



Full Length Article

Gas Exchange and Leaf Anatomy of Cotton Subjected to Application of Calcium Silicate and Mepiquat Chloride under Water Stress

Dayane Bortoloto da Silva¹, Amanda Pereira Paixão-Daruichi¹, Noemi Cristina de Souza Vieira¹, Aline Redondo Martins^{2*} and Enes Furlani Junior¹

¹Department of Phytotechnics, Food Technology and Social Economy, São Paulo State University, Ilha Solteira 15.385-000, Brazil

²Department of Biology and Animal Science, São Paulo State University (UNESP - FEIS), Ilha Solteira, 15.385-000, Brazil

*For correspondence: aline.martins@unesp.br

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Abstract

Silicate fertilization can give crops better conditions to withstand edaphoclimatic and biological adversities, with the result of increased production and better quality. The objective of this work was to study leaf anatomy and gas exchange in herbaceous cotton as a function of calcium silicate doses, mepiquat chloride (MC) use and water availability. The experiment was carried out in a greenhouse. The experimental design was completely randomized, in a $4 \times 2 \times 2$ factorial design, with 4 replications. The factors studied were four doses of calcium silicate (CS) as foliar fertilizer at the rate of 0, 100, 200 and 400 g/ha with and without the foliar spray of MC. Two soil water levels i.e., full field capacity (FC) and 1/3 FC (water stress). The foliar spray of CS and MC increased net photosynthesis rate, transpiration, and stomatal conductance but decreased the internal concentration of CO₂ of leaf. The thickness of the epidermis of the abaxial leaf increased linearly with CS doses, thereby increasing the protective barrier against water stress. © 2023 Friends Science Publishers

Keywords: Cotton; Greenhouse; Growth regulator; Silicate fertilization; Water deficit

Introduction

One of the main factors that can influence plant development is water deficit. Water stress is considered the most important environmental factor, capable of interfering with plant metabolism. The capacity of plants to tolerate drought exists as a function of several anatomical, morphological and physiological characteristics, which interact allowing the maintenance of growth and development processes (Taiz and Zeiger 2013).

The herbaceous cotton (*Gossypium hirsutum* L. var. latifolium Hutch.) is considered a relatively drought-tolerant plant, especially when compared to crops such as soybean, rice, corn etc. However, the lack of water during critical growth periods compromises plant growth, development and productivity (Luo *et al.* 2014). As it is a plant with an indeterminate growth habit, the cotton plant may present excessive vegetative growth and consequent imbalance in the source/drain ratio, resulting in lower yields. In this sense, it is essential to apply growth regulators due to the development of this culture (Ferrari *et al.* 2014).

Mepiquat chloride (MC) stands out among the most used plant regulators in modern Brazilian cotton farming. It inhibits the natural production of gibberellins, reducing excess vegetative growth without affecting productivity

(Lamas *et al.* 2013). Despite the benefits arising from the application of growth regulators, the management of these products is still a challenge due to high temperatures and water deficit (Echer and Rosolem 2017). According to Paixão *et al.* (2017) there is evidence that the regulator may interfere with exchanges gases, modifying the photosynthetic rate of cotton plants. Currently, numerous studies 150 report the beneficial effects of silicon (Si) in some economically important crops. According to Filho (2010), Si plays an important role in plant-environment relations, as it can provide crops with better conditions to withstand climatic, edaphic and biological adversities with the final result of increased production and better quality of crops.

Among the benefits provided by Si, we highlighted increase in cell wall resistance and regulation of evapotranspiration, improved photosynthetic rate, in addition to improving leaf architecture (Basagli *et al.* 2003). The foliar use of Si can be a viable alternative to minimize the harmful effect of biotic and abiotic stresses on crops (Lima *et al.* 2011; Sheng and Chen 2020). This is because the element can optimize some desirable morphophysiological and biochemical processes by significantly increasing the yield of some cultivated species, notably by the accumulation and polymerization of silicates in epidermal cells forming a silicon-cuticle double layer that substantially reduces

transpiration, converging to lower water consumption (Peixoto *et al.* 2011). Thus, it is believed that Si represents an interesting alternative, with great potential to be used in the improvement of Brazilian agricultural production. This study aimed to analyze the doses of calcium silicate (CS) with the use of MC in different water conditions, through leaf anatomical and gas exchange, to verify the possible changes that occur in leaf providing information about silicate fertilization for cotton.

Materials and Methods

Experiment location

The experiment was carried out in a Pad and Fan greenhouse, with a maximum temperature of 30°C, at Universidade Estadual Paulista, Ilha Solteira – SP, located at 20°43'09"S and 51°33'79"W, with an altitude of about 335 m.

Experimental design

The experimental design was completely randomized in a 4 × 2 × 2 factorial scheme, with 4 replications. The factors studied were four doses of CS *i.e.*, 0, 100, 200 and 400 g ha⁻¹, with (10, 20 and 30% of the 120 mL dose) and without the use of MC and two levels of soil water [full field capacity (FC) and 1/3 field capacity (1/3 FC)].

Installation and conduct of experiment

Pots polyethylene of 15 liters were used, and all pots were first filled with 1 kg of gravel n°2 (for drainage) and 14 kg of soil on top. The soil used in the installation of the experiment was the typical Dystrophic RED LATOSOL, from the 0 to 0.20 m layer, it was corrected and fertilized based on chemical analysis: pH (CaCl₂) = 5.1; Presin = 2 mg dm⁻³; M.O. = 17 g dm⁻³; K⁺ = 0.7 mmol/dm³; Ca⁺² = 9 mmol/dm³; Mg⁺² = 8 mmol/dm³; H+Al = 16 mmol/dm³; Al = 0 mmol/dm³; CTC = 33.7 mmol/dm³; V=53%.

Four cotton seeds of the TMG 81WS genotype were sown per pot on December 29, 2018. The seedlings emerged six days after planting, two thinning were performed, the first at 15 days after emergence (DAE) and the second at 25 DAE, leaving one plant per pot until the end of the experiment. CS was sprayed on the plant leaves with the aid of a sprayer, and divided equally into three times, at 30, 45 and 60 DAE. The growth regulator applied was MC at three concentrations (10, 20 and 30% of the 120 mL dose), after the application of CS. To determine the FC, the pots were initially saturated with water and then covered with polyethylene bags to prevent evaporation from the soil in the pots and left to rest for a period of no less than 20 h, until stabilization was closer to the FC. The soil moisture was read daily using a portable moisture meter, which shows the water retained in the soil, thus determining the amount of water to be placed in each pot.

Variables measured

Measurements of gas exchanges, Infra-Red Gas Analyzer, were performed at 80 DAE, using the LC-Pro equipment (ADC – Bioscientific Ltd., Hoddesdon, UK) and the conditions for measurements were made under 1000 μmol m⁻² s⁻² photosynthetically active radiation (PAR), provided by LED lamps; 380 ppm CO₂ and chamber temperature of 28°C. Measurements were performed on a sunny day, between 8:00 and 12:00 h, taking the 2nd or 3rd adult leaf, completely expanded from the apex of the branch. The data were recorded for internal concentration of CO₂ (C_i), transpiration rate (E), stomatal conductance (g_s) and photosynthetic rate (A).

For the evaluation of anatomical characters of the leaf blade, leaves were collected from the middle third of the plant. The material was fixed with FAA 70 (formaldehyde + glacial acetic acid + 70% alcohol) for 48 h and subsequently preserved in 70% alcohol. The leaf samples were sectioned freehand with a steel blade, in the middle region of the mesophyll. The samples were dehydrated in an ascending ethylic series, dried by the drying process at the critical point of CO₂. The samples were mounted on aluminum “stubs”, metallized with a 30–40 nm gold layer and observed under a scanning electron microscope (model LEO 435VP, Germany). Images of the transverse view of the epidermis were digitally generated. The images were processed with the Digimizer 5.3.5 Software and the thickness of the epidermis, adaxial and abaxial and of the palisade and spongy parenchyma was determined. The total thickness of the leaf blade was treated from the sum of the epidermis with the parenchyma.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and F values were derived. The means were compared using the Tukey test at 5% probability. For finding the significant regressions, regression models that best fitted the effects obtained from doses of CS statistical software SISVAR 5.6 were applied.

Results

The water conditions significantly altered (P < 0.01) the leaf anatomy of the cotton plant, where the plants submitted to 1/3 CC presented greater total thickness of the leaf, due to the greater thickness of the abaxial and adaxial epidermis, and of palisade and spongy parenchyma, the differences reaching 15.30% for the epidermis and 23.45% for the spongy parenchyma, when comparing the plants that were not subjected to water deficit (Fig. 1A). A significant (P < 0.05) effect of MC was observed only in the spongy parenchyma and the total thickness of the leaf blade, where it is possible to verify differences of 15.34 and 9.57%, respectively when comparing the plants without the application of the regulator (Fig. 1B).

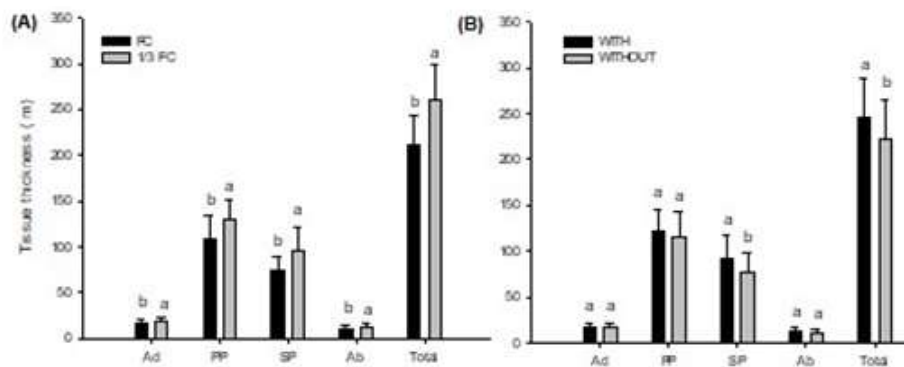


Fig. 1: Leaf-blade tissue thickness (µm), the adaxial epidermis (Ad), palisade parenchyma (PP), spongy parenchyma (SP), the abaxial epidermis (Ab) and a total thickness of herbaceous cotton, under FC and 1/3 FC (A) as well as with and without application of MC (B)

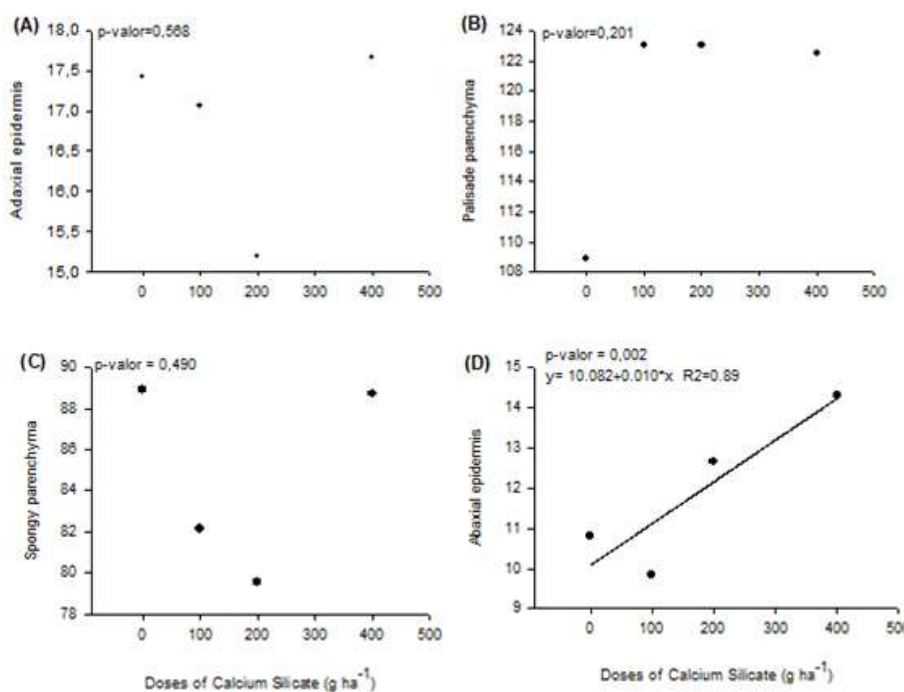


Fig. 2: Thickness of abaxial epidermis in herbaceous cotton leaves as a function of CS doses

The CS applied to cotton significantly ($P < 0.01$) increased the thickness of the abaxial epidermis; the increase occurring linearly with CS doses (Fig. 2). Using scanning electron microscopy (Fig. 3), it was possible to verify the presence of a thicker cuticle on the lower surface of the leaves of plants treated with CS. According to the ANOVA (Table 1), different water conditions had significant ($P < 0.05$) effect on g_s and net photosynthesis (A) of herbaceous cotton. MC significantly ($P < 0.01$) influenced E, g_s and A. CS doses did not ($P > 0.05$) affect any variable nor the interaction between the studied factors.

The g_s was significantly ($P < 0.01$) affected by water conditions, where there was an increase in g_s in treatments submitted to water stress (1/3 CF). Comparing the g_s of the plants introduced to the lowest and highest water content in

the soil, there was a decrease of 34.78%. Lower soil water levels were also responsible for the increase in net photosynthesis (Table 1), which was 61.70% higher when compared to treatments that were not subjected to low water availability. The application of MC significantly ($P < 0.01$) increased E, g_s and A. Compared with the use of the product, there was a difference of 25% in E, 43.18% for g_s and A, the greatest difference (87.51%) was observed, which was for plants that did not receive the foliar sprays (Table 1).

Discussion

In environments where water deficit occurs frequently, morphophysiological mechanisms are severely affected, causing plants to quickly adapt to the new condition (Santos

Table 1: Summary of analysis of variance for the internal concentration of CO₂ (Ci), transpiration rate (E), stomatal conductance (gs), net photosynthesis (A), of herbaceous cotton plants, under different hydric conditions (HC), mepiquat chloride (MC), calcium silicate (CS) levels

FV	p>F			
	Ci	E	Gs	A
Hydric conditions (HC)	0.422 ^{ns}	0.099 ^{ns}	0.006 ^{**}	0.001 ^{**}
Mepiquat chloride (MC)	0.425 ^{ns}	0.001 ^{**}	0.001 ^{**}	0.001 ^{**}
Calcium silicate (CS).	0.552 ^{ns}	0.694 ^{ns}	0.851 ^{ns}	0.941 ^{ns}
Linear regression	0.530 ^{ns}	0.977 ^{ns}	0.773 ^{ns}	0.658 ^{ns}
Quadratic regression	0.928 ^{ns}	0.512 ^{ns}	0.428 ^{ns}	0.804 ^{ns}
HC × MC	0.917 ^{ns}	0.274 ^{ns}	0.221 ^{ns}	0.264 ^{ns}
HC × CS	0.703 ^{ns}	0.462 ^{ns}	0.431 ^{ns}	0.712 ^{ns}
MC × CS	0.984 ^{ns}	0.993 ^{ns}	0.820 ^{ns}	0.698 ^{ns}
HC × MC × CS	0.352 ^{ns}	0.968 ^{ns}	0.775 ^{ns}	0.432 ^{ns}
C.V (%)	16.55	32.09	41.44	52.53
Hydric conditions				
FC	302.87 a	2.31 a	0.46 b	10.21 b
1/3 FC	292.96 a	2.64 a	0.62 a	16.51 a
Mepiquat chloride				
WITH	292.93 a	2.75 a	0.63 a	17.42 a
WITHOUT	302.90 a	2.20 b	0.44 b	9.29 b

ns, ** and * – non-significant and significant at 1 and 5% by the F Test, respectively

Means followed by the same letter, in the column, do not differ from each other by Tukey's test, at a 5% probability level

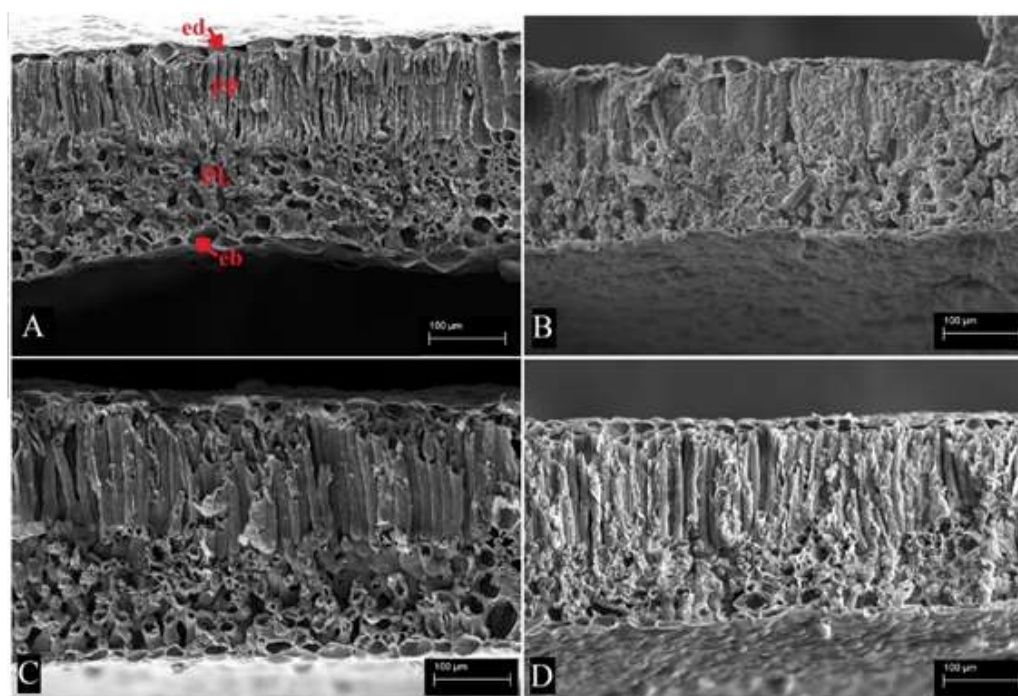


Fig. 3: Cross sections in scanning electron microscopy of the cotton leaf showing the adaxial epidermis (ed), palisade parenchyma (pp), sponge parenchyma and abaxial epidermis (eb) as a function of doses of CS (A) 0, (B) 100, (C) 200, (D) 400 g ha⁻¹)

and Carlesso 1998; Tombesi *et al.* 2018). When the plants are subjected to water deficit from the beginning of the cycle, plant adaptation occurs more easily, as was the case in the present study, where the water deficit started at 30 DAE and remained throughout the development of the cotton plant, causing the plant to adapt to the conditions of the water. The E was not significantly affected with different water stress conditions, which may be a result of a greater transpiration capacity under water stress conditions. According to

Tombesi *et al.* (2018), this strategy can be evaluated as positive, as it reduces the evapotranspiration and can indirectly reduce the long-term damage associated with the energy loss via evapotranspiration.

Leaf anatomy of the herbaceous cotton plant showed plasticity for water deficit, changing the thickness of the palisade and spongy parenchyma (Fig. 1A). These resulting from low availability of water are involved in the protection of the plants to avoid excessive loss of water by transpiration

and, therefore, an important mechanism of tolerance to water deficit (Castro *et al.* 2009).

The spongy parenchyma is specialized for the temporary storage of water (Beltrão *et al.* 2011), while the palisade parenchyma is well-developed and specialized for the photosynthetic process (Castro *et al.* 2009), making the plant show good efficiency in the use of water, thus being able to survive better in an environment of water stress. The thickness of the palisade parenchyma is one of the adaptations responsible for greater photosynthesis in plants subjected to 1/3 CF (Batista *et al.* 2010). Adaptations to water stress can be physiological, anatomical, and morphological, but these responses vary according to the species and cultivar (Devi and Reddy 2018).

In the plants where MC was applied, it is possible to observe the greater thickness of the spongy parenchyma and the total thickness of the plants (Fig. 1B). The application of MC is responsible for making plants more compact and with smaller leaves (Stewart *et al.* 2001) changing the morphology and physiological characteristics of the leaves (Leal *et al.* 2020). These changes occur as a way for plants to compensate because the smallest size of the leaf increases the thickness of the leaf and even the plants being more compact manage to be photosynthetically efficient.

The increase in abaxial thickness with CS doses (Fig. 2) is linked to the fact that Si is an element that accumulates in the aerial part, next to the cuticle, mainly in the endoplasmic reticulum, in intercellular spaces and cell walls (Taiz and Zeiger 2013). The deposition of silica in the cell wall makes the plant more resistant to the action of fungi and insects. It is also possible to verify that, as the deposition of Si in the leaf increased, they became more upright, which can contribute to an improvement in the architecture of the plants, thus allowing better penetration of light into the canopy of the plant and improving photosynthesis (Junior *et al.* 2021). However, this was not observed in the study, where CS did not influence leaf photosynthesis (Table 1).

Understanding how each plant will respond to water deficit is extremely important, due to climate change, where periods of low water rainfall are becoming increasingly common. Knowing these answers facilitates the search for techniques that can help us, such as the use of nutritional elements such as Si, which has been shown to be a tool for different cultures as a protector against stress, both biotic and abiotic (Lima *et al.* 2011; Peixoto *et al.* 2011; Junior *et al.* 2021), as revealed from this study.

Conclusion

Low water availability increased A, gs, and the thickness of the adaxial epidermis, palisade parenchyma, spongy parenchyma, and abaxial epidermis. The use of MC increased the total thickness of cotton leaves. Moreover, CS increased the thickness of the abaxial epidermis of the leaves, resultantly increasing the protective barrier against water stress.

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Author Contributions

DBS, ARM and EFJ designed the experiments, DBS collected and analyzed the data, DBS, APPD, NCSV interpreted the results and revised the manuscript, all authors did the final revision of the manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

References

- Devi MJ, VR Reddy (2018). Transpiration response of cotton to vapor pressure deficit and its relationship with stomatal traits. *Front Plant Sci* 9:1572
- Basagli MA, JC Moraes, GA Carvalho, CC Ecole, RDC Gonçalves-Gervásio (2003). Efeito da aplicação de silicato de sódio na resistência de plantas de trigo ao pulgão-verde *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). *Neotrop Entomol* 32:659–663
- Batista LA, RJ Guimarães, FJ Pereira, GR Carvalho, EMD Castro (2010). Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. *Rev Ciênc Agron* 41:475–481
- Beltrão NEM, MIP Oliveira, SP Sousa Júnior, GG Brito, GD Cardoso (2011). Ecofisiologia do Algodoeiro (*Gossypium hirsutum* L. r. latifolium Hutch.). In: *Ecofisiologia das Culturas de Algodão, Amendoim, Gergelim, Mamona, Pinhão-manso e Sisal*, pp: 65–123. Beltrão NE de M, MIP de Oliveira (Eds.). Embrapa Informação Tecnológica, Brasília, Campina Grande, Embrapa Algodão, Brasília
- Castro ED, FJ Pereira, R Paiva (2009). *Histologia Vegetal: Estrutura e Função de Órgãos Vegetativos*. UFLA, 9, Lavras, Brazil
- Echer FR, CA Rosolem (2017). Plant growth regulation: a method for fine-tuning mepiquat chloride rates in cotton. *Pesq Agropec Trop* 47:286–295
- Ferrari JV, E Furlani Júnior, S Ferrari, AP Luques, DM dos Santos, FDM Benke (2014). Growth and cotton yield over row spacing and growth regulators. *Rev Bras de Ciênc Agra* 9:359–364
- Lamas FM, AO Barcellos, JC Bogiani (2013). *Pontos a serem considerados no manejo de regulador de crescimento na cultura do algodoeiro*. Embrapa Agropecuária Oeste-Comunicado Técnico. (INFOTECA-E), Brasília
- Leal AJ, GL Piati, RC Leite, MS Zanella, CR Osorio, SF Lima (2020). Nitrogen and mepiquat chloride can affect fiber quality and cotton yield. *Rev Bras Eng Agríc e Ambient* 24:238–243
- Filho ODL (2010). Aspectos gerais sobre o silício em solos, plantas e animais. RODRIGUES, FA. Silício na agricultura. *Anais do V Simpósio de Silício na Agríc* 5:47–60

- Lima MDA, VFD Castro, JB Vidal, J Enéas-Filho (2011). Aplicação de silício em milho e feijão-de-corda sob estresse salino. *Rev Ciênc Agron* 42:398–403
- Luo H, H Zhang, H Han, Y Hu, Y Zhang, W Zhang (2014). Effects of water storage in deeper soil layers on growth, yield, and water productivity of cotton (*Gossypium hirsutum* L.) in arid areas of northwestern China. *Irrig Drain* 63:59–70
- Paixão AP, E Furlani Junior, SS Hiraki, LHMD Machado, LS Camargos, FT Carvalho (2017). Crescimento, fotossíntese e atividade enzimática de genótipos de algodoeiro herbáceo submetidos ao cloreto de mepiquat. *Rev Cult Agron* 26:540–553
- Peixoto ML, JC Moraes, AA Silva, FA Assis (2011). Efeito do silício na preferência para oviposição de *Bemisia tabaci* biótipo b (Genn.) (Hemiptera: Aleyrodidae) em plantas de feijão (*Phaseolus vulgaris* L.). *Ciênc e Agrotec* 35:478–481
- Santos RF, R Carlesso (1998). Déficit hídrico e os processos morfológico e fisiológico das plantas. *Rev Bras de Eng Agríc e Ambient* 2:287–294
- Sheng H, S Chen (2020). Plant silicon-cell wall complexes: Identification, model of covalent bond formation and biofunction. *Plant Physiol Biochem* 155:13–19
- Junior JPS, R Mello Prado, MB Soares, JLF da Silva, VH de Farias Guedes, MM dos Santos Sarah, JO Cazetta (2021). Effect of different foliar silicon sources on cotton plants. *J Soil Sci Plant Nutr* 21:95–103
- Stewart AM, KL Edmisten, R Wells, DL Jordan, AC York (2001). Wick applicator for applying mepiquat chloride on cotton: I. Rate response of wick and spray delivery systems. *J Cotton Sci* 5:9–14
- Taiz L, E Zeiger (2013). *Fisiologia Vegetal*. Editora Artemed, Porto Alegre, Brazil
- Tombesi S, T Frioni, S Poni, A Palliotti (2018). Effect of water stress “memory” on plant behavior during subsequent drought stress. *Environ Exp Bot* 150:106–114