

Original Article

# Pyruvic acid as attenuator of water deficit in cotton plants varying the phenological stage

Ácido pirúvico como atenuante do déficit hídrico em algodoeiros variando a fase fenológica

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#### **Abstract**

The lack of water during crop growth causes damage to any production system, especially when it occurs during the initial establishment or beginning of the reproductive stage. Although cotton can be properly managed in regions with water limitation, its yield is affected at different levels according to the genetics of the cultivar adopted. Exogenous application of some organic components has shown a stress-mitigating effect and can be a valuable procedure to enhance the yield of water stress-sensitive cultivars. The objective of this work was to evaluate the benefits of exogenous application of pyruvic acid ( $100 \, \mu\text{M}$ ) in cotton plants under water deficit varying the phenological stage of the crop. The experiment was conducted in a greenhouse, where the plants were grown in pots and subjected to seven days of water suspension, initiated individually in stages V2 and B1. Each pot contained two plants. The treatments adopted were: T1 - control, T2 - water suppression; and T3 - water suppression + pyruvate application. The design was randomized blocks in a factorial scheme ( $3 \times 3$ ) with three replicates. The reductions in gas exchange and growth of the cultivars BRS Seridó, CNPA 7MH and FM 966 were more significant in the reproductive stage, especially for FM 966, which was more sensitive. Pyruvate application reduced the effects of water suppression on boll production by 31% in BRS Seridó and 34% in CNPA 7MH and FM 966.

Keywords: Gossypium hirsutum L., water stress mitigation, production, gas exchange.

#### Resumo

A falta d'água durante o crescimento da cultura traz prejuízos em qualquer sistema de produção, especialmente quando ocorre durante o estabelecimento inicial ou início da fase reprodutiva. O algodoeiro, apesar de ter larga habilidade para manejo em regiões com limitação hídrica, tem o rendimento afetado, com níveis diferenciados em função da genética do cultivar adotado. A aplicação exógena de alguns componentes orgânicos tem demonstrado efeito mitigador do estresse podendo ser um aditivo valioso para impulsionar a produtividade de cultivares sensíveis ao estresse hídrico. Neste trabalho, objetivou-se avaliar os benefícios da aplicação exógena de ácido pirúvico (100 µM) em algodoeiros sob déficit hídrico variando a fase fenológica da cultura. O ensaio foi conduzido em casa de vegetação, onde as plantas foram cultivadas em vasos e submetidas a sete dias de suspensão hídrica, iniciadas, individualmente, nas fases V2 e B1. Cada vaso conteve duas plantas. Os tratamentos adotados foram: T1- controle, T2 - supressão hídrica; T3- supressão hídrica + aplicação de piruvato. O delineamento foi em blocos casualizados em esquema fatorial (3 × 3) com três repetições. Foi observado que as reduções nas trocas gasosas e crescimento das cultivares BRS Seridó, CNPA 7MH e FM 966 foram mais expressivas na fase reprodutiva, especialmente da última que se mostrou mais sensível. A aplicação de piruvato mitigou os efeitos da supressão hídrica sobre a produção de capulhos 31% na BRS Seridó e 34% em CNPA 7MH e FM 966.

Palavras-chave: Gossypium hirsutum L., mitigação do estresse hídrico, produção, trocas gasosas.

# 1. Introduction

Cotton (*Gossypium hirsutum* L.) is a commodity widely cultivated in various parts of the world. It is the most important source of natural fiber, being a product

of high importance in the textile industry in both developed and developing countries (Hussain et al., 2022; Barros et al., 2022; James, 2018). Since the mid-1990s, the

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competitiveness of cotton fibers has increased considerably among producing countries, so that current commercial cultivars have served this market because they have several genetic attributes that allow greater production and environmental adaptation (Barros et al., 2022).

Brazil is one of the world's largest producers of cotton fibers, with a production of 5.5 million tons, meeting the internal and external demands of the textile industry (IBGE, 2021). The current commercial cultivars have high technical quality, with wide environmental adaptation and high yield (Barros et al., 2022). Despite this robustness, drought problems occur throughout the agricultural region, with indefinite durations, affecting the growth and development of the cultivars differently, depending on their genetic basis.

At the cellular level, water suppression during the phenological cycle of cotton affects gas exchange, water relations, synthesis of compatible solutes, among other events, with negative consequences on plant growth and development (Ul-Allah et al., 2021; Niu et al., 2018). The impacts of drought on growth generate different effects, which are lighter until the appearance of the first floral bud and more drastic during flowering and fruit development, leading to high percentages of shedding of floral buds and young bolls. In the boll opening stage, water need is reduced, but the water stress caused in this phase directly affects fiber quality (Hussain et al., 2020; Iqbal et al., 2017; Zonta et al., 2017).

After perceiving water stress, plants release a series of metabolites that act coordinately to minimize damage to their cells, storing amino acids, sugars, mineral ions, hormones and proteins, to try to deal with stress (Hamidi et al., 2024). The agility of response to minimize the production of reactive species and accelerate the synthesis and accumulation of compatible osmoprotectants and solutes is what differentiates a drought-sensitive from a drought-tolerant genotype (Barbosa et al., 2021; Osakabe et al., 2014; Lisar et al., 2012).

Reports in the literature have shown that supplementation of organic solutes, such as ascorbic acid, salicylic acid, tocopherol, pyruvate, among others, in plants under water stress contributes to mitigating cell damage, especially in drought-sensitive plants (Barbosa et al., 2021; Karimian et al., 2015; Sadiq et al., 2017; Aziz et al., 2018).

For pyruvate, a molecule involved in the Krebs cycle, studies report its influence on the activation of stomatal movements, promoted by mitochondrial pyruvate carrier proteins (MPCs), which activate anion channels to trigger stomatal closure in response to drought (Li et al., 2014; Wang et al., 2014; Shen et al., 2017).

One of the main results in this segment was reported by Wang et al. (2014), who applied exogenous pyruvate in *Arabidopsis* plants subjected to water stress and found a negative regulator protein acting on stomatal opening and signaling of ABA-induced guard cells, called NRGA1 (Negative Regulator of Guard cell ABA signaling 1). This protein is a transcription factor responsible for activating pyruvate accumulation and signaling for ABA action, triggering stomatal closure. Shen et al. (2017) incubated leaves of *Arabidopsis* at various concentrations of pyruvate and found an increase in the anion channel

current of the guard cells, which induced stomatal closure at  $100 \, \mu M$ . With peanuts, Barbosa et al. (2021) found the mitigating effect of exogenous pyruvate in plants grown under water stress, favoring their growth, gas exchange and photosynthesis rate especially in the drought-sensitive cultivar, at low concentration.

The objective of this work was to evaluate the benefits of exogenous application of pyruvic  $acid (100 \, \mu M)$  in cotton plants under water deficit varying the phenological stage of the crop.

#### 2. Material and Methods

The experiment was conducted in a greenhouse, in Campina Grande, PB, Brazil (06° 48' 50" S and 37° 56' 31" W, 550 m), from Nov/2019 to Mar/2020. Three commercial cotton cultivars were used in the study, BRS Seridó, CNPA 7MH and FM 966, the first being drought-tolerant and the last being drought-sensitive (Vasconcelos et al., 2018). Seeds of the cultivars were sown in pots (25 L), containing soil (*Neossolo Regolítico* – Entisol) previously fertilized with urea, single superphosphate and potassium chloride, in the amounts of 37.5, 5.6 and 6.2 g in each pot, respectively. A hose was fixed at the bottom of each pot, connected to a plastic container (2 L) to collect the drained water, in order to determine the water depth to be applied in each irrigation event.

After emergence, two plants were maintained per pot. Irrigation was performed daily, maintaining the soil at pot capacity, determined by the method of capillary saturation followed by drainage.

The volume applied in each irrigation event was estimated by means of water balance, according to the terms of Equation 1.

$$C_W = Va - Vd \tag{1}$$

Where:

C<sub>w</sub>: Water consumption (mL);

Va: Volume of water applied to plants in the previous day (mL); and

Vd: Volume drained, quantified in the morning of the next day (mL).

At 15 and 28 days after emergence (DAE), when the plants had 2-3 true leaves (stage V2) and were producing the first floral bud (stage B1), respectively (Marur and Ruano, 2001), they were subjected to seven days of water suppression in individual trials. At the end of these periods, physiological evaluations were performed. Control plants were irrigated daily. The treatments adopted for each trial were: T1 - control, T2 - water stress of seven days; T3 - water stress + application of pyruvate (PVT, Merck, 8201700500). The design was randomized blocks in a factorial scheme (3 × 3) with five replicates. The PVT concentration was 100  $\mu$ M, based on the study conducted by Shen et al. (2017).

PVT (100  $\mu$ M) was applied by spraying (50 mL) on the leaves for three consecutive days, starting from the fourth day after the beginning of water suppression. In plants of treatments T1 and T2, pyruvate application on the leaves

was carried out with water (Barbosa et al., 2021). During applications, the base of each pot of plants was protected with a plastic sheet to avoid drift or flow of solution to the root zone. At the end of the water suppression, soil moisture contents were recorded in the two studied stages (Table 1).

Physiological parameters were evaluated at the end of the stress period at 22 and 35 DAE. Stomatal conductance (gs) (mol m-² s-¹), transpiration (E) (mmol H<sub>2</sub>O m-² s-¹), CO<sub>2</sub> assimilation rate (A) (µmol m-² s-¹) and internal CO<sub>2</sub> concentration (Ci) (µmol mol-¹) were estimated with an Infrared Gas Analyzer (IRGA, ADC BioScientific Ltd, LC-Pro model), in the third apical leaf of the plant. Air temperature and CO<sub>2</sub> concentration data were collected under ambient conditions, and luminosity was adjusted to 1200 µmol m-² s-¹ of radiation. Instantaneous carboxylation efficiency (A/Ci) was estimated using the gas exchange data.

Growth and production analyses were performed at 120 DAE in plants subjected to stress in stages V2 and B1, with records of stem height and diameter, and the number and weight of bolls per plant. The data were subjected to the homogeneity test and subsequent analysis of variance by the F test, using Sisvar software (Ferreira, 2019). Tukey test ( $p \le 0.05$ ) was adopted to classify the means.

### 3. Results and Discussion

Significant ( $p \le 0.01$ ) statistical differences were found in all variables for treatments and genotypes, as well as significant G x T interaction for most variables, indicating differentiated response of the germplasm as a function of exogenous application of PVT.

The gas exchange data of the cultivars evaluated in stage V2 are presented in Figure 1. Despite the changes observed in the cultivars due to the stress period (T2), the changes in gas exchange were mild, not exceeding 24%, even in the most sensitive cultivar (FM 966), which corroborates Iqbal et al. (2017) and Zonta et al. (2017). It is possible to notice, however, an alleviation in the status of the plant resulting from the spraying of PVT (T3), based on the reduction in the percentages of each variable, compared to the control (T1).

In general, it was found that the tolerant cultivar, BRS Seridó, benefited more from the application of 100  $\mu M$  of PVT when water stress was applied at the beginning of the vegetative stage, based on the statistically similar means of most gas exchange variables in treatments T1 and

T3 (Figure 1). These results indicate that the application of PVT contributed to restoring the gas exchange of plants, minimizing the effects of water stress in the beginning of growth. The most visible response of this cultivar was observed in the instantaneous carboxylation efficiency (A/Ci), which was reduced by 29% in T2, compared to the mean of the control, and restored in T3, with an increase of 32% compared to T2 (Figure 1E). The intracellular CO<sub>2</sub> concentration (Ci) of this cultivar increased 18% in T2, indicating lower utilization of CO<sub>2</sub> in the photosynthetic process, but was restored in T3 (Figure 1E). This affected the CO2 assimilation (Figure 1D), which, despite having decreased 16% with water restriction, was restored in the treatment with PVT, indicating that its application contributed for plants to continue to perform photosynthesis in a reasonable way, when water stress was imposed at the beginning of growth.

In the sensitive cultivar, FM 966, the benefits promoted by PVT application were subtle, based on the A/Ci ratio, which was reduced by 24% in T2, compared to T1, and increased 18% in T3, compared to the mean of T2 (Figure 1E). This is an indication that there was improvement in the carbon capture and fixation process in plants under stress and PVT application, although it was mild, as there were no effects on  $\rm CO_2$  assimilation (Figure 1D), which was not restored in T3.

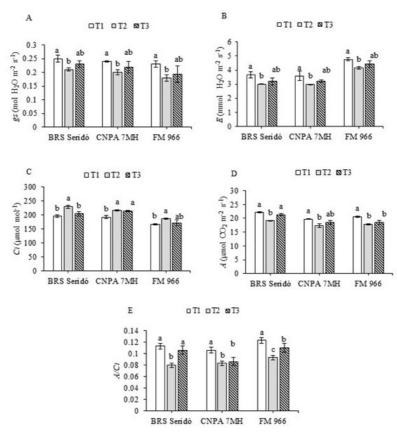
The average growth and production of the cultivars, subjected to seven days of total water suppression, established at the beginning of growth (V2), are presented in Figure 2. It was observed that, except for stem diameter, which did not differ between T2 and T3, most variables showed reduction under water stress, being totally reestablished with the addition of PVT. In general, the main benefits for the cultivars were observed in plant height and weight of bolls. BRS Seridó, CNPA 7MH and FM 966 plants had their heights reduced by 13%, 20% and 17%, respectively, returning more quickly to the control condition, with minimum differences of 8%, 13% and 9%, respectively, compared to the means of T3 and T1. According to Barbosa et al. (2021), who studied the mitigating effect of PVT on stressed peanut plants; the closer the mean of T3 is to the mean of the control (T1), the more effective the effect of mitigation (Barbosa et al., 2021).

Regarding the number of bolls per plant, the benefits of PVT application (T3) were visible only for BRS Seridó, in which the application favored the total restoration of this variable in T1, after a reduction of 22% caused by water suppression (T2).

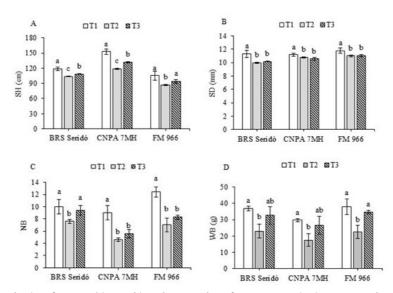
**Table 1.** Soil moisture recorded at the end of the water suppression period in stages V2 and B1.

Treatments	Vegetative stage			Flowering stage		
	BRS Seridó	CNPA 7MH	FM 966	BRS Seridó	CNPA 7MH	FM 966
	Soil moisture (%)			Soil moisture (%)		
T1	22.97	21.50	18.16	20.8	20.8	17.0
T2	16.02	16.24	15.77	13.68	8.18	7.18
T3	15.63	15.45	14.94	11.49	8.83	6.3

T1: control; T2: water stress of seven days; T3: water stress of seven days + pyruvate application.



**Figure 1.** Gas exchange in cotton cultivars subjected to seven days of water suppression in the initial growth stage (V2) and treated with pyruvate (100  $\mu$ M). Means followed by the same letter do not differ statistically between treatments (Tukey,  $p \le 0.05$ ). Stomatal conductance (gs), transpiration (*E*), internal CO<sub>2</sub> concentration (*Ci*), CO<sub>2</sub> assimilation rate (A) and instantaneous carboxylation efficiency (A/Ci). T1: control; T2: water stress; T3: water stress + pyruvate application.



**Figure 2.** Growth and production of cotton cultivars subjected to seven days of water suppression in stage V2 and treated with pyruvate (100  $\mu$ M). Means followed by the same letter do not differ statistically between treatments (Tukey, p $\leq$ 0.05). Stem height (SH), stem diameter (SD), number of bolls (NB), weight of bolls (WB). T1: control; T2: water stress; T3: water stress + pyruvate application.

For weight of bolls (WB), the effects of PVT were promising for all cultivars, especially the sensitive one, FM 966, which had losses of 44% due to water suppression and reestablished the mean of the control, as there was no statistical difference between T1 and T3 for this cultivar.

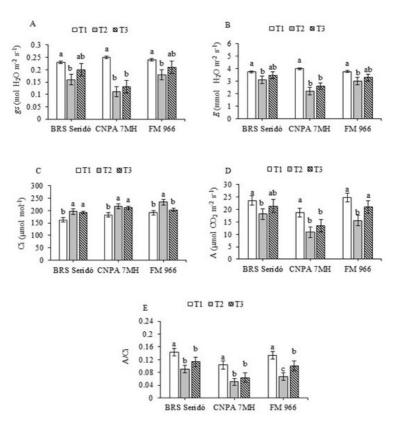
Despite the relevance of these results, it is worth mentioning that these cultivars faced a water stress which lasted seven days, established at the beginning of growth, and were later rehydrated until the end of the cycle. As the cultivars have an average cycle of 150 days, these results demonstrate that, in general, the application of PVT (100  $\mu$ M) in plants under water stress in stage V2 promotes moderate benefits, so it is necessary to estimate the potential economic benefits to enable its use under field conditions.

Figure 3 shows the means of gas exchange of the cultivars subjected to water stress in stage B1. Water restriction in this stage compromised the physiological parameters of the plants with greater intensity than the stress in the vegetative stage, causing losses greater than 50% in some variables, compared to the means of the control (T1). Water stress-mitigating effects were recorded in plants treated with PVT for BRS Seridó and FM 966, being more significant in the latter, in which the application of PVT

favored carbon fixation by plants, restoring by 34% the carboxylation efficiency (A/Ci), which was reduced by 50% in T2, compared to the mean of T1 (Figure 3E). As a consequence, the internal CO<sub>2</sub> concentration (Ci) and the CO<sub>2</sub> assimilation rate (A), which were reduced by 22% and 37% due to water stress, were fully restored after the application of PVT (Figure 3C-3D), proving the benefits of PVT application at 100  $\mu$ M for the photosynthetic process of the plants.

Stomatal conductance was also favored by PVT application, with restoration of 25, 18.8 and 16.6% in BRS Seridó, CNPA 7MH and FM 966, respectively, when compared to T2. A similar trend was observed for *E*, whose restoration was equal to 7.3, 19.0 and 9.27%, respectively, in BRS Seridó, CNPA 7MH and FM 966. As stomatal conductance is reduced, transpiration is also reduced successively. Open stomata enable carbon absorption and exit, and their closure saves water and reduces the risk of dehydration (Taiz et al., 2017).

In the early cultivar CNPA 7MH, there was no benefit of PVT, at least at the concentration used, possibly because, for being a hybrid resulting from the cross *G. gossypium* var. latifolium x Marie Gallant, this cultivar has fixed a set of characteristics of adaptation to a dry environment,



**Figure 3.** Gas exchange in cotton cultivars subjected to seven days of water suppression in the reproductive stage (B1) and treated with pyruvate (100  $\mu$ M). Means followed by the same letter do not differ statistically between treatments (Tukey,  $p \le 0.05$ ). Stomatal conductance (gs), transpiration (E), internal CO<sub>2</sub> concentration (Ci), CO<sub>2</sub> assimilation rate (A) and instantaneous carboxylation efficiency (A/Ci). T1: control; T2: water stress; T3: water stress + pyruvate application.

allowing intrinsic adjustments when the signs of water suppression are identified by the plant (Rodrigues et al., 2016).

The means related to growth and production components at 120 DAE of cotton plants subjected to water suppression in stage B1 are presented in Figure 4. It was found that the benefits of applying PVT to restore plant height, stem diameter and number of bolls were subtle or nonexistent, similar to those seen in Figure 3, except for weight of bolls, which was restored by 31%, 33% and 42%, considering the means of T2 for BRS Seridó, CNPA 7MH and FM 966, indicating once again that, at the concentration adopted, pyruvate application is more beneficial for the drought-sensitive cultivar.

Studies on supplementation of pyruvate as a possible water stress mitigator in commercial crops are limited. The first approach on the subject was carried out by Shen et al. (2017), with *Arabidopsis*, and by Barbosa et al. (2021), with peanuts.

Cotton plants have a C3 photosynthetic metabolism, with high photorespiration rate and physiological complexity. This indicates that, when  $\rm CO_2$  limitation occurs, the RuBisCO enzyme reacts with  $\rm O_2$ , leading to biological and molecular changes (Santos et al., 2022). At the physiological level, the reduction in stomatal conductance, as a way to reduce water loss by transpiration, leads to the reduction of most gas exchange parameters. As a result of the reduction in  $\rm CO_2$  diffusion, the photochemical and biochemical phases become unbalanced, which affects the activity of the electron transport chain in chloroplasts and mitochondria, leading to the formation of ROS (Foyer, 2018; Denaxa et al., 2020). As the continued production

of ROS triggers a set of oxidative effects, and even cell death, phenotypic effects become more pronounced, such as growth retardation, yellowing and shedding of leaves and reproductive structures, finally affecting the biological production of the plant.

As every cellular response involves a chain of events, the metabolism in tolerant plants is expected to be faster, giving them better adjustment in situations of internal adversity (Marcelino et al., 2022; Braz et al., 2019). In sensitive plants, these responses are slower, and supplementation of organic substances that are involved in osmotic adjustment or energy supply generally provides more effective contributions since they act as a support to overcome the negative effects of water restriction, while hydration is not reestablished (Barbosa et al., 2021; Shen et al., 2017). This explains the low response of PVT utilization in the resistant cultivar CNPA 7MH and the best utilization of the organic compound in the sensitive cultivar FM 966.

Another aspect that should be considered in this study is related to the stages in which the PVT application was performed. In drought-prone environments, planting is carried out during the first rains, allowing the soil to have enough moisture to ensure at least the first 20 days of emergence, for cultivars with cycle of around 150 days. To ensure production, however, it is necessary that, at the time of the emergence of the first flowers (45-50 DAE), plants have enough moisture to establish at least 50% of the production, since water suppression at this stage can increase the shedding of flower buds by 50%, on average, in a semi-arid climate (Carvalho et al., 2019; Coutinho et al., 2015). Thus, a drought period during this phase will directly

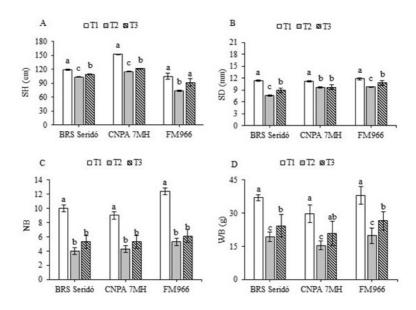


Figure 4. Growth and production of cotton cultivars subjected to seven days of water suppression in stage B1 and treated with pyruvate (100  $\mu$ M). Means followed by the same letter do not differ statistically between treatments (Tukey, p<0.05). Stem height (SH), stem diameter (SD), number of bolls (NB), weight of bolls (WB). T1: control; T2: water stress of seven days; T3: water stress of seven days + pyruvate application.

interfere in the formation and production of cotton bolls. Although the canopy is abundant in this phase, it would be interesting to assess the impact of PVT supplementation on plants under stress. As the average yield of bolls of the cultivars of this study is 2.2 t ha<sup>-1</sup> in a semi-arid environment (Coutinho et al., 2015; Vidal Neto and Freire, 2013), an additional input of 30-40%, as seen in Figure 4, represents a significant gain, which could stimulate the use of PVT in the field, considering the low concentration, price of the product (U\$ 100) and absence of toxicity to the environment. Evidently, subsequent validation tests will be necessary to attest to the cost/benefit ratio for adopting this process.

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