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# Foliar application of potassium nitrate induces tolerance to water deficit in pre-flowering sorghum plants

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**ABSTRACT.** The objective of this study was to evaluate the ability of foliar application of potassium nitrate (KNO<sub>3</sub>) to induce water deficit tolerance in sorghum plants (*Sorghum bicolor* cv. P898012) subjected to water deficit at pre-flowering. The experiment was conducted under greenhouse conditions with 4 treatments: field capacity (FC), water deficit (WD), field capacity + KNO<sub>3</sub> (FC + KNO<sub>3</sub>), and water deficit + KNO<sub>3</sub> (WD + KNO<sub>3</sub>). Two foliar applications of 3% (m/v) KNO<sub>3</sub> were made, the first on day zero of stress and the second on the fifth day. All analyses were performed after 12 days of stress (end of stress). Foliar application of KNO<sub>3</sub> to irrigated plants led to increases in relative chlorophyll content, photosynthetic rate, stomatal conductance, transpiration, and carboxylation efficiency. It also induced increases in leaf concentrations of P, Mg, S, Cu, and Fe, in addition to height growth. Under water deficit conditions, plants treated with KNO<sub>3</sub> presented higher relative chlorophyll content, leaf area, photosynthetic rate, stomatal conductance, transpiration, carboxylation efficiency, and higher levels of P, K, Mg, S, Cu, and Fe than those not treated with KNO<sub>3</sub>. The morphometry of the root system was not altered by the treatments. In addition, plants treated with KNO<sub>3</sub> under water deficit conditions showed higher growth and a grain yield 32.2% higher than those that did not receive KNO<sub>3</sub>. These results demonstrated that KNO<sub>3</sub> applied to the leaves induced water deficit tolerance in sorghum plants subjected to severe water stress at pre-flowering.

**Keywords:** mineral nutrition; drought resistance; leaf gas exchange; chlorophyll fluorescence; WinRhizo; *Sorghum bicolor*.

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## Introduction

Research has shown that climate change in recent years has altered rainfall patterns worldwide and has threatened food production in environments where water is increasingly scarce. Therefore, the major challenge for this half of the century is to produce more food with reduced water resources (Foley et al., 2011); this will generate selection pressure, requiring crops that are highly adapted to water deficit. Among these food crops, sorghum (*Sorghum bicolor* Moench L.), which originated in tropical Africa and is a food source for more than 500 million people in 98 countries, is a cereal with great adaptation to drought, when compared to other crops (Pennisi, 2009).

It is now known that some sorghum genotypes are tolerant to water deficit at the pre-flowering stage and others at the post-flowering stage (Emendack, Burke, Sanchez, Laza, & Hayes, 2018). However, due to the poor rainfall distribution, studies and techniques that search for tolerant genotypes are necessary because although sorghum shows good tolerance to water deficit, losses due to water stress recur (Elhag & Zhang, 2018).

In sorghum, drought at pre-flowering leads to an increase in the production of reactive oxygen species (ROS) and a reduction in the function of plant photosystems, thus causing a decrease in energy in photosynthetic processes (Guo, Tian, Liu, Wang, & Sui, 2018). In addition, water deficit leads to a delay in flowering, leaf curling, flower abortion, and reduction in panicle and plant size and weight (Borrell et al., 2014). This occurs because of a reduction in stomatal conductance. This reduction is one of the mechanisms by which plants reduce water loss when the water absorption capacity in the soil is lower than the losses to the atmosphere. However, by decreasing water loss by transpiration, the reduction in stomatal conductance inevitably restricts CO<sub>2</sub> input into the mesophyll (Lawlor & Cornic, 2002; Souza et al., 2013; Lavinsky,

Magalhães, Ávila, Diniz, & Souza, 2015).

In addition, it is emphasized that a reduction in gas exchange strongly inhibits the absorption and transport of nutrients. This is partly due to the reduction of up to 70% in transpiration during drought (Lavinsky et al., 2015), since the transport of minerals is governed by the transpiration chain via the xylem (White, 2001). Associated with this is a reduction in the photosynthetic rate and, therefore, the energy load of the plant. In maize plants, for example, severe water stress may lead to an average reduction of 80% in the photosynthetic rate (Ávila et al., 2016) and consequently a severe energy deficit. Therefore, this low energy load induces an entropy in the system, compromising the absorption of nutrients, among other processes, since many of the nutrient carriers are energy-dependent channels (Grossman & Takahashi, 2001).

In addition to the tools of traditional and modern plant breeding, the foliar application of mineral and organic substances has contributed to mitigating the effect of water deficit on plants (Gimeno et al., 2014; Liu et al., 2015; Reis et al., 2018; Ávila et al., 2019). However, few studies have explored the mechanisms and effects of foliar application of potassium nitrate ( $\text{KNO}_3$ ) on morphophysiological and agronomic characteristics, and whether these substances actually lead to an increase in production.

Therefore, recent studies have drawn attention to mineral nutrition against stress factors (Saud et al., 2017; Wang et al., 2019). In this context, it has been observed that well-nourished plants show better physiological performance under stress conditions (Zhong et al., 2017). Therefore, it has been observed that soil mineral supplementation with nitrogen compounds such as  $\text{KNO}_3$  has led to improvements in osmotic adjustment, the enzymatic antioxidant system (Khammari, Galavi, Ghanbari, Solouki, & Poorchaman, 2012), photosynthesis, stomatal conductance, transpiration, and nutrition in plants under water stress (Gimeno et al., 2014).

In addition, it has been verified that  $\text{KNO}_3$  supplementation can positively influence the 'stay green' function, which is characterized by the maintenance of photosynthetic pigments as well as the photosynthetic process (Borrell et al., 2014). However, many of the nitrogen compounds applied, including  $\text{KNO}_3$ , are in the form of salts and, therefore, in dry soils, they may contribute even more to soil water deficit (Gimeno et al., 2014). Thus, an alternative would be to explore the use of nitrogen compounds through foliar application, in an attempt to maintain the nitrogen status in the leaf under optimum conditions; this could mitigate the effects of water deficit, inducing physiological improvements in the plants (Bahrami-Rad & Hajiboland, 2017; Ul-Allah et al., 2020). Few papers report this alternative with  $\text{KNO}_3$ , showing the novelty of this approach. The objective of this study was to evaluate the ability of  $\text{KNO}_3$  via foliar application to induce tolerance to water deficit in sorghum plants subjected to stress at pre-flowering.

## Material and methods

### Growing conditions, experimental design, and application of $\text{KNO}_3$

The experiment was conducted in a greenhouse at Embrapa Milho e Sorgo (19°28' S, 44°15'08" W, 732 m altitude) and the plant material used was the pre-flowering drought-sensitive sorghum cultivar, P898012, which presents the 'stay green' characteristic in its phenotype. The design was completely randomized, with four treatments and six replicates. The treatments were field capacity (FC), water deficit (WD), field capacity + potassium nitrate (FC +  $\text{KNO}_3$ ), and water deficit + potassium nitrate (WD +  $\text{KNO}_3$ ). The treatments FC and FC +  $\text{KNO}_3$  had the soil water tension maintained close to -18 KPa throughout the cycle, whereas treatments WD and WD +  $\text{KNO}_3$  were imposed when the plants reached pre-flowering. In the treatment with water deficit, the soil had its water pressure reduced to -138 KPa, corresponding to the application of 50% of the available water, maintained at that level for a period of twelve days (Souza et al., 2014).

The plants were grown in plastic pots containing 20 kg of oxisol. The water content in the soil was monitored daily between 9 a.m. and 3 p.m. with the aid of GB Reader N1535 (Measurement Engineering, Australia) moisture sensors installed in the center of each pot with a screw thread at a depth of 20 cm. These sensors detect soil water tension, based on electrical resistance, and are coupled to digital meters. Water replenishment through irrigation was based on the readings obtained with the sensor, and the water returned to field capacity during the period before the treatments. Water replenishment was calculated with the aid of a spreadsheet, according to the water retention curve of the soil. Correction and basal and cover fertilization were based on soil chemical analyses and the sorghum crop demand.

$\text{KNO}_3$  (Vetec Química, Sigma-Aldrich®) was applied to the leaves at a concentration of 3% ( $\text{m v}^{-1}$ ) with the aid

of a pressurized CO<sub>2</sub> applicator, calibrated for a flow of 200 L ha<sup>-1</sup> at day zero of stress and on the fifth day of stress.

### Physiological and morphological analyses

All analyses were performed after twelve days of stress (end of stress) on the first leaf below the flag leaf. The leaf water potential was determined using a Scholander pressure pump (3005 Soil Moisture Equipment Corp., Santa Barbara CA, USA) at noon (midday,  $\Psi_{md}$ ). The relative chlorophyll content and/or SPAD index was determined using a chlorophyll meter (SPAD 502, Minolta, Japan). The variable quantum efficiency of photosystem II ( $F_v/F_m$ ) was obtained using a pulse-modulated fluorometer (PEA-Pocket chlorophyll Fluorimeter, Hansatech Instruments, Norfolk, England).

Leaf gas exchange was evaluated with an LI 6400 infrared gas analyzer (IRGA – LI-COR, Lincoln, NE, USA) equipped with a camera (LI-6400-40, LI-COR Inc.). The variables evaluated were photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), concentration of intercellular CO<sub>2</sub> (Ci), intrinsic water use efficiency (A/gs), and carboxylation efficiency (A/Ci). The measurements were taken between 9 a.m. and 11 a.m. under a photosynthetically active artificial radiation of 1,500  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at leaf level, with 21% O<sub>2</sub> and 400  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  air.

The concentrations of macro- (N, P, K, Mg, Ca, and S) and micronutrients (Zn, Fe, Mn, and Cu) in the leaf were determined according to the methodology described by Silva (2009).

The WinRhizo computer system (WinRhizo Pro, Regent Inc. Instr., Canada) was used to perform morphometric evaluations of the root system at the end of stress, in which the volume and surface area of the roots were measured by diameter classes, as described below: very fine roots ( $\varnothing < 0.5 \text{ mm}$ ), fine roots ( $0.5 \varnothing < 2.0 \text{ mm}$ ), and thick roots ( $\varnothing > 2.0 \text{ mm}$ ) (Magalhães, Souza, & Cantão, 2011). After 12 days of stress, total leaf area measurements were taken using a leaf area meter (Li-Cor Inc.).

### Agronomic analysis

At the end of the crop cycle, plant height (PH) was measured with a graduated ruler and plant diameter (PD) was measured with a caliper. The plants then had their panicles collected and subjected to forced air drying at 70°C for 72 hours. Subsequently, with the aid of a digital analytical balance, grain dry biomass was obtained.

### Statistical analysis

After obtaining the data, preliminary statistical tests were applied to match the results to the analysis of variance (ANOVA), using the statistical software Sisvar. The Scott-Knott test at 95% ( $p < 0.05^{**}$ ) significance was used to test any and all differences between treatments. The functional relationship between photosynthesis and stomatal conductance, carboxylation efficiency, and  $F_v/F_m$  ratio was determined by simple linear regression analysis, using the BioEstat 5.0 statistical software, at a significance level of 99% ( $p < 0.01^*$ ).

## Results and discussion

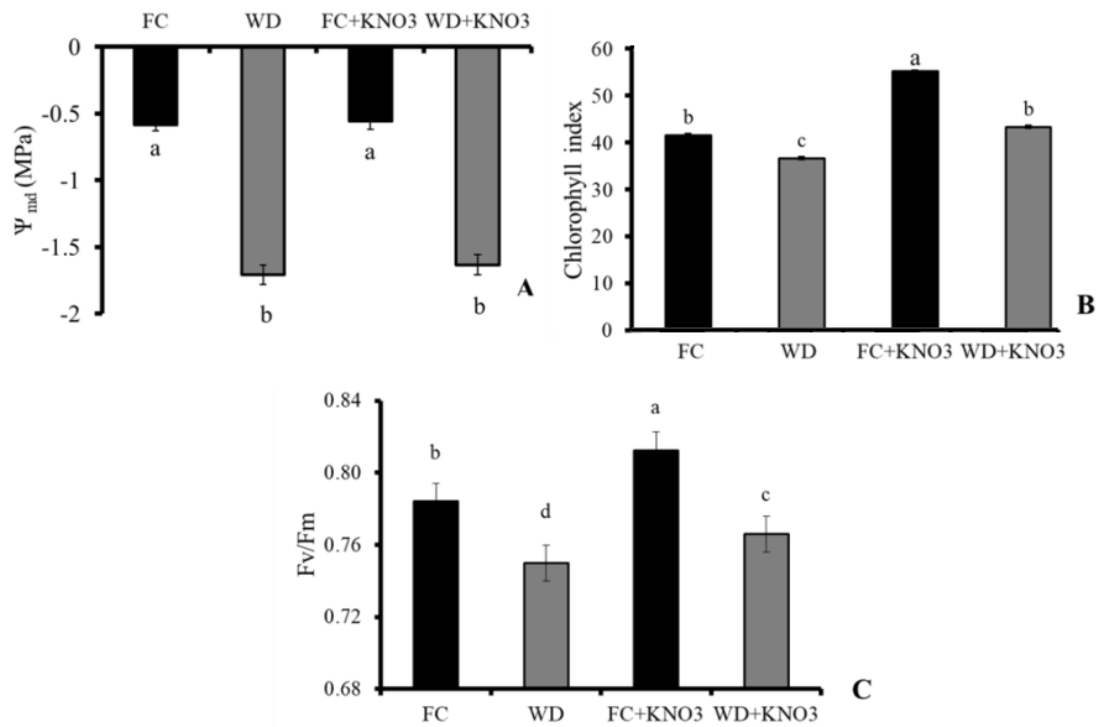
After 12 days of treatment, all plants that were under water deficit (WD) presented a reduction in leaf water potential in relation to those that remained with irrigation at the field capacity level (FC), regardless of the treatment (Figure 1A).

The 12-day WD treatment significantly reduced the relative chlorophyll content (Figure 1B). However, KNO<sub>3</sub> induced an increase in the chlorophyll biosynthesis of the plants at FC and guaranteed the maintenance of the plants under WD since, under FC, plants treated with KNO<sub>3</sub> had higher chlorophyll indices than those not treated. In addition, among the plants that were under stress conditions, those treated with KNO<sub>3</sub>, presented a higher relative chlorophyll content and had values of this variable similar to those that were under FC.

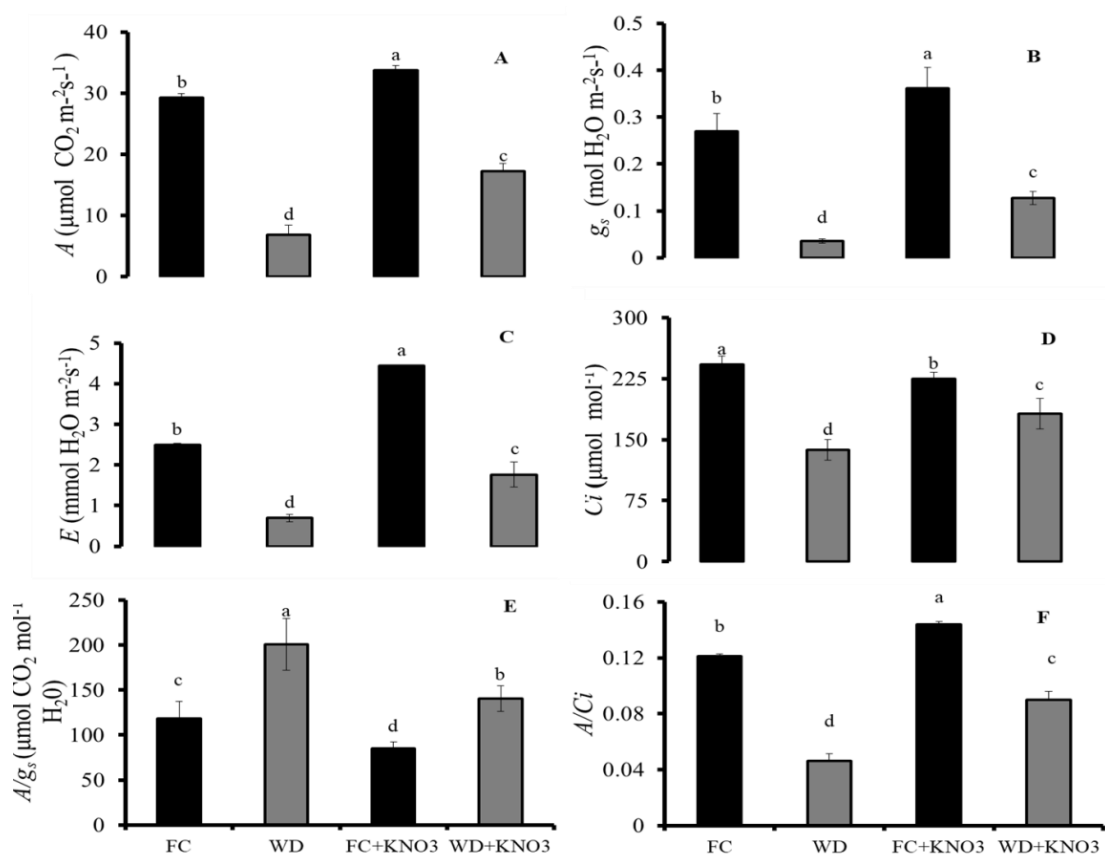
The concentration of intercellular CO<sub>2</sub> (Ci) was higher in plants under FC, regardless of the treatment (Figure 2D). However, under this water condition, the plants that did not receive KNO<sub>3</sub> had higher internal carbon levels than those treated with KNO<sub>3</sub>. In contrast, under WD, the plants that received the treatment with KNO<sub>3</sub> had higher internal carbon values than those not treated. Under drought conditions, plants increased their intrinsic water use efficiency (A/gs), when compared to the controls under normal irrigation (Figure 2E). However, plants not treated with KNO<sub>3</sub> showed higher intrinsic water use efficiency at the two water levels in the soil.

The photosynthetic rate was positively correlated ( $r = 0.87$ ) with stomatal conductance, so that as stomatal conductance increased, significant increases occurred in photosynthesis (Figure 3A). In this context, it is noted that the reduction in stomatal conductance induced by drought contributed significantly to lower photosynthesis rates in plants

under stress. A similar behavior was observed for the relationship between carboxylation efficiency and photosystem II efficiency (Figure 3B), since the higher the  $F_v/F_m$  ratio, the greater the carboxylation efficiency ( $r = 0.88$ ).

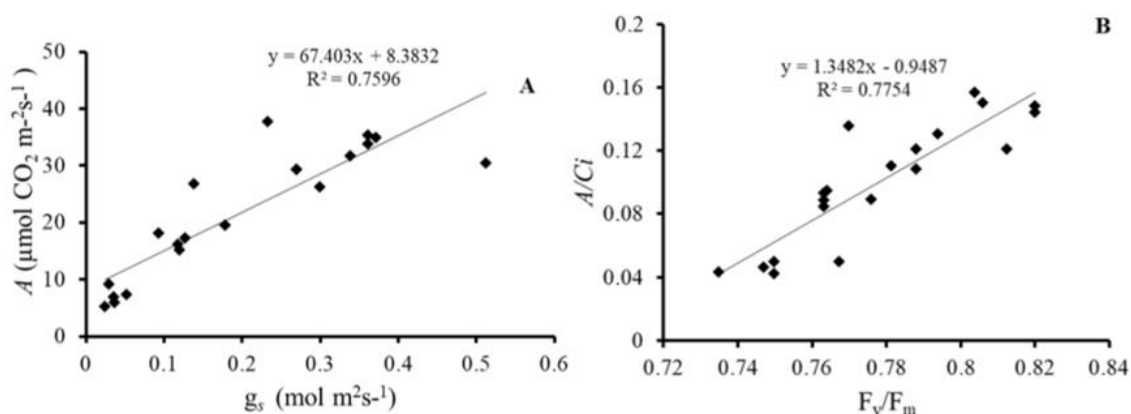


**Figure 1.** Leaf water potential (midday,  $\Psi_{md}$ ) (A), SPAD index (B), and  $F_v/F_m$  ratio (C) in sorghum plants subjected to water stress and treated with KNO<sub>3</sub> at pre-flowering. FC = field capacity; WD = water deficit; FC + KNO<sub>3</sub> = field capacity + KNO<sub>3</sub>; WD + KNO<sub>3</sub> = water deficit + KNO<sub>3</sub>. Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability. The bars represent the standard error of the mean of six replicates.



**Figure 2.** Photosynthetic rate (A), A, stomatal conductance ( $g_s$ ), B; transpiration rate (E), C; concentration of intercellular CO<sub>2</sub> ( $C_i$ ), D;

intrinsic water use efficiency ( $A/g_s$ ),  $E$ ; and carboxylation efficiency ( $A/C_i$ ),  $F$  in sorghum plants subjected to water stress and treated with KNO<sub>3</sub> at pre-flowering. FC = field capacity; WD = water deficit; FC + KNO<sub>3</sub> = field capacity + KNO<sub>3</sub>; WD + KNO<sub>3</sub> = water deficit + KNO<sub>3</sub>. Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability. The bars represent the standard error of the mean of six replicates.



**Figure 3.** Relationship between stomatal conductance ( $g_s$ ) and photosynthesis ( $A$ ) – A, and between carboxylation efficiency ( $A/C_i$ ) and efficiency of photosystem II ( $F_v/F_m$ ) – B, in sorghum plants subjected to water stress and treated with KNO<sub>3</sub> at pre-flowering. Linear correlation, significant at 1% probability.

The  $F_v/F_m$  ratio, which represents the quantum efficiency of photosystem II (Figure 1C), exhibited a behavior similar to that of the chlorophyll index, showing the close interconnection between these two variables. It was therefore observed that WD led to an increase in chlorophyll fluorescence, regardless of the treatment. However, the plants treated with KNO<sub>3</sub> had lower fluorescence levels than their controls under both water conditions.

Plants grown under FC presented higher photosynthetic rate ( $A$ ; Figure 2A), stomatal conductance ( $g_s$ ; Figure 2B), transpiration ( $E$ ; Figure 2C), and carboxylation efficiency ( $A/C_i$ ; Figure 2F) than those grown under WD conditions. However, it is important to note that KNO<sub>3</sub>-treated plants showed higher values for these parameters than those that did not receive KNO<sub>3</sub> under both water conditions.

Regarding mineral nutrition, in general, water deficit reduced leaf macro- and micronutrients; however, foliar application of KNO<sub>3</sub> attenuated the effects of drought (Table 1). Nitrogen was higher in plants at FC than in those under WD. There was no effect of KNO<sub>3</sub> on the levels of nitrogen in FC plants, but plants under WD and treated with KNO<sub>3</sub> exhibited higher values of nitrogen in their leaves than those that did not receive KNO<sub>3</sub>.

Under FC, phosphorus content was higher, regardless of KNO<sub>3</sub>, and the plants treated with KNO<sub>3</sub> had higher levels of phosphorus than those not treated. A similar behavior was observed under water stress, since plants treated with KNO<sub>3</sub> presented higher values than those that did not receive the treatment. Potassium was higher only in the plants under WD + KNO<sub>3</sub>, while there were no differences in potassium for the other treatments (Table 1).

**Table 1.** Concentration of macro- and micronutrients in the leaves of sorghum plants subjected to water deficit and treated with KNO<sub>3</sub> at pre-flowering.

	FC	WD	FC + KNO <sub>3</sub>	WD + KNO <sub>3</sub>
N (%)	4.59a	3.83 c	4.60 a	3.97 b
P (g kg <sup>-1</sup> )	3.47 b	2.25 d	4.27 a	2.96 c
K (g kg <sup>-1</sup> )	24.18 b	24.81 b	24.29 b	27.26 a
Ca (g kg <sup>-1</sup> )	10.15 a	7.66 b	9.58 a	8.38 b
Mg (g kg <sup>-1</sup> )	3.07 b	2.78 c	3.43 a	2.96 b
S (g kg <sup>-1</sup> )	2.29 b	1.98 c	2.56 a	2.04 c
Zn (mg kg <sup>-1</sup> )	150.45 a	107.64 b	149.09 a	113.64 b
Cu (mg kg <sup>-1</sup> )	11.43 b	9.32 c	12.02 a	8.76 c
Fe (mg kg <sup>-1</sup> )	186.40 b	158.40 c	238.97 a	179.70 b
Mn (mg kg <sup>-1</sup> )	187.53 c	256.63 a	197.50 c	241.25 b

FC = field capacity; WD = water deficit; FC + KNO<sub>3</sub> = field capacity + KNO<sub>3</sub>; WD + KNO<sub>3</sub> = water deficit + KNO<sub>3</sub>. Means followed by the same letter in the line do not differ statistically by the Scott-Knott test at 5% probability.

The levels of calcium in the leaves were affected only by the water levels, since plants under FC presented higher levels of this macronutrient. Treatment with KNO<sub>3</sub> induced an increase in magnesium content in plants under FC and the maintenance of their contents in plants under stress, with plants under WD + KNO<sub>3</sub> showing

values similar to those of plants under FC + KNO<sub>3</sub>. Plants of the WD + KNO<sub>3</sub> treatment showed the lowest magnesium content in their leaves. Sulfur content was reduced in plants under drought, regardless of the treatment. During normal irrigation conditions, KNO<sub>3</sub> led to increases in its contents. Among the plants under stress, there were no changes in sulfur content (Table 1).

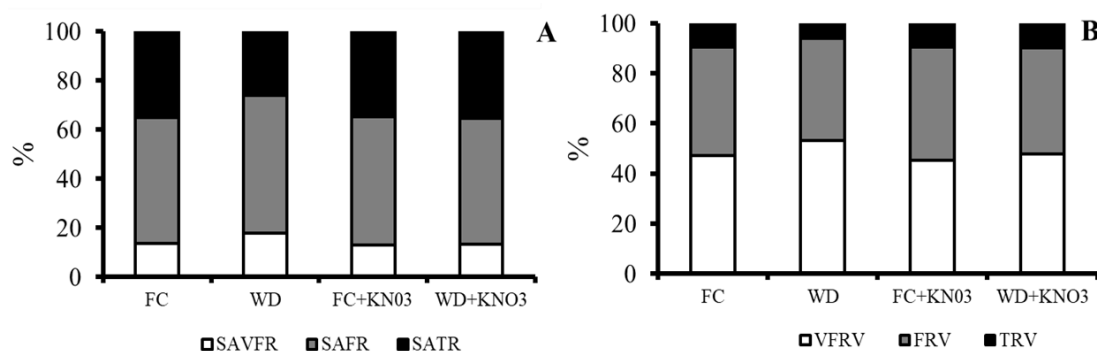
The concentrations of copper and iron presented similar behavior. Plants under stress reduced their levels regardless of the presence of KNO<sub>3</sub>. However, plants at FC treated with KNO<sub>3</sub> had higher values of both nutrients. Iron differed from copper, which had similar levels during drought in plants under stress, as values of iron were higher in plants under WD + KNO<sub>3</sub> than those under WD, and similar to those in FC + KNO<sub>3</sub>. Zinc was reduced with drought, independently of KNO<sub>3</sub>, but within the same soil water level, its content did not vary after KNO<sub>3</sub> treatment. Regarding manganese, it was verified that WD increased its levels independently of the treatment; under drought conditions, plants of the WD treatment showed higher levels of this element, while under irrigation, there were no differences among treatments (Table 1).

Unlike the variables analyzed in the shoot, where both drought and treatment with KNO<sub>3</sub> had a strong influence, the morphometry of the root system was not altered by these two factors. However, it is important to observe that the surface area of the root system (Figure 4A) consists of an average of 15% of very fine roots (SAVFR), 65% of fine roots (SAFR), and 30% of thick roots (SATR). The volume of the root system (Fig. 4B) had results similar to those of surface area, differing only in the volume of fine (VFR) and very fine roots (VVFR), which occupy, on average, 90% of the entire volume of the root system.

KNO<sub>3</sub> positively influenced plant height (PH; Table 2), since plants under FC + KNO<sub>3</sub> exhibited the highest plant height among all treatments, and plants under WD + KNO<sub>3</sub> showed similar heights to those in FC, demonstrating that KNO<sub>3</sub> attenuated the effects of stress on plant growth. Among all the treatments studied, only plants that were under WD showed lower height. Plant diameter (PD) was lower in all WD plants. Within the same water level in the soil, there were no differences in this variable among treatments.

Leaf area (LA) was higher in plants under FC, followed by FC + KNO<sub>3</sub> and WD + KNO<sub>3</sub>, which did not differ from each other. Among all the treatments, the plants under WD not treated with KNO<sub>3</sub> presented the lowest LA. Thus, it is possible to note that KNO<sub>3</sub> guaranteed the maintenance of plant LA during stress. Drought significantly affected grain dry biomass (GDB), since all the plants that were under this water condition reduced their biomass in relation to the plants under FC, independently of KNO<sub>3</sub>. At FC, there were no differences among treatments. However, during WD, plants treated with KNO<sub>3</sub> produced a 32.2% higher final grain yield than plants that did not receive KNO<sub>3</sub> (Table 2).

Foliar application of KNO<sub>3</sub> led to drought tolerance in sorghum plants subjected to water stress at pre-flowering, and also yielded physiological improvements in plants under irrigation, since the plants of the treatments FC + KNO<sub>3</sub> and WD + KNO<sub>3</sub> presented higher growth in height in relation to their respective FC and WD controls. In addition, plants under WD + KNO<sub>3</sub> showed an increase of 32.2% in the final grain yield, when compared to those not treated with KNO<sub>3</sub>.



**Figure 4.** Root morphometry of sorghum plants subjected to water stress and treated with KNO<sub>3</sub> at pre-flowering. SAVFR: surface area of very fine roots; SAFR: surface area of fine roots; SATR: surface area of thick roots – A. VVFR: Volume of very fine roots; VFR: Volume of fine roots; VTR: Volume of thick roots – B. FC = field capacity; WD = water deficit; FC + KNO<sub>3</sub> = field capacity + KNO<sub>3</sub>; WD + KNO<sub>3</sub> = water deficit + KNO<sub>3</sub>.

**Table 2.** Plant height (PH), plant diameter (PD), leaf area (LA), and grain dry biomass (GDB) of sorghum plants subjected to water deficit and treated with KNO<sub>3</sub> at pre-flowering.

	FC	WD	FC + KNO <sub>3</sub>	WD + KNO <sub>3</sub>
PH (cm)	1.14 b	1.06 c	1.28 a	1.14 b

PD (mm)	12.47 a	10.54 b	12.27 a	11.45 b
LA (m <sup>2</sup> )	13.60 a	9.36 c	12.41 b	11.57 b
GDB (g)	30.11 a	12.65 c	29.97 a	18.39 b

FC = field capacity; WD = water deficit; FC + KNO<sub>3</sub> = field capacity + KNO<sub>3</sub>; WD + KNO<sub>3</sub> = water deficit + KNO<sub>3</sub>. Means followed by the same letter in the line do not differ statistically by the Scott-Knott test at 5% probability.

It is important to emphasize that the sorghum cultivar P898012 has a “stay-green” phenotype, and that materials with this phenotype are sensitive to water deficit at pre-flowering (Burke et al., 2013). One of the effects of water deficiency in these strains is accelerated leaf senescence, which directly impacts the photosynthetically active leaf area, grain growth, and yield (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). Therefore, KNO<sub>3</sub> contributed to the maintenance of the functional ‘stay-green’ phenotype, since it delayed senescence, maintaining the plants with a greater photosynthetically active area after stress, compared to plants under WD. Thus, the increase of 32.2% in the dry biomass of grains in plants under WD + KNO<sub>3</sub> in relation to the plants under WD is directly related to the increase in leaf area, since it increases the productive area of photoassimilates, which are highly essential to sustain grain filling (Avila et al., 2016).

The increase in growth of plants under FC + KNO<sub>3</sub> and in the growth and yield of grains in plants under WD + KNO<sub>3</sub> can be attributed to the improvement in the photosynthetic process promoted by KNO<sub>3</sub>. Therefore, in the photochemical apparatus, it increased the levels of chlorophyll in plants under irrigation and maintained those in the plants under stress. This positive feedback on the levels of photosynthetic pigments, especially during drought, may have contributed to a higher photochemical efficiency, since these pigments are constituents of the antenna complexes of photosystems I and II; therefore, they are responsible for the absorption and transfer of solar energy for the photosystems (Mirkovic et al., 2017). In addition, it is known that the continuous transfer of energy from the antenna complexes to the photochemistry is of paramount importance to reduce the loss of energy by non-photochemical pathways such as fluorescence, and also to avoid the oxidation and degradation of photosynthetic pigments (Kalaji et al., 2016; Mirkovic et al., 2017).

Chlorophyll ‘a’ fluorescence is an indication of the integrity and efficiency of photosystem II. Thus, lower fluorescence indicates a lower stress level and a higher photochemical efficiency, which results in higher formation of photochemical products, which are substrates for carbon fixation in the Calvin cycle (Kalaji et al., 2016). In this context, it was observed that KNO<sub>3</sub> increased the plants’ photochemical efficiency in the FC + KNO<sub>3</sub> and WD + KNO<sub>3</sub> treatments, compared with FC and WD, respectively.

It is important to note that the stomatal pathway contributed significantly to the reduction in the photosynthetic rate of plants under stress, since the reduction in photosynthetic rate was correlated with the reduction in stomatal conductance, which led to a reduction in internal carbon levels in the mesophyll and transpiration. However, in addition to improving the photochemical process, KNO<sub>3</sub> contributed to leaf biophysical processes (gas exchange), which are directly related to photosynthesis, as they increase the transpiration and stomatal conductance of irrigated plants and mitigate the effects on plants under stress. In tobacco under foliar potassium application, the same responses were observed for gas exchange for both irrigated plants and those under drought (Bahrami-Rad & Hajiboland, 2017).

Transpiration is a process of great importance for plants, as it is responsible for events such as leaf cooling, allowing leaves to maintain stomatal opening throughout the day (Maurel, Verdoucq, & Rodrigues, 2016). At the same time, greater stomatal conductance may favor the entry of atmospheric CO<sub>2</sub> into the leaf mesophyll, leading to a higher formation of organic compounds, which will later be transported, decarboxylated, and assimilated in the sheath cells during the photosynthetic process (Lavinsky et al., 2015).

This can be observed in this study, as plants at FC exhibited higher values of stomatal conductance, internal carbon, and photosynthesis than those under stress. In addition, plants under WD + KNO<sub>3</sub>, which presented higher photosynthesis rates, also exhibited greater stomatal conductance and internal carbon levels when compared to WD treatments. Plants treated with KNO<sub>3</sub> under both water conditions also had higher carboxylation efficiency, indicating a higher activity of the Rubisco enzyme (Farooq et al., 2009) in relation to their controls under the same water conditions. This higher carboxylation efficiency explains the lower internal carbon levels in plants under FC + KNO<sub>3</sub>, compared to the FC treatment because the greater activity of Rubisco intensifies CO<sub>2</sub> consumption.

In relation to the concentration of intercellular CO<sub>2</sub>, it was higher in plants treated with KNO<sub>3</sub> under WD, when compared to those not treated. This may have occurred because these plants had 25% greater stomatal conductance than those not treated. Thus, plants treated with KNO<sub>3</sub> absorbed more carbon, but CO<sub>2</sub> accumulated because of the reduction in carboxylation efficiency that was caused by the reduction in the



efficiency of photosystem II. Therefore, it is suggested that the improvements in these coupled processes may have favored the Calvin cycle activity and led to improvements in the photosynthesis of plants treated with  $\text{KNO}_3$  in relation to their controls within each soil water level.

The higher photosynthesis and stomatal conductance in treatments FC +  $\text{KNO}_3$  and WD +  $\text{KNO}_3$ , in relation to their respective FC and WD controls, explains the lower intrinsic water use efficiency, when this parameter is based on the ratio of photosynthetic rate/conductance. Therefore, the greater the stomatal conductance, the lower is the intrinsic water use efficiency. These data corroborate the findings of Gimeno et al. (2014), who observed that citrus plants subjected to water stress and treated with  $\text{KNO}_3$  had higher photosynthetic rate, stomatal conductance, and transpiration than those not treated with  $\text{KNO}_3$ . However, these authors did not observe an increase in the root system or water-use efficiency. Therefore, they attributed the higher transpiration rate and stomatal conductance to osmotic adjustment, since plants treated with  $\text{KNO}_3$  significantly increased proline and potassium levels in the leaves. In fact, in the present study, plants under WD +  $\text{KNO}_3$  had the highest levels of potassium in the leaves among all treatments. Thus, it is suggested that this osmotic adjustment may have favored plant water recovery during the night and, therefore, contributed to gas exchange the next day.

Foliar application of  $\text{KNO}_3$  also contributed to plant mineral nutrition since, under normal irrigation conditions, plants at FC +  $\text{KNO}_3$  showed higher concentrations of P, Mg, S, Cu, and Fe than the plants at FC that were not supplemented with  $\text{KNO}_3$ . Furthermore, under water deficit conditions, plants treated with  $\text{KNO}_3$  exhibited higher concentrations of N, P, K, Mg, S, Cu, and Fe than those not treated with  $\text{KNO}_3$ . Therefore, it is important to observe that the absorption and transport of minerals occurs by mass flow through the xylem, from the transpiratory flow (Garg, 2003).

Thus, the higher concentration of nutrients shown by plants treated with  $\text{KNO}_3$ , within each soil water level, is a result of the greater transpiration from these plants, with plants from the treatment FC +  $\text{KNO}_3$  having a transpiration rate 45.63% greater than those at FC. This difference is even greater under stress conditions, with plants from WD +  $\text{KNO}_3$  having 60.61% more transpiration than those from WD. Moreover, when the treatments FC and FC +  $\text{KNO}_3$  were compared with WD and WD +  $\text{KNO}_3$ , the plants that were under FC had a higher transpiration rate, and, in general, higher nutrient content in its leaves.

The higher concentrations of nitrogen under WD +  $\text{KNO}_3$ , compared to WD, may be correlated with the maintenance of chlorophyll content and lower fluorescence observed in these plants, since nitrogen is a structural component of chlorophyll (Peterson, Blackmer, Francis, & Schepers, 1993) and its metabolism is a process of high energy consumption. Thus, it is suggested that when the Calvin cycle is not able to consume all the energy generated in photochemistry, the nitrogen metabolic activity could consume some of this excess energy (Carelli, Fahl, & Ramalho, 2006). In addition, it has been observed that nitrogen metabolism may be associated with water stress tolerance in rice, as it contributes to the protection of the photosynthetic apparatus through activation of the antioxidant system and osmotic adjustment (Zhong et al., 2017).

The increase in relative chlorophyll content in plants under FC +  $\text{KNO}_3$  and its maintenance in plants under WD +  $\text{KNO}_3$  is directly related to the magnesium content, since this element constitutes the central nucleus of chlorophylls (Rissler, Collakova, DellaPenna, Whelan, & Pogson, 2002) and presented the same pattern of levels of this molecule. Magnesium may have contributed to the greater carboxylation efficiency of the plants of the treatments FC +  $\text{KNO}_3$  and WD +  $\text{KNO}_3$  in relation to their respective controls, as, together with  $\text{CO}_2$ , it forms a complex of activation of Rubisco (Farooq et al., 2009). Iron is another element that is closely linked to the synthesis of chlorophylls, acting as an enzymatic catalyst in this synthesis (Chen & Barak, 1982). In addition, iron is a fundamental element in the transfer of electrons through iron-sulfurous proteins in respiration, photochemistry, and nitrogen assimilation (Vigani & Briat, 2016). Improvements in height growth of plants treated with  $\text{KNO}_3$  under FC and WD may be linked to increase and maintenance, respectively, in phosphorus concentration, since this element is fundamental in energy metabolism and, therefore, in grain growth and production of the plant, mainly under stress conditions, where the plant undergoes an energy deficit (Liu et al., 2015).

Although there were no differences among treatments, it was important to characterize the roots by different diameter classes, as the fine and very fine roots were the ones that presented the greatest surface area and volume. This is directly related to the absorption capacity of water and minerals, since fine and very fine roots present higher kinetic absorption than thick ones, as thick roots are commonly in advanced stages of suberization (Liu et al., 2010), which gives greater rigidity to these roots, whose main function is plant

support.

## Conclusion

The results obtained in this study indicate that KNO<sub>3</sub> induces tolerance to water deficit in sorghum plants subjected to severe water stress at pre-flowering.

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