

EXOGENOUS CELLULAR SIGNALING MODULATE COTTON PLANT RESPONSES TO DROUGHT

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Abstract: Climate change has increasingly constrained agricultural productivity, particularly water deficit. In this context, the application of exogenous signaling molecules appears promising for activating plant defense mechanisms. This study aimed to evaluate the effects of cell signaling compounds on the performance of cotton plants (*Gossypium hirsutum* L.) under water deficit conditions. The experiment was conducted in a greenhouse using a completely randomized design (CRD), with eighteen treatments and four replicates. Treatments consisted of combinations of signaling molecules (salicylic acid, jasmonic acid, hydrogen peroxide, and melatonin) at four concentrations, along with two controls. The variables analyzed included photosynthetic pigments, relative water content, number of floral buds, and dry biomass. Under water deficit, all signaling molecules increased pigment accumulation and the number of floral buds. Jasmonic acid at 50 and 100 μM increased the carotenoid and relative water content. However, only foliar application of melatonin at 400 μM significantly increased leaf and stem dry mass, thereby improving relative drought tolerance. The findings indicate that foliar spraying of signaling molecules is a viable strategy to enhance cotton resilience to water deficit.

Keywords: Bioregulators, *Gossypium hirsutum* L., plant defense, water restriction.

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SINALIZADORES CELULARES EXÓGENOS MODULAM AS RESPOSTAS DE PLANTAS DE ALGODÃO AO DÉFICIT HÍDRICO

Resumo: As mudanças climáticas, especialmente o déficit hídrico, têm limitado a produtividade agrícola. Assim, o uso de sinalizadores exógenos pode ser promissor para ativar defesas. Esse estudo teve como objetivo avaliar o efeito de sinalizadores celulares, em quatro doses distintas, no desempenho de plantas de algodão (*Gossypium hirsutum* L.) sob déficit hídrico. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado (DIC), com dezoito tratamentos e quatro repetições. Os tratamentos consistiram na combinação de sinalizadores (ácido salicílico, ácido jasmônico, peróxido de hidrogênio e melatonina) em quatro doses e dois controles. As variáveis analisadas incluíram pigmentos fotossintéticos, teor relativo de água, número de botões flores e biomassa seca. Sob déficit hídrico, todos os sinalizadores estudados promoveram incrementos no acúmulo de pigmentos, e no número de botões florais. O tratamento com ácido jasmônico nas doses 50 e 100 μM promoveu maior acúmulo de carotenoides e teor relativo de água. Contudo, apenas a pulverização com melatonina a 400 μM aumentou a massa seca das folhas e caules e, conseqüentemente, a tolerância relativa ao estresse. Conclui-se que o uso de sinalizadores celulares representa uma alternativa viável para aumentar a resiliência do algodoeiro ao déficit hídrico.

Palavras-chave: Bioreguladores, defesa de plantas, *Gossypium hirsutum* L., restrição hídrica.

INTRODUCTION:

Cotton (*Gossypium hirsutum* L.) accounts for approximately 35% of global fiber and oil production and is cultivated in over 100 countries (Zafar et al., 2023). In the Brazilian Cerrado, agricultural production faces climatic challenges such as high temperatures, irregular rainfall, and prolonged droughts periods that significantly reduce productivity. The yield losses may reach up to 67% of cotton production, with a global production drop of 73% (Santos et al., 2020; Khattak et al., 2023).

Drought is one of the main limiting factors in agriculture, affecting key physiological processes like photosynthesis, respiration and stomatal regulation. Under prolonged stress, energy-producing organelles such as mitochondria and chloroplasts are disrupted, leading to the overproduction of reactive oxygen species (ROS), which cause oxidative damage to lipids,

proteins, and DNA (Omidbakhshfard et al., 2020). To cope with stress, plants activate defense mechanisms involving gene expression and metabolic reprogramming. Key signaling molecules include ABA, Ca²⁺, proline, glycine betaine, and polyamines (Ozturk et al., 2021). Antioxidant responses also play a central role, involving enzymes like SOD, CAT, and GPX, and non-enzymatic compounds such as carotenoids, ascorbate and flavonoids (Qamer et al., 2021).

Drought tolerance results from the interplay of multiple genes and signaling pathways. Upon sensing stress, plant cells activate molecular cascades that regulate physiological and morphological adjustments to maintain growth and limit damage. In this context, the exogenous application of signaling molecules has emerged as a promising strategy to induce cross-tolerance in plants. Compounds such as salicylic acid, jasmonic acid, melatonin, H₂O₂, brassinosteroids, and polyamines have shown protective effects in several crops (Kerchev et al., 2020). Although cotton exhibits adaptive responses such as changes in photosynthesis and root–shoot morphology under drought (Zahid et al., 2021), information underlying plant defense modulated by exogenous signals are poorly explored.

Our investigative study aimed to evaluate the foliar application of four signaling compounds (salicylic acid, jasmonic acid, melatonin, and H₂O₂) at different concentrations on a drought-sensitive cotton cultivar (FM911 GLTB) (Sousa et al., 2025). Growth and physiological parameters were assessed to investigate defense mechanisms induced under water deficit conditions.

MATERIALS AND METHODS

Experimental site, cultivation conditions, and treatments

The experiment was conducted in a greenhouse, and analyses were performed at the Department of Crop Science, Center for Agricultural Sciences, Federal University of Piauí (UFPI), in Teresina, Brazil. Trials were carried out using 11 dm³ plastic pots with soil, and the cotton cultivar FM 911 GLTP was selected due to its sensitivity to drought (Sousa et al., 2025).

Plants were grown under drought conditions by maintaining soil moisture at 50% of field capacity (FC). The experiment followed a completely randomized design (CRD) with eighteen treatments and four replicates. Treatments included a drought treatment without foliar spraying (T0 - negative control), and plants sprayed with salicylic acid (T1 - 500 µM, T2 - 1000 µM, T3 - 1500 µM, T4 - 2000 µM) (Barros et al., 2019), jasmonic acid (T5 - 50 µM, T6 - 100 µM, T7

- 150 μM , T8 - 200 μM) (Muhammad et al., 2021), hydrogen peroxide (T9 - 500 μM , T10 - 1000 μM , T11 - 1500 μM , T12 - 2000 μM) (Ishibashi, 2011), and melatonin (T13 - 100 μM , T14 - 200 μM , T15 - 300 μM , T16 - 400 μM) (Hu et al., 2020; Jiang et al., 2021). Applications were divided into two time-points: 48 and 24 h before the imposition of water deficit, when plants reached the vegetative stage V3 (21 days after sowing). A separate group of plants was maintained under full irrigation at 90% FC, serving as the absolute control.

Pots were weighed daily using an electronic scale to monitor water loss by evapotranspiration, and water was replenished to maintain the desired soil moisture levels (Xu et al., 2009). Plant harvest was performed at the onset of the reproductive stage, 45 days after the drought imposition.

Relative water content and dry biomass

Relative water content (RWC) was measured using 1.0 cm^2 leaf discs, following the method proposed by Čatský (1960). Using the following equation:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

where: FW = fresh weight; DW = dry weight; TW = turgid weight. Plant material was dried in a forced-air oven at 65 $^{\circ}\text{C}$ for three days to determine dry mass.

Photosynthetic pigments

The extraction of photosynthetic pigments, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl total), and carotenoids, was performed by incubating leaf discs in dimethyl sulfoxide (DMSO) solution saturated with calcium carbonate (CaCO_3), kept in the dark. Pigment concentrations were determined by spectrophotometry, with absorbance readings at 665, 649, and 480 nm, using the equations described by Wellburn (1994).

Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) using the F-test at a 5% significance level ($p \leq 0.05$). Means were compared using the Scott-Knott clustering test. Statistical analyses were performed using the Sisvar software.

RESULTS AND DISCUSSION

Cotton plants from treatments T5 and T6 exhibited higher relative water content (RWC) than those of absolute control, suggesting improved leaf water retention under drought conditions (Figure 1). In contrast, plants treated with T9 and T10 showed the lowest RWC values, indicating that these treatments resulted in greater stress-induced impairment of leaf water status.

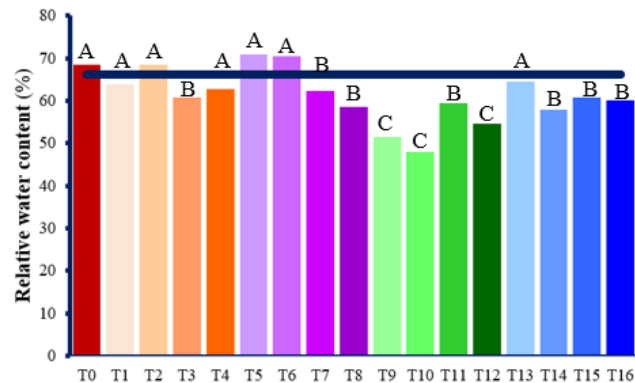


Figure 1: Relative water content of cotton plants subjected to different treatments with cellular signaling molecules after 45 days to drought imposition (50% field capacity - FC). In graph, black line represents the values of well-irrigated plants at 90% FC (absolute control). Different capital letters represent significant difference due to cell signaling treatment, according to Scott-Knott test ($p \leq 0.05$).

It is important to highlight that the treatments with hydrogen peroxide and high levels of jasmonic acid and melatonin decreased the RWC as compared to well-irrigated plants, whereas jasmonic acid at T5 and T6 induced higher RWC. The data suggest a beneficial action of T5 and T6 in maintaining the plant water status under stress conditions, indicating a possible protective action on water balance or on water retention mechanisms, such as stomatal closure, accumulation of osmotically active solutes, or preservation of cell membrane integrity (Al-Yasi et al., 2020; Verbeke et al., 2022; Akter et al., 2023; Juenger and Verslues, 2023). Thus, although no significant differences were observed in comparison to the negative control, the treatments may have contributed to mitigating the negative effects of water deficit by preserving physiological parameters relevant to plant performance.

The accumulation of photosynthetic pigments was similar between well-irrigated and drought-stressed control plants (Figure 2), but varied significantly in response to the cellular signaling treatments. In general, increased levels of Chl a, Chl b, Chl total, and carotenoids were observed in drought-stressed plants treated with almost all signaling molecules, with notable effects in treatments T6, T9, T10 and T14. Elevated photosynthetic pigments are critical for photosynthetic performance and plant growth. Also, the accumulation of carotenoids suggests the activation of metabolic pathways related to antioxidant defense and

photoprotection, indicating that the signaling molecules may positively modulate the plant defense system under water deficit conditions. Photoprotection under drought conditions can be mediated by secondary metabolites such as xanthophylls from the violaxanthin cycle (VAZ), zeaxanthin, and flavonoids, which dissipate excess light energy through non-photochemical quenching (NPQ) and reduce oxidative stress in chloroplasts. These compounds can also act as antioxidants, stabilize thylakoid membranes, and neutralize reactive oxygen species (Gori et al., 2021).

Several studies have already demonstrated the beneficial effects of the exogenous application of cellular signaling molecules in improving physiological parameters and inducing drought tolerance, corroborating the findings of the present study. Similar benefits have been reported in basil sprayed with methyl jasmonate (Lopes et al., 2025), sesame treated with salicylic acid and jasmonate (Kochi et al., 2025), maize subjected to melatonin application (Zhao et al., 2021), and rice treated with salicylic acid and melatonin (Urmi et al., 2023).

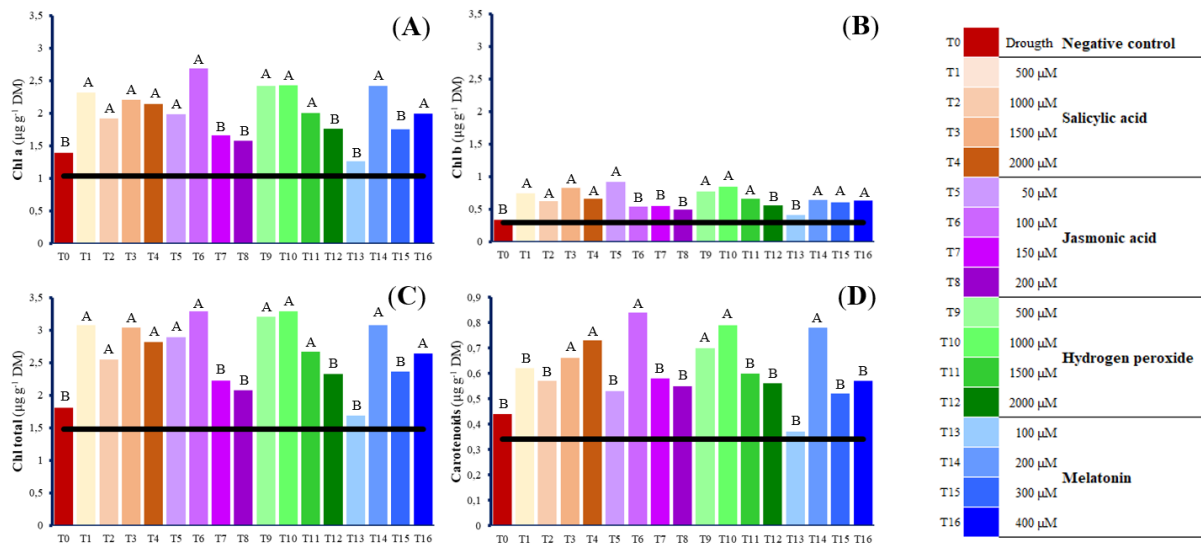


Figure 2: Contents of chlorophyll a (Chl a, A), chlorophyll b (Chl b, B), total chlorophyll (Chl total, C) and carotenoids (D) of cotton plants subjected to different treatments. Additional details as Figure 1.

Drought (T0) significantly reduced the number of flower buds (Figure 3A), as well as the dry mass of leaves (Figure 3B) and stems (Figure 3C), compared to the well-watered control (black line in the graphs). Treatments T2, T3, T7, T8, T9, and T13 showed higher average numbers of flower buds in cotton plants, suggesting a stimulatory effect on reproductive development even under drought conditions. However, only melatonin at 400 µM (T16) promoted a greater average increase (but no significant) in biomass accumulation and improved the relative drought tolerance of cotton plants compared to the negative control. Despite these observed increases, the signaling treatments did not result in statistically significant differences.

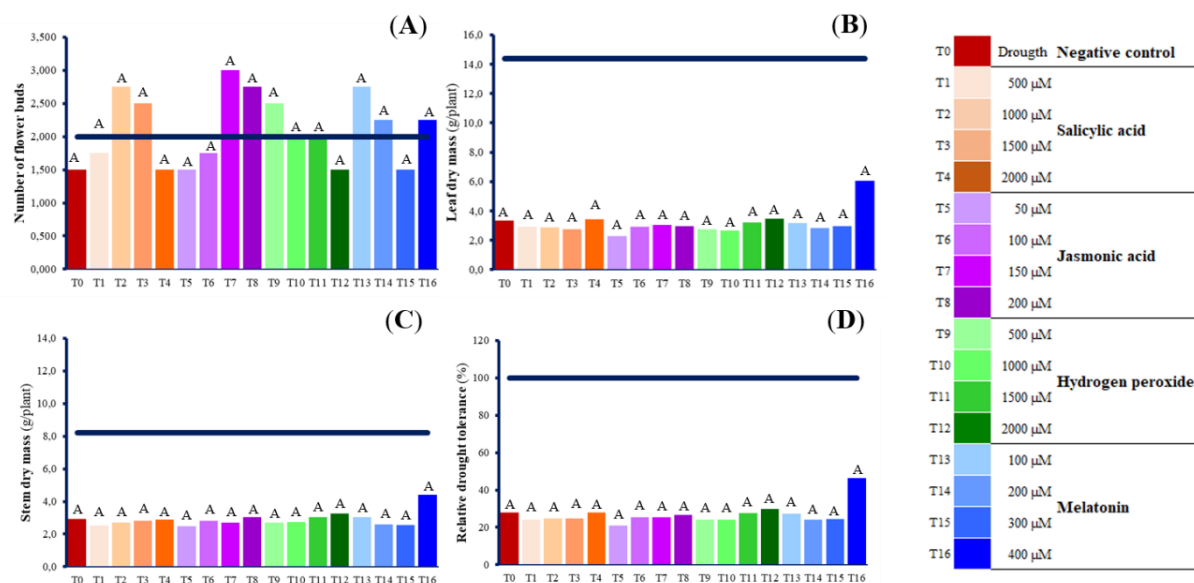


Figure 3: Number of floral buds (A), dry mass of leaf (B) and stem (C), and relative tolerance to drought (D) of cotton plants subjected to different treatments. Additional details as Figure 1.

CONCLUSIONS

Foliar application of salicylic acid, jasmonic acid, hydrogen peroxide, and melatonin enhances the accumulation of photosynthetic pigments and contributes to the maintenance of relative water content in cotton plants under water deficit. These findings indicate that such signaling molecules may activate metabolic pathways associated with photoprotection and antioxidant defense. However, further studies in a long-term and field are required to validate their potential as promising strategies for improving drought tolerance in cotton cultivars.

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