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ORIGINAL PAPER

Silicon Supplementation Improves Tolerance to Water Deficiency in Sorghum Plants by Increasing Root System Growth and Improving Photosynthesis

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Abstract

Purpose This study was conducted to assess the effects of silicon treatments on architecture and morphometry of root systems of sorghum plants grown at two different soil water levels and to elucidate whether physiological improvements caused by silicon were related to morphometric modifications of the root system.

Methods Plants of the sorghum genotype BRS332 which is sensitive to drought at pre-flowering stage were used in this study. These plants were grown in a greenhouse, either at field capacity or under water deficiency, and were treated with silicon or were untreated. Leaf water potential was evaluated at noon, and gas exchange, photosynthetic pigment levels, relative aquaporin expression, root system morphometry, and grain yield were assessed.

Results Silicon treatments mitigated the effects of water deficiency on leaf potential, photosynthesis, instantaneous carboxylation efficiency, and morphometry of the root system. These positive effects contributed to a higher grain yield, and thus indicated higher tolerance to drought. The beneficial effects of silicon also occurred in plants grown at field capacity. Silicon treatments did not increase the relative expression of aquaporin genes. However, we observed that expression of aquaporin TIP4 responded more strongly to drought than that of aquaporins PIP1;6 and PIP1;3/1;4.

Conclusion We conclude that silicon supplementation increases the tolerance of sorghum plants to drought by increasing growth of the root system and mitigating adverse effects of drought on photosynthesis.

Keywords Aquaporins · Gas exchange · Photosynthetic pigments · Nutrition · Drought · WinRhizo

1 Introduction

Climate change causes a global increase in droughts, thus drought-adapted crops are required in order to guarantee consistent crop production in the future [1]. Hence, more drought-resistant crops such as sorghum may be an alternative to maize to ensure consistent productivity in regions that are prone to water scarcity [2].

Drought is characterized by soil water deficiency and a high atmospheric vapor-pressure deficiency [3]. Under this condition, plants experience a hydraulic dysfunction due to the loss of water at a rate that exceeds water uptake by the roots [4]. Therefore, as a first response to leaf water signaling, plants reduce stomatal conductance through changes in turgor pressure and osmotic potential [5]. This response is of paramount importance since it reduces perspiration and water loss to the atmosphere [6]. However, with the reduction in stomatal conductance, CO_2 entry into the leaf mesophyll also decreases, which compromises photosynthetic activity, initially due to stomatal limitation [7]. When drought stress becomes more severe, photosynthesis will be inhibited due to different biochemical failures, frequently including oxidative damage [8]. As a consequence, these morphophysiological changes directly affect grain production, particularly when drought occurs at the pre-

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flowering stage, as it reduces the photoassimilate flow for panicle formation and grain filling [9].

The down-regulation in transpiration and photosynthetic rates induced by water deficiency negatively affect the nutritional state of plants, as nutrient uptake and transport occur by mass flow in a process that strongly depends on transpiration [10]. Furthermore, numerous macro- and micronutrient transport channels are energy-dependent and therefore require photosynthesis in order to maintain homeostasis of the cellular charge [11–14].

Water deficiency also affects morphometry and root system architecture [9]. Previous studies have shown that plants are more tolerant to drought when these changes function to reduce the relative surface area of roots near the soil surface and increase longitudinal growth [15]. This phenomenon is intuitive, as soil layers near the surface experience the highest rate of evaporation and thus dry out more rapidly [15]. Therefore, carbon investment in growth of deep roots is a strategy to improve water absorption efficiency, contributing to higher water used efficiency and improved water distribution in tissues [16, 17].

It has been shown that silicon can ameliorate adverse effects of drought by improving hydraulic conductivity, maintenance of higher transpiratory and photosynthetic rates, increased aquaporin pools, and maintenance of photosynthetic pigment levels [18–24]. Aquaporins are channel proteins, and those of plants are commonly assigned to four families: aquaporins located on the plasma membrane (plasma membrane intrinsic proteins [PIPs]), aquaporins located on the tonoplast (tonoplast intrinsic proteins [TIPs]), nodulin-26 aquaporins (nodulin-26-like intrinsic proteins [NIPs]), and small intrinsic proteins (SIPs). Aquaporins belonging to the TIP and PIP families are relatively active in water transport mechanisms, whereas most aquaporins belonging to the NIP and SIP families are not [25, 26].

Silicon can potentially affect growth and architecture of the root system, as numerous effects of silicon that ameliorate drought stress are linked to the water status of plants, which strongly depends on root system plasticity and efficacy of soil water acquisition. However, few studies have been performed to examine the effects of silicon on root system architecture. Thus, the objective of the present study was to assess the effects of silicon on gas exchange, grain yield, and morphometry and aquaporin content of the root system of sorghum plants grown at two soil water levels.

2 Material and Methods

2.1 Growing Conditions, Plant Material, and Experimental Design

Plants of the sorghum cultivar BRS3 32 which at the preflowring stages is sensitive to drought, were grown in a greenhouse at Embrapa Milho e Sorgo (19°28’ S, 44°15′08” W, 732 m above sea level). The experimental design was completely randomized, with four treatments and six replicates of each treatment. The treatment conditions were water at field capacity, water deficiency, water at field capacity plus silicon, and water deficiency plus silicon. Soil water tension in field capacity treatments was maintained close to −18 KPa throughout the experiment and at −138 KPa in water deficiency treatments, corresponding to a supply with 50% of the available water when the plants reached the pre-flowering stage; this level was maintained for a period of 12 days [27]. Silicon was supplied by fertigation from the commercially available product Silício Foliar, which is a potassium silicate compound containing 13% K_2O and 26.59% SiO_2. According to the manufacturer’s instructions, a 2 mM silicon solution was prepared which was then applied to the soil at a dose of 250 mL per day, for 17 days (from five days before to 12 after beginning of drought stress). The amount of Si supplied was independent of the water supply. Plants were grown in plastic pots containing 20 kg oxisol. Soil water content was monitored daily between 9 a.m. and 3 p.m. using moisture sensors (GB Reader N1535; Measurement Engineering, Australia) installed in the center of each pot using a screw thread at a depth of 20 cm. These sensors detect soil water tension measured by electrical resistance and are coupled to digital meters. Irrigation was performed based on the water tension measurements, and water tension was maintained at field capacity before the treatments. Water replenishment calculations were performed using a spreadsheet, according to a soil water retention curve. Corrections and basal and cover fertilizations were carried out based on soil chemical analyses and according to crop requirements.

2.2 Physiologic and Morphometric Analyses

All biophysical analyses were performed on the first leaf below the flag leaf. After 12 days of drought stress, leaf gas exchange was assessed using an LI 6400 infrared gas analyzer (IRGA - LI-COR, Lincoln, NE, USA), equipped with a 3-cm^2 camera (LI-6400-40, LI-COR Inc.). Measurements were performed between 9 and 11 a.m. under photosynthetically active artificial radiation of 1500 μmol photons m^{-2} s^{-1} at the leaf level, in an atmosphere of 21% O_2 and 400 μmol CO_2 mol^{-1}. The assessed parameters were foliar photosynthesis rate, stomatal conductance, transpiration, intercellular CO_2 concentration and instantaneous carboxylation efficiency. Water use efficiency was calculated as foliar photosynthesis rate divided by transpiration rate.

Leaf water potential was determined at noon using a Scholander pressure pump. Dry biomass was recorded at the end of the experiment; after this, the plant material was dried, ground, and subjected to nitroperchloric digestion. The concentrations of macronutrients (N, P, K, Mg, Ca, and S),

micronutrients (Zn, Fe, Mn, and Cu), and silicon were measured at the Plant Chemical Analysis Laboratory at Embrapa Maize and Sorghum using an inductively-coupled argon plasma and combustion method with an FP-528 nitrogen determinator (Leco, USA) [28].

To determine the levels of photosynthetic pigments (chlorophyll *a* and *b* and carotenoids), the middle third of the first leaf below the flag leaf was collected, wrapped in aluminum foil, and stored on ice. Subsequently, 0.1 g leaf tissue was fragmented into parts of approximately 3 mm and immersed in 20 mL 80% (*v*/v) acetone for 24 h at −4 °C in a light-protected environment. After this, measurements were made according to Linchtenthanler and Buschmann [29].

To measure relative expression of the genes *PIP1;6*, *PIP1;3/1;4*, and *TIP4;2*, 10-cm fragments of the root tip of plants of each replicate were collected, washed using distilled water, and stored in liquid nitrogen. Subsequently, 200 mg of each sample were homogenized using a liquid nitrogen mortar, and a 100-mg sample was used for RNA isolation and cDNA transcription using the High-Capacity kit (ThermoFisher, Waltham, USA), according to the manufacturer's instructions.

Following isolation, RNA integrity was tested by electrophoresis using 2% agarose gels (m/v) in a Bio-RAD cuvette. The test was performed using 5 μL water, 2 μL bromophenol blue, 1 μL red gel, and 2 μL RNA extract. A voltage of 110 V was applied for 15 min. RNA purity and concentration of the extract were determined using a 2-μL aliquot. The relative expression of the genes was quantified by qPCR, according to Lana et al. [30]. A *PIP1;6* fragment was amplified using the primers F-5′-TGACGGTGCTGACGGTGAT-3′ and R-5′-GGAGGAGCCCGAAGGTGAC-3′, a *PIP1;3/1;4* fragment was amplified using the primers F-5'-AATCGGGT TCGCGGTGTT-3′ and R-5′-CCAGGCATGGTTCT GGTTGTA-3′, and a *TIP4;2* fragment was amplified using the primers F-5′-GCCGGGTTCATCTACGAGTCT-3′ and R-5′-CTGACTGCCCTGCCCACA-3′. The gene Atcin1 was used as a reference gene and was amplified using the primers F-5′-TGTTCCCTGGGATTGCTG-3′ and R-5′-GCCG GACTCATCGTACTCA-3′ [22].

Leaf area was measured using a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA). The WinRhizo computer system (WinRhizo Pro, Regent Inc. Instr., Canada), was used to record root volume and surface area using the following categories: very fine roots (diameter less than 0.5 mm), fine roots (diameter more than 0.5 and less than 2.0 mm), and thick roots (diameter more than 2.0 mm) [31].

2.3 Agronomic Analyses and Tolerance Index

At the time of harvesting, plant height was measured using a graduated ruler. Subsequently, the plants were partitioned into vegetative parts and reproductive organs. Panicle length was measured using a graduated ruler. The sample material was subsequently subjected to forced air drying at 70 °C for 72 h. Dry biomass of panicle, grains, and vegetative biomass (leaves, stem, and roots) were recorded using a digital analytical balance. The harvest index (HI) was determined based on the total dry matter mass of grains and total plant mass using the following formula:grain dry weight/(plant dry weight + grain dry weight) × 100 [27–32].

The drought tolerance index was calculated as dry matter biomass of grains under water deficiency/total plant mass at field capacity [33].

2.4 Statistical Analyses

The data were tested by an analysis of variance using SISVAR software. A Scott-Knott test was used to test differences between treatments; statistical significance is reported at $p < 0.05$.

3 Results

3.1 Leaf Water Potential

Water deficiency at the pre-flowering stage reduced plant water potential, regardless of the treatment (Fig. 1). Plants grown at field capacity and treated with silicon showed higher leaf water potential than untreated plants. The same effect was observed in treated and untreated plants grown under water deficiency.

3.2 Photosynthetic Pigments and Leaf Gas Exchange

Water deficiency significantly affected photosynthetic pigment levels (Table 1). The contents of chlorophyll *a* and total

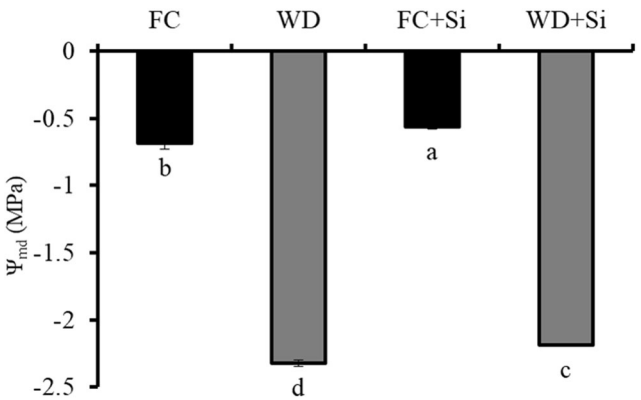

Fig. 1 Leaf water potential at noon (Ψmd) in sorghum plants submitted to different water conditions, treated with silicon or untreated. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters indicate statistically significant differences according to a Scott-Knott test

chlorophyll were reduced in all water deficiency treatments, compared to controls grown at field capacity. Silicon treated plants grown at field capacity showed a higher content of chlorophyll *a* and total chlorophyll, compared to untreated plants. Under water deficiency, no differences in chlorophyll *a* content were observed between treatments. Chlorophyll *b* was reduced under water deficiency, but no effects of the silicon treatment were observed. Carotenoid levels were reduced in all treatments under water deficiency, compared to those in plants grown at field capacity (Table 1).

Drought reduced photosynthetic rates and instantaneous carboxylation efficiency of silicon-treated and untreated sorghum plants. Plants treated with silicon had higher photosynthetic rates and instantaneous carboxylation efficiency than their respective controls, under each water condition (Fig. 2). Stomatal conductance and transpiration were also reduced by water deficiency; however, these parameters were not influenced by silicon treatments under field capacity. Under water deficiency, silicon treatments increased stomatal conductance and transpiration. Intercellular CO_2 concentrations were higher in plants grown at field capacity and without silicon, compared to the other treatments, among which no significant differences were observed. All plants grown under water deficiency increased water use efficiency, compared to the controls grown at field capacity. Moreover, plants grown under water deficiency exhibited greater water use efficiency when untreated than when treated with silicon. Plants grown at field capacity showed no difference in this parameter between treatments (Fig. 2).

3.3 Macro- and Micronutrients

Water deficiency significantly reduced macro- and micronutrient levels in leaves, i.e. lower levels of nitrogen, phosphorus, calcium, magnesium, and copper were observed in plants grown under water deficiency, regardless of the silicon treatments (Table 2). Potassium levels were not affected by water deficiency and silicon treatments. Sulfur concentrations were higher in plants grown at field capacity, regardless of the silicon treatments. Zinc concentrations were affected by water deficiency and silicon treatments, with higher concentrations in plants grown at field capacity and, among these plants, those treated with silicon had higher zinc levels. Under water deficiency, zinc concentrations did not differ between treatments. Iron was reduced only in plants at grown field capacity and treated with silicon, whereas no difference was observed among the other treatments. Manganese was reduced under water deficiency and did not differ between silicon treatments and controls. Silicon levels were higher in plants treated with silicon and were not affected by water deficiency (Table 2).

Table 1 Content of chlorophyll a (μg g-1 Fresh Mass), chlorophyll b (μg g-1 FM), total chlorophyll (μg g-1 FM), and carotenoids (μg g-1 FM) in leaves of sorghum plants subjected to drought stress and treated with silicon at the pre-flowering stage. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters in each line indicate statistically significant differences according to a Scott-Knott test

	FC	WD	FC + Si	WD + Si
Chlorophyll *a*	1377.08 b	678.56 c	1555.23 a	721.96 c
Chlorophyll *b*	480.26 a	244.87 b	469.37 a	262.63 b
Total chlorophyll	1857.34 b	923.48 c	2024.60a	984.60 c
Carotenoids	718.52 a	476.83 c	734.25 a	517.71 b

3.4 Aquaporins and Root Morphometry

Water deficiency increased the relative expression of the *TIP 4;2* gene (Fig. 3-A), which codes for a tonoplast aquaporin; however, under both soil water conditions, silicon treatments reduced its relative expression. Relative expression of the gene *PIP1;6* (Fig. 3-B), which belongs to a family of membrane aquaporins, was reduced by both water deficiency and silicon treatment. Furthermore, plants treated with silicon under both soil water conditions showed lower values of relative gene expression, compared to the control treatments. Relative expression of the gene *PIP1;3/1;4* was increased in plants grown under water deficiency (Fig. 3-C) and in plants not treated with silicon, compared to the controls. In contrast, plants under drought stress and treated with silicon showed lower relative expression, compared to those grown at field capacity and treated with silicon. It is also worth noting that, regardless of the water condition, plants treated with silicon showed lower relative expression of the gene *PIP1;3/1;4* than those not treated with silicon.

The surface area of very fine roots (Fig. 4-A) was reduced in plants grown under water deficiency without the silicon treatment. Silicon contributed to the maintenance of the total surface area of very fine roots under water deficiency, as this parameter did not differ between drought-stressed plants treated with silicon and plants grown at field capacity. The surface area of fine roots (Fig. 4-B) was lower in plants grown under water deficiency, regardless of the treatment. In contrast, plants treated with silicon produced larger surface areas of fine roots, compared to drought-stressed plants and plants not treated with silicon. The surface area of thick roots (Fig. 4-C) showed the same pattern as the surface area of very fine roots and was reduced only in plants grown under water deficiency that had not received silicon.

The volume of very fine roots was lower in drought-stressed plants than in plants grown at field capacity (Fig. 4-D); however, no differences between treatments were observed. Plants grown under water deficiency and plants that were not treated with silicon showed a lower volume of fine roots, compared to the controls (Fig. 4-E). The volume of

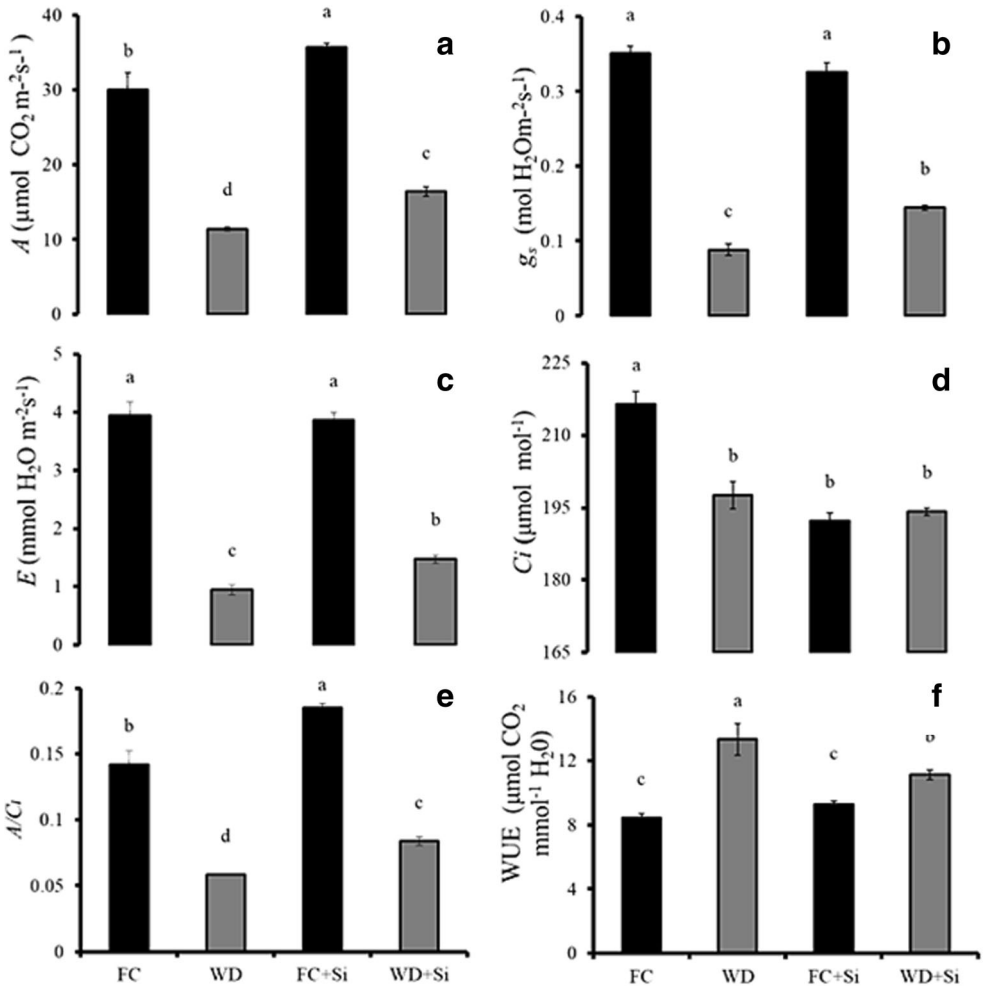

Fig. 2 Effects of drought stress and silicon treatments on gas exchange in sorghum plants at the pre-flowering stage. **(A)** A = photosynthetic rate ([A] μmol CO_2 m^{-2} s^{-1}); **(B)** g_s = stomatal conductance (mol H_2O m^{-2} s^{-1}); **(C)** E = respiration rate (mol H_2O m^{-2} s^{-1}); **(D)** Ci = intercellular CO_2 concentration; **(E)** A/Ci = instantaneous carboxylation efficiency; **(F)** EUA = water use efficiency (mol CO_2 $mmol^{-1}$ H_2O); FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters indicate statistically significant differences according to a Scott-Knott test. Error bars indicate the standard error of the mean of six replicates

thick roots was lower in drought-stressed plants not treated with silicon than in the other treatments, among which no significant difference was observed (Fig. 4-F). Plants grown under water deficiency showed longer root lengths compared to plants grown under field capacity, regardless of silicon treatments (Fig. 4-G). However, plants treated with silicon produced longer roots than those not treated with silicon, under both water conditions. Furthermore, plants grown at field capacity and treated with silicon showed root lengths similar to those of plants grown under water deficiency and not treated with silicon.

Table 2 Macro- and micronutrients in leaves of sorghum plants subjected to water deficiency and treated with silicon at the pre-flowering stage. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters in each line indicate statistically significant differences according to a Scott-Knott test

	FC	WD	FC + Si	WD + Si
N (%)	3.70 a	3.54 b	3.87 a	3.55 b
P (g/Kg)	3.85 a	3.06 b	3.93 a	2.82 b
K (g/Kg)	13.7 a	12.83 a	13.64 a	13.09 a
Ca (g/Kg)	7.53 a	6.60 b	7.16 a	6.13 b
Mg (g/Kg)	4.40 a	3.77 b	4.27 a	3.57 b
S (g/Kg)	3.77 a	1.99 b	2.60 b	1.99 b
Zn (mg/Kg)	64.03 b	51.15 c	76.47 a	52.15 c
Cu (mg/Kg)	8.35 a	6.05 b	8.28 a	5.81 b
Fe (mg/Kg)	212.06 a	222.45 a	155.90 b	194.01 a
Mn (mg/Kg)	127.34 b	159.14 a	136.08 b	160.70 a
Si (mg/Kg)	299.00 b	271.00 b	417.70 a	382.50 a

3.5 Growth and Yield

Water deficiency significantly affected growth parameters and grain yield. However, besides ameliorating the effects of drought stress, silicon treatments led to improved performance in plants grown at field capacity (Table 3). Reduced height growth was observed in plants of all treatments grown under water deficiency, compared to controls grown at field capacity. Nevertheless, it is important to note that height growth of plants under drought stress and treated with silicon did not differ significantly from that of plants not treated with silicon.

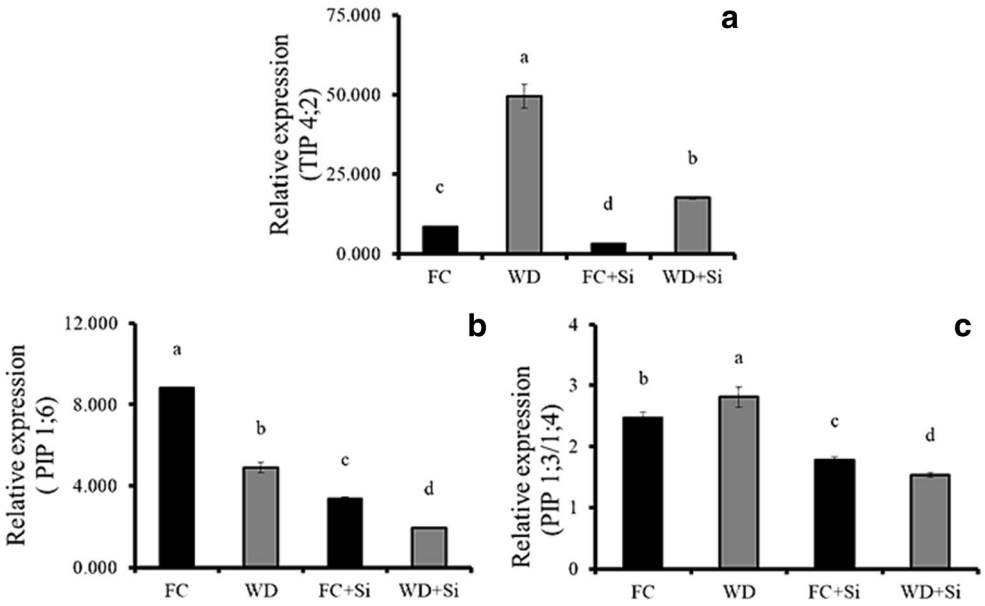

Fig. 3 Relative expression of aquaporins **(A)** TIP4;2; **(B)** PIP1;6; **(C)** PIP1;3/1;4 in sorghum roots subjected to water stress and treated with silicon at the pre-flowering stage. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters indicate statistically significant differences according to a Scott-Knott test. Error bars indicate the standard error of the mean of six replicates

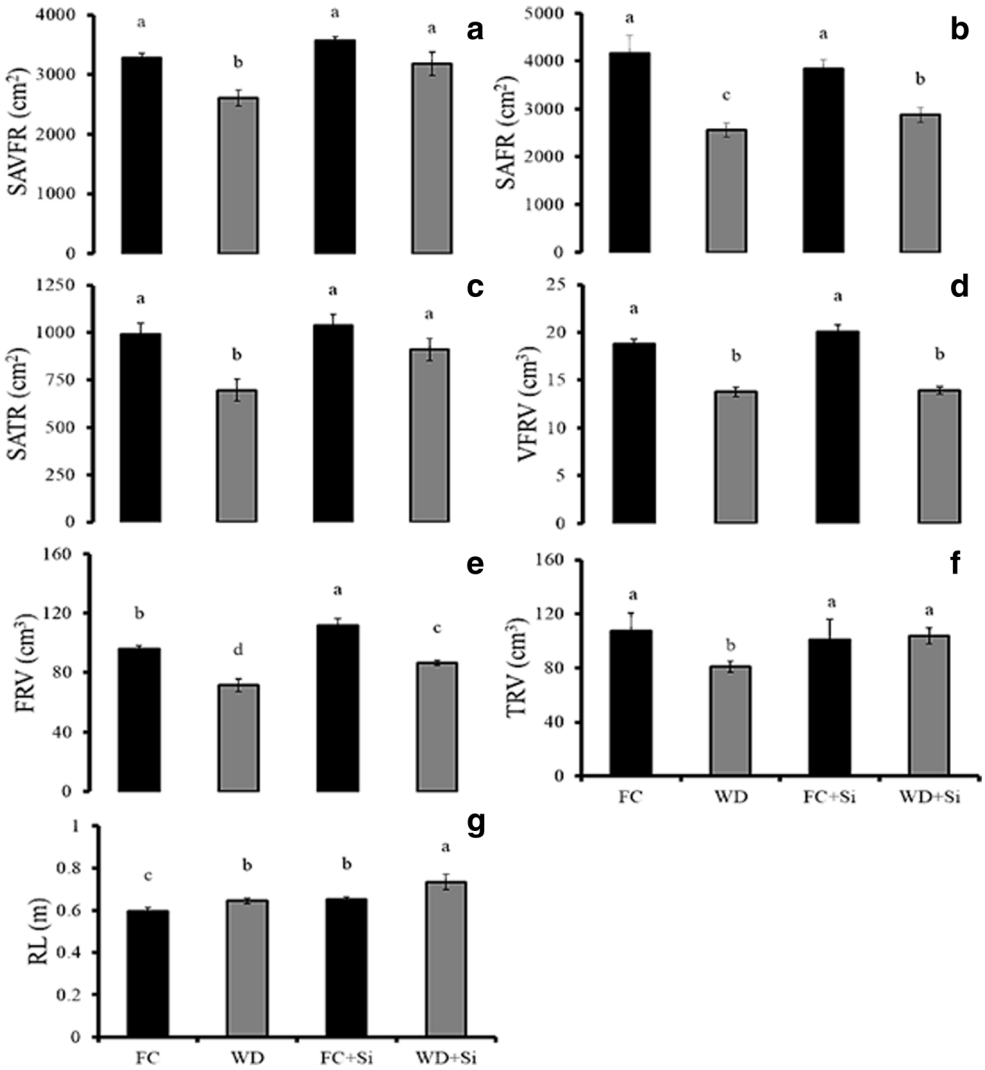

Fig. 4 Root morphometry of sorghum plants subjected to drought stress and treated with silicon at the pre-flowering stage. **A** - Surface area of very fine roots (SAVFR) cm^2; **A**, surface area of fine roots (SAFR) cm^2 - **B**, surface area of thick roots (SATR) cm^2 - **C**, volume of very fine roots (VVFR) cm^3 - **D**, volume of fine roots (VFR) cm^3 - **E**, volume of thick roots (VTR) cm^3 - **F**, and root length (m) - **G**. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters indicate statistically significant differences according to a Scott-Knott test. Error bars indicate the standard error of the mean of six replicates

Table 3 Plant height (PH), plant dry matter (PDM), leaf area (LA), panicle length (PL), panicle dry matter (PADM), grain dry matter (GDM,) and harvest index (HI) of sorghum plants subjected to water stress and treated with silicon at the pre-flowering stage. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters in each line indicate statistically significant differences according to a Scott-Knott test

	FC	WD	FC + Si	WD + Si
PH (m)	1.07b	0.99c	1.28a	1.06b
PDM (g)	87.14b	95.66a	79.47c	75.79c
LA (m^2)	0.49a	0.26c	0.48a	0.36b
PL (cm)	30.25b	22.83c	34.41a	29.91b
PADM (g)	76,63b	24.20d	90.96a	32.32c
GDM (g)	69.68b	19.91d	79.27a	26.27c
HI	0.42b	0.16d	0.46a	0.24c

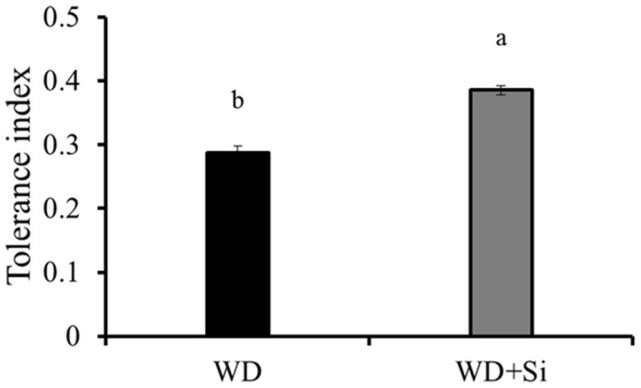

Fig. 5 Drought tolerance index of sorghum plants submitted to water stress and fertilized with silicon at the pre-flowering stage. FC – field capacity; WD – water deficiency; FC + Si – grown at field capacity and treated with silicon; WD + Si – grown under water deficiency and treated with silicon. Different letters indicate statistically significant differences according to a Scott-Knott test. Error bars indicate the standard error of the mean of six replicates

Furthermore, we observed that silicon-treated plants grown at field capacity grew tallest among all treatments (Table 3).

The amount of plant dry matter (root, stem, and leaves) was higher in plants grown under water deficiency and not treated with silicon, followed by plants grown at field capacity and not treated with silicon. Plants treated with silicon had the lowest values of vegetative dry biomass, and did not differ between water conditions (Table 3). Leaf area was reduced in all water deficiency treatments, compared plants grown at field capacity. However, under water deficiency, the leaf area of plants treated with silicon was 27.77% higher than in those not treated with silicon. At field capacity, no differences between treatments were observed (Table 3).

Silicon contributed to the formation and growth of the floral organs: plants grown at field capacity and treated with silicon produced the longest panicles among all treatments (Table 3). Plants treated with silicon and grown under water deficiency produced longer panicles than untreated plants, which did not differ from panicles of plants grown at field capacity and not treated with silicon.

The dry matter content of panicles and grains was affected by water deficiency and by silicon trestments: both parameters were reduced in water deficient plants of both treatments compared to controls grown at field capacity (Table 3). However, under both water conditions, plants treated with silicon produced higher values of panicle and grain dry matter. Under field capacity, this increase was 15.27% for panicle dry matter and 12.09% for grain dry matter. Under water deficiency conditions, panicle dry matter increased by 25.12% and grain dry matter increased by 24.21%. The harvest index was significantly lower in plants grown water deficiency (Fig. 5). However, plants treated with silicon had a higher harvest index than those not treated with silicon, under both water conditions. Therefore, silicon supplementation increased the tolerance of sorghum plants to drought by 25.5%.

4 Discussion

Water deficiency reduced growth and yield of sorghum plants; silicon treatments, however, can ameliorate this effect. Drought-stressed plants treated with silicon grew to the same height as non-stressed plants, and grain yield was increased by 24.21% compared to untreated plants. In plants grown at field capacity, silicon treatments elicited higher growth and increase in grain yield by 12.09%, indicating that, besides increasing tolerance of sorghum plants to drought, silicon treatments improved productive performance of plants grown at field capacity.

Growth reduction caused by water deficiency is in part due to the plants' reduced water potential. Plants with low water potential exhibit low cellular turgor, which compromises, cell expansion, among other functions, as it reduces activity of expansins in the cell walls and reduce the pressure that is necessary for cells to expand after loosening [33, 34]. Therefore, higher water potential in plants treated with silicon under both water conditions may have helped maintain their cellular turgor and growth, because Si has the ability to function as a secondary messenger under both water conditions by modifying hydroxyl linkages in protein groups linked to water turgor maintenance cell signaling [22, 23].

Higher photosynthesis rate observed in silicon-treated plants grown at field capacity and under water deficiency is most likely explanation for the increased growth, panicle length, and panicle and grain dry matter content. Besides the higher photosynthetic rate, it is important to consider the larger total leaf area of plants grown under water deficiency and treated with silicon, compared to untreated plants under drought stress, which facilitated increased production of photoassimilates.

In maize, genotypes that maintain higher photosynthetic rates following drought during the pre-flowering stage

produce higher total yields, due to the high energy demand during the reproductive stage to sustain grain formation and filling [35], which also applies to sorghum. Silicon treatments may thus increase total grain yield due to increased photosynthesic activity elicited by changes in mesophyll conductance and primary metabolism and due to maintenance of tilacoid membrane proteins [21, 36].

In cereals, silicon is essential for the formation of panicles, and silicon application can elicit over-expression of the *Lsi6* gene that is responsible for silicon transport [16], possibly to ensure panicle silicon concentrations that are equivalent to those during the growing season. Silicon application following panicle formation have the tendency to produce stronger sink in sorghum plants, and this contributed to greater exportation of flag leaf sugars to grains, preventing negative feedback by the product in photosynthesis [16]. These insights will help understand the mechanisms underlying increased production parameters such as panicle dry mass and harvest index (HI) in sorghum plants treated with silicon, and lower values of vegetative dry biomass, because with a stronger sink, plants have allocated more carbon from their reserves for grain filling.

It has been shown previously that silicon improves hydraulic conductivity in plants, particularly under water deficiency [22]. These changes are of paramount importance for maintaining stomatal movement and for plant growth [37]. In the present study, although water deficiency generally reduced photosynthetic rate, stomatal conductance, transpiration, and carboxylation efficiency, we observed that drought-stressed plants treated with silicon showed higher values of these variables than untreaded drought-stressed plants. In fact, several studies demonstrated that silicon can ameliorate adverse effects of drought on photosynthesis and stomatal conductance [19–24]. In the present study, the beneficial effects of silicon treatments during water deficiency was observed, considering that the ability to maintain high photosynthetic activity is an important characteristic of drought tolerance in plants [38]. Furthermore, plants grown at field capacity and treated with silicon showed higher photosynthesis rates than untreated plants grown at field capacity. Increased photosynthetic rates due to silicon treatments were associated with high stomatal conductance values, as plants grown at field capacity produced the same values of stomatal conductance and transpiration regardless of silicon treatments; however, the silicon-treated plants also showed higher values of carboxylation efficiency and lower internal carbon values. Hence, we suggest that the same amount of carbon is introduced into the leaf mesophyll, however, as carboxylation activity increased, more CO_2 was consumed.

Application of silicon to plants may support antioxidant systems, which may explain the increased chlorophyll *a* and total chlorophyll levels in silicon-treated plants grown at field capacity [39], suggesting improved light absorption. Under water deficiency, these effects on chlorophylls were not observed; however, the levels of carotenoids were increased in silicon-treated plants. Carotenoids are accessory pigments, photoprotectors, and antioxidants, thus maintaining their levels is extremely important during stress, as they can prevent photoinhibition [40, 41].

Reduced gas exchange caused by drought stress may be directly associated with nutrient levels in the leaves, as, in general, drought-stressed plants showed lower mineral concentrations in leaves than plants grown at field capacity. Therefore, we suggest that the average reduction in transpiration by 68.3% in drought-stressed plants may have negatively affected absorption and transport of minerals, as these processes are governed by the transpiratory current [10]. Moreover, this effect may be associated with reduced photosynthesis and, therefore, with lower energy metabolism. Hence, a lower energy supply may induce systemic entropy and adversely affect nutrient absorption, as numerous nutrient carriers are energy-dependent channels [11]. High silicon content in leaves of silicon-treated plants were due to its exogenous supply.

Improved root system morphometry in silicon-treated plants observed in the current study may explain the higher values of leaf water potential [42]. Thus, we suggest that improved root performance of drought-stressed plants treated with silicon may have favored water status (water potential), maintenance of photosynthesis, transpiratory rate, and stomatal conductance [16, 43]. This adaptive response is of paramount importance for increasing the efficiency of soil water capture. The soil surface layer dries faster than the layers beneath [44], therefore plants reduce the amount of roots near the surface and invest in root growth in deeper layers [6]. This is in line with the results of the present study, as, in general, root architecture of plants grown under water deficiency was modified, compared to plants grown at field capacity. Thus, we confirmed that root systems of drought-stressed plants generally had smaller surface areas and volumes, but greater length. It is important to emphasize the positive effect of silicon on the root system, as the surface area of very fine roots, of fine roots, and of thick roots, as well as the volume of fine roots and of thick roots was kept stable and longitudinal root growth was mostly stimulated.

It has been reported that increased protein channel activity may contribute to plant performance under water deficiency [6]. Here, we confirmed that expression of *TIP 4*, which encodes an intrinsic tonoplast aquaporin, was increased in plants grown under water deficiency, indicating that this aquaporin plays a key role during drought stress. This increase is possibly related to the attempt to maintain vacuole water levels and, therefore, maintain pressure within the cell.

Furthermore, *PIP1;6* expression was also increased in plants grown under water deficiency; however, this effect was observed only non-silicon treated plants. In contrast, *PIP1;3/1;4* expression was reduced in all plants under drought stress, regsardless of the treatment, and was even more reduce in silicon-treated plants. In line with Shi et al. [45] who

examined the effects of silicon on tomato plants under drought stress, we did not observe positive effects of silicon treatments on expression of the aquaporin genes *SlPIP1*;*3*, *SlPIP1;5*, and *SlPIP2;6*.

The results of the present study regarding *PIP* gene expression contrasted with those of Liu et al. [22], who observed increased *PIP1;6* and *PIP1;3/1;4* expression in plants subjected to osmotic stress and treated with silicon. However, it is important to highlight methodological differences regarding the assessment of gene expression between the two studies. Thus, we suggest the existence of endogenous and/or exogenous factors that affect the regulation of expression of genes that code for these proteins. This is likely, as root hydraulic conductance is influenced by several internal and external factors, such as xylem vessel embolism, root anatomy, water availability, salts in soil water, temperature, and cell properties [46–48], directly affecting the activity and/or abundance of aquaporins [49]. Therefore, we suggest that the observed lower expression of aquaporin genes in silicon-treated plants may be linked to other processes influenced by silicon, which warrants further examination.

5 Conclusions

Silicon treatments mitigated the effects of water deficiency on leaf potential, photosynthesis, instantaneous carboxylation efficiency, and morphometry of the root system of sorghum plants. These positive effects contributed to a higher grain yield and, therefore, to improved drought tolerance. The positive effects of silicon also occurred in plants grown at field capacity, demonstrating that silicon can improve the productive performance of sorghum grown under drought conditions and at field capacity.

The silicon treatment did not increase relative expression of the aquaporins *PIP1;6* and *PIP1;3/1;4*; however, aquaporin *TIP 4* appeared to be more responsive to drought than aquaporins *PIP1;6* and *PIP1;3/1;4*.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This study did not involve any human participants or animals.

References

1. Pennis E (2009) How Sorghum withstands heat and drought. Science. 323:573. https://doi.org/10.1126/science.323.5914.573
2. Bonfim-Silva EM, da Silva TJA, Cabral CEA, Kroth BE, Rezende D (2011) Desenvolvimento inicial de gramíneas submetidas ao estresse hídrico. Rev Caatinga 24:180–186
3. Dai A (2011) Drought under global warming: a review. Wires Climate Change. https://doi.org/10.1002/wcc.81
4. Stanton KM, Mickelbart MV (2014) Maintenance of water uptake and reduced water loss contribute to water stress tolerance of *Spiraea alba* Du Roi and *Spiraea tomentosa* L. Hortic Res. https://doi.org/10.1038/hortres.2014.33
5. Nikinmaa E, Hölttä T, Hari P, Kolari P, Mäkelä A, Sevanto S, Vesala T (2013) Assimilate transport in phloem sets conditions for leaf gas exchange. Plant Cell Environ. https://doi.org/10.1111/pce.12004
6. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (eds) Sustainable Agriculture. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2666-8_12
7. Lavinsky AO, Magalhães PC, Ávila R, Gomes Jr CC, Carneiro NP (2015) Analysis of maize photosyntheis parameters and whole plant oxidative damage under long-term drought. Adv Crop Sci Tech. https://doi.org/10.4172/2329-8863.S1-007
8. Noctor G, Mhamdi A, Foyer CH (2014) The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol. https://doi.org/10.1104/pp.113.233478
9. Magalhães PC, Souza TC, Lavinsky AO, Albuquerque PEP, Oliveira LL, Castro EM (2016) Phenotypic plasticity of root system and shoots of Sorghum bicolor under different soil water levels during pre-flowering stage. Aust J Crop Sci 10(1):81–87
10. White PJ (2001) The pathways of calcium movement to the xylem. J Exp Bot. https://doi.org/10.1093/jexbot/52.358.891
11. Grossman A, Takashi H (2001) Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. Annu Rev Plant Physiol Plant Mol Biol 52:163–210. https://doi.org/10.1146/annurev.arplant.52.1.163
12. Chen Y, Barak P (1982) Iron nutrition of plants in calcareous soils. Adv Agron 35:217–240. https://doi.org/10.1016/S0065-2113(08)60326-0
13. Peterson TA, Blackmer TM, Francis DD, Schepers JS (1993) G93-1171 using a chlorophyll meter to improve N management. Historical Materials from University of Nebraska-Lincoln Extension, 1353
14. Rissler HM, Collakova E, DellaPenna D, Whelan J, Pogson BJ (2002) Chlorophyll biosynthesis. Expression of a second chl I gene of magnesium chelatase in *Arabidopsis* supports only limited chlorophyll synthesis Plant Physiol. https://doi.org/10.1104/pp.010625
15. Broedel E, Tomasella J, Cândido LA, von Randow C (2017) Deep soil water dynamics in an undisturbed primary forest in Central Amazonia: differences between normal years and the 2005 drought. Hydrol Process. https://doi.org/10.1002/hyp.11143
16. Lavinsky AO, Detmann KC, Reis JV, Ávila RT, Sanglard ML, Pereira LF, Sanglard LMVP, Rodrigues FA, Araújo WL, DaMatta FM (2016) Silicon improves rice grain yield and photosynthesis specifically when supplied during the reproductive growth stage. J Plant Physiol. https://doi.org/10.1016/j.jplph.2016.09.010
17. Zhan A, Schneider H, Lynch J (2015) Reduced lateral root branching density improves drought tolerance in maize. Plant Physiol. https://doi.org/10.1104/pp.15.00187
18. Camargo MS, Bezerra BKL, Holanda LA, Oliveira AL, Vitti AC, Silva MA (2019) Silicon fertilization improves physiological responses in sugarcane cultivars grown under water deficit. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-019-0012-1

19. Cao BL, Xu K, Shi J, Xin GF, Liu CY, Li X (2013) Effects of silicon on growth, photosynthesis and transpiration of tomato. Plant Nutr Fert Sci 19:354–360
20. Chen W, Yao X, Cai K, Chen J (2011) Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. Biol Trace Elem Res. https://doi.org/10.1007/s12011-010-8742-x
21. Detmann KC, Araújo WL, Martins SC, Sanglard LM, Reis JV, Detmann E, Rodrigues FA, Nunes-Nesi A, FernieAR DMFM (2012) Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice. New Phytol. https://doi.org/10.1111/j.1469-8137.2012.04299.x
22. Liu P, Yin L, Deng X, Wang S, Tanaka K, Zhang S (2014) Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in *Sorghum bicolor* L. J Exp Bot. https://doi.org/10.1093/jxb/eru220
23. Saud S, Li X, Chen Y, Zhang L, Fahad S, Hussain S, Sadig A, Chen Y (2014) Silicon application increases drought tolerance of *Kentucky bluegrass* by improving plant water relations and morphophysiological functions. Sci World J. https://doi.org/10.1155/2014/368694
24. Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol. https://doi.org/10.1016/j.jplph.2010.04.011
25. McElrone AJ, Bichler J, Pockman WT, Addington RN, Linder CR, Jackson RB (2007) Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. Plant Cell Environ. https://doi.org/10.1111/j.1365-3040.2007.01714.x
26. Sakurai J, Ishikawa F, Yamaguchi T, Uemura M, Maeshima M (2005) Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol. https://doi.org/10.1093/pcp/pci172
27. Souza TC, Castro EM, Magalhaes PC, Lino LDO, Alves ET, Albuquerque PEP (2013) Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. Acta Physiol Plant. https://doi.org/10.1007/s11738-013-1355-1
28. Silva FC (2009) Manual de análises químicas de solos, plantas e fertilizantes. Rio de Janeiro: Embrapa Solos
29. Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current protocols in Food analytical Chemistry New York: John Wiley and Sons. https://doi.org/10.1002/0471142913.faf0403s01
30. Oliveira CA, Marriel IE, Gomes EA, Lana UGP, Scotti MR, Alves VMC (2009) Bacterial diversity in the rhizosphere of maize genotypes contrasting for phosphorus use efficiency. Pesq Agropec Bras. https://doi.org/10.1590/S0100-204X2009001100015
31. Magalhães PC, Souza TC, Cantão FRO (2011) Early evaluation of root morphology of maize genotypes under phosphorus deficiency. Plant Soil Environ. https://doi.org/10.17221/360/2010-PSE
32. Durães FOM, Magalhães PC, Oliveira AC (2002) Genetical harvest index and possibilities of the physiological genetics to improve maize yield. Rev bras Milho e sorgo. https://doi.org/10.18512/1980-6477/rbms.v1n1p33-40
33. Tardieu F, Reymond M, Hamard P, Granier C, Muller B (2000) Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature. J Exp Bot. https://doi.org/10.1093/jexbot/51.350.1505
34. Zhou S, Han YY, Chen Y, Kong X, Wang W (2015) The involvement of expansins in response to water stress during leaf development in wheat. J Plant Physiol. https://doi.org/10.1016/j.jplph.2015.05.012
35. Avila R, Magalhaes PC, Alvarenga AA, Lavinsky ADO, Campos CN, Souza TC, Gomes Júnior CC (2016) Drought-tolerant maize genotypes invest in root system and maintain high harvest index during water stress. Rev bras Milho e sorgo. https://doi.org/10.18512/1980-6477/rbms.v15n3p450-460
36. Wang Y, Zhang B, Jiang D, Chen G (2019) Silicon improves photosynthetic performance by optimizing thylakoid membrane protein components in rice under drought stress. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2018.11.022
37. Sutka M, Li G, Boudet J, Boursiac Y, Doumas P, Maurel C (2011) Natural variation of root hydraulics in *Arabidopsis* grown in normal and salt stress conditions. Plant Physiol. https://doi.org/10.1104/pp.110.163113
38. Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crop Res. https://doi.org/10.1016/j.fcr.2007.07.004
39. Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci. https://doi.org/10.1016/j.plantsci.2005.02.023
40. Kreslavski VD, Zorina AA, Los DA, Fomina IR, Allakhverdiev SI (2013) Molecular mechanisms of stress resistance of photosynthetic machinery. In: Rout G., Das A. (eds) Molecular stress physiology of plants. Springer, India. https://doi.org/10.1007/978-81-322-0807-5_2
41. Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. https://doi.org/10.1155/2012/217037
42. Souza TC, Magalhães PC, Castro EM, Carneiro NP, Padilha FA, Gomes Júnior CC (2014) ABA application to maize hybrids contrasting for drought tolerance: changes in water parameters and in antioxidant enzyme activity. Plant Growth Regul. https://doi.org/10.1007/s10725-013-9881-9
43. Souza TC, Magalhães PC, Castro EM, Duarte VP, Lavinsky AO (2016) Corn root morphoanatomy at different development stages and yield under water stress. Pesq Agropec Bras. https://doi.org/10.1590/S0100-204X2016000400005
44. Lynch JP, Chimungu J, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. J Exp Bot. https://doi.org/10.1093/jxb/eru162
45. Shi Y, Zhang Y, Han W, Feng R, Hu Y, Guo J, Gong H (2016) Silicon enhances water stress tolerance by improving root hydraulic conductance in *Solanum lycopersicum* L. Front Plant Sci. https://doi.org/10.3389/fpls.2016.00196
46. Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ (2005) The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. Plant Physiol. https://doi.org/10.1104/pp.104.051045
47. Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries N, Maurel C (2005) Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression Plant Physiol. https://doi.org/10.1104/pp.105.065029
48. Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ (2002) Plasma membrane aquaporins play a significant role during recovery from water deficit. Plant Physiol. https://doi.org/10.1104/pp.009019
49. Luu DT, Maurel C (2005) Aquaporins in a challenging environment: molecular gears for adjusting plant water status. Plant Cell Environ. https://doi.org/10.1111/j.1365-3040.2004.01295.x