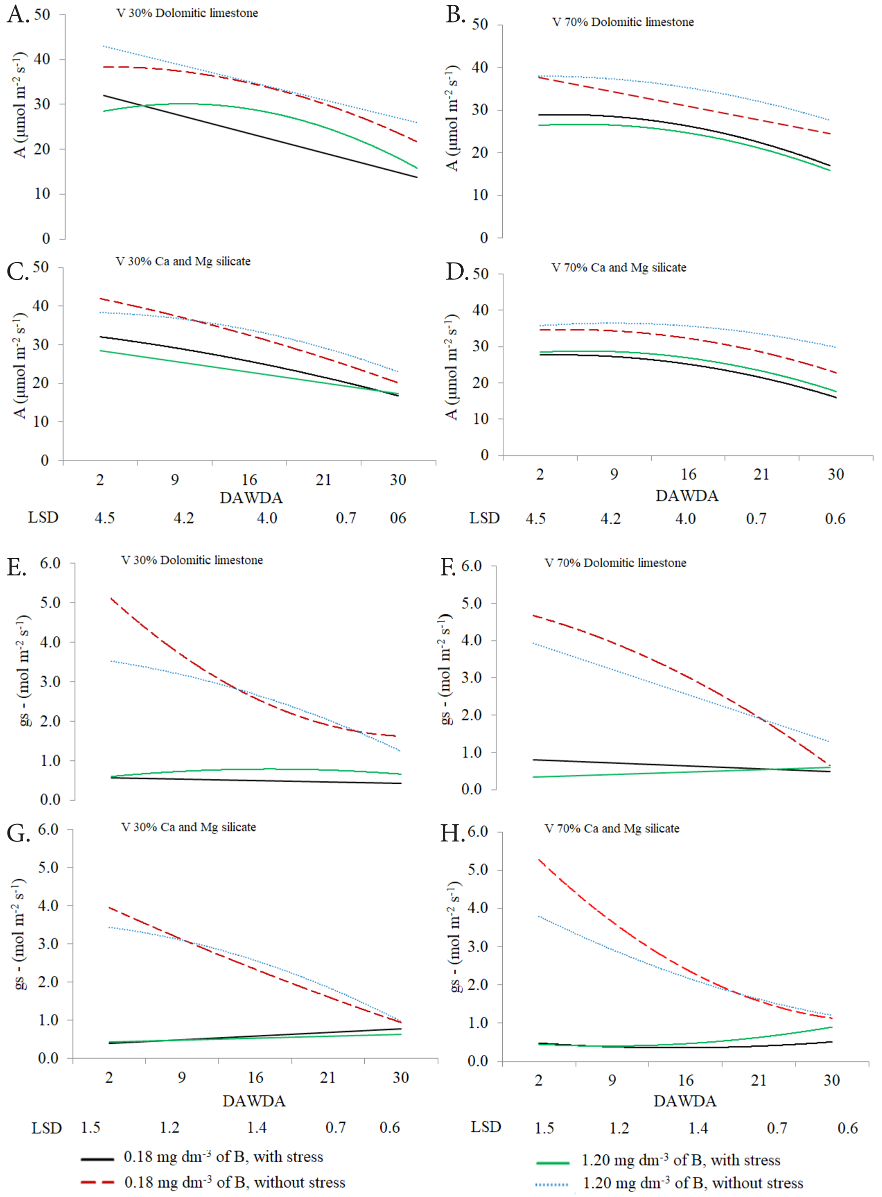
Sausen (2007), in castor bean, along 31 days of water deficit. Roza (2010) reported that in greenhouse experiments the efficiency of PSII, expressed by the F_V/F_m ratio, was not altered, which indicates greater tolerance to water deficit. However, for plants cultivated under uncontrolled environmental conditions (field), the F_v/F_m ratio influences the PSII under water deficit conditions.

The F_v/F_m ratio values obtained in this study are within those recommended as normal (between 0.750 and 0.850) in plants under ideal conditions of water regime. The decrease in this ratio indicates a reduction in the photochemical efficiency of PSII (Silva et al., 2015). The F_v/F_m ratio ranged from 0.800 to 0.840 (Figure 2), indicating that the photosynthetic apparatus remained intact, i.e., it did not exhibit photoinhibition damage in the PSII reaction center, which is often attributed to damage in the protein D_1 (Cassana et al., 2008).

In the other light-adapted photochemical variables, significant changes were observed between plants grown at water potentials of -10 and -60 kPa from the first reading at three days after water deficit application (56 DAS), similarly to what occurred in the gas exchanges. The values of Y, ETR and Q_P at water potential of -60 kPa were significantly lower than those of the soil maintained at field capacity.

The effective photochemical quantum yield of PSII (Y) was higher in plants cultivated at water potential of -10 kPa, reflecting in the difference of electron transport rate in photosystem II (ETR) and in the photochemical and non-photochemical coefficients (Figure 2). Higher non-photochemical coefficient (Q_N and NPQ) of plants under stress probably indicates that they showed greater dissipation of light energy absorbed in the form of thermal energy, in detriment of the use of this energy in photochemical effect, i.e., production of chemical energy in the form of ATP and NADPH (Zanandrea et al., 2006). However, the photochemical quenching coefficient (Q_P) represents the proportion of photon energy captured by the open PSII reaction centers and dissipated through electron transport.

Two days after water deficit induction, plants that were grown under base saturation of 30% showed higher A (Figures 3A, B, C and D). It was also found that the lowest level of B in



Means of photosynthetic rate and stomatal conductance of each period after water deficit application were compared based on least significant difference (LSD)

Figure 3. Photosynthetic rate (A, B, C, D) and stomatal conductance (E, F, G and H) during 30 days after water deficit application (DAWDA)

the soil was efficient to guarantee a greater response in terms of A and g_s (Figure 3), and the highest level of B reduced stomatal opening, regardless of the water regime.

These results differ from those obtained by Castro et al. (2006), in which the levels of B in the soil, below 0.27 mg dm^{-3}, were not sufficient to meet the nutritional requirements of sunflower plants, regardless of the water deficit application phases and water conditions. However, according to Foloni et al. (2010), in corrected soils whose B contents are below 0.26 mg dm^{-3}, sunflower has high responsiveness to B fertilization.

At the achene development stage (21 DAWDA), however, there was effect of the interaction of Base saturation versus B versus Water deficit. Thus, it was possible to observe (Figures 3B and D) that base saturation at 70% and the highest level of B led to the highest value of photosynthetic rate at the water potentials of -10 and -60 kPa. The nutritional demand in terms of B becomes more intense at the beginning of the reproductive stage and may also become more critical during the formation of seeds. In addition, B acts in the absorption and metabolism of cations, especially Ca, and in the absorption of water (Silva et al., 2016).

At the stage of achene development and filling (21 and 30 DAWDA), the highest level of B favored higher g_s in plants under water deficit (Figures 3F and G). Bogiani et al. (2013) explained that lower g_s in plants grown with lower doses of B may be attributed to the reduction in the frequency and number of stomata present in the leaf, in case of deficiency, and also to the accumulation of starch in the leaves, due to the lower transport of carbohydrates caused by B deficiency. Rosolem & Leite (2007) found lower number of stomata and reduction in their functioning in leaves of coffee subjected to B deficiency.

Sunflower plants cultivated in soil corrected with Ca and Mg silicate showed higher g_s under water deficit during the achene filling stage, corresponding to the 30th day after water deficit (Figures 3G and H). According to Gunes et al. (2008), sunflower cultivars which received Si application reduced stomatal conductance and, consequently, increased WUE_i.

Zanão Júnior (2011) found that applications of potassium silicate doses led to a reduction in transpiration rate and increase in A and g_s, with the increment in the applied doses of Si. This same study also revealed the ability of sunflower to accumulate Si in the trichomes and cells of the epidermis, through the anatomical study of the leaves, which can help this plant tolerate abiotic stresses.

The lowest Q_P values occurred in plants under water deficit (Figures 4A, B, C and D). It can be highlighted that, at the achene filling stage (30 DAWDA), base saturation of 30% led to higher Q_P efficiency by the interaction of the silicate-based corrective in plants grown at water potential of -10 kPa. The interaction of the dolomitic corrective versus water potential of -10 kPa led to higher Q_P with base saturation at 70%. Thus, Ca and Mg silicate under full irrigation conditions improved the performance of chlorophyll fluorescence, with the lowest level of base saturation.

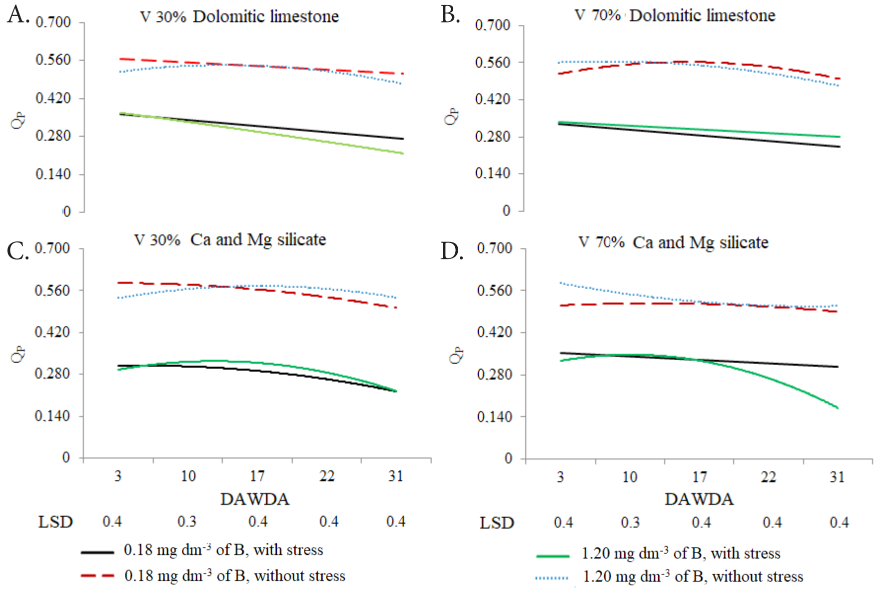
There was a reduction in leaf water potential at 22 days after water deficit application (Table 2). Carneiro (2011) also found similar values of water potential (-0.6 MPa) in plants under control irrigation. The cultivars M735 and MG2 showed potentials of -1.20 and -1.72 MPa, respectively, after 12 days of stress (Carneiro, 2011).

In some studies on sunflower cultivars, the critical water potential for photosynthetic rate was approximately -2.1 MPa

Table 2. Leaf water potential of sunflower determined in the achene development stage after 22 days of water deficit

WR	Leaf water potential (MPa)	
I	-0.61 A	
S	-2.08 B	
CV (%)	15.66	
SB	**C_1**	**C_2**
$V_{30\%}$	-1.40 a A	-1.30 a B
$V_{70\%}$	-1.45 a A	-1.10 b A
CV (%)	11.6	

SB - Base saturation; $V_{30\%}$ - Base saturation at 30% ; $V_{70\%}$ - Base saturation at 70%; C_1 - Dolomitic corrective; C_2 - Silicate corrective; WR - Type of water regime; I - Irrigation (water potential of -10 kPa); and S - Stress (water potential of -60 kPa).
Means followed by the same lowercase letters in rows and uppercase letters in columns do not differ statistically by Tukey test at 0.05 probability level



Means of photochemical quenching coefficient of each period after water deficit application were compared based on least significant difference (LSD)

Figure 4. Photochemical dissipation (A, B, C, D) during 31 days after water deficit application (DAWDA)

(Mojayad & Planchon, 1994). The reduction of leaf water potential led to decreased stomatal conductance, which represented the primary cause of reduction in photosynthetic rate and transpiration rate under water deficit conditions. This reduction is explained by the decrease in CO_2 availability in the substomatal chambers of the leaves, caused by the lower angle of stomatal opening (Gonçalves et al., 2009).

Base saturation of 70% was favorable to attenuate the reduction of leaf water potential when it interacted with Ca and Mg silicate. This significant difference may be induced by increased proline content due to lower soil acidity (Carlin & Santos, 2009). Also, because sunflower plants have the ability to increase proline content under water deficit conditions, Si increases the production of this solute in sunflower (Gunes et al., 2008). The increase in proline content is related to leaf osmotic adjustment with the reduction of water potential. Proline accumulates as a compatible solute to stabilize membranes and maintain the conformation of proteins, which prevents cytosol dehydration (Kishor et al., 2005).

CONCLUSION

The supply of Si and B can reduce the damage to sunflower caused by water deficit.

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