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CROP PRODUCTION

Pyruvate supplementation in cotton under water restriction varying the phenological phases

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ABSTRACT. Cotton is one of the largest agricultural commodities that generate various sources of foreign exchange and employment worldwide. However, water deficiency is an environmental factor that limits the production of this crop, especially in semi-arid regions. We evaluated pyruvate supplementation to mitigate the effects of water stress on colored cotton. Experiments were conducted in a greenhouse. We studied two forms of pyruvate supplementation (SP1- via seed and foliar and SP2- only via foliar); three conditions of irrigation management of the plants: water restriction in the vegetative phase (VE), flowering (FL), and vegetative and flowering (VE/FL); and additional treatment (total irrigation throughout the crop cycle and without pyruvate supplementation). The experimental design included a randomized block in a 2 × 3 + 1 factorial scheme. The factors resulted in seven treatments with three replications, with a total of 21 experimental units. Gas exchange, enzyme activity, and production of components were evaluated. Water restriction in the vegetative phase does not cause losses in BRS Jade cotton when supplemented with pyruvate. However, in the flowering and vegetative phases plus flowering, it reduces gas exchange and production components and increases the activity of antioxidant enzymes in relation to plants under full irrigation. Supplementation with pyruvate via seed plus foliar (SP1) was better for BRS Jade cotton grown under water restriction.

Keywords: *Gossypium hirsutum* L.; hydric restriction; gas exchange; antioxidant enzymes.

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Introduction

Colored cotton, in addition to its great socioeconomic potential, is environmentally friendly because the yarns produced do not undergo chemical bleaching and dyeing, which prevents the discharge of a large amount of waste into the environment (Barbosa et al., 2019). The species is considered less demanding in terms of water and requires good rainfall distribution. Its water requirement is 550-600 mm (Zwart & Bastiaanssen, 2004; Farahani, Oweis, & Izzi, 2008), and the most sensitive phase of the crop begins at flowering and intensifies at full peak bloom (Hake & Grimes, 2010; Zonta, Brandão, Rodrigues, & Sofiatti, 2017).

For all crops worldwide, climate change increases the frequency and intensity of abiotic stressors, particularly water stress (Ul-Allah, Rehman, Hussain, & Farooq, 2021). Under such conditions, plants alter at all levels of cellular organization, inducing the accumulation of abscisic acid (ABA), which is involved in stomatal closure, with a reduction in water loss through transpiration and gas exchange processes. In addition, there is an excessive production of reactive oxygen species, caused by the exit of electrons to the cellular environment, from the activity of electron transport in chloroplasts, mitochondria, and other organelles, because the plant is in the process of defense against stress (Sharma, Jha, Dubey, & Pessarakli, 2012; Taiz, Zeiger, Moller, & Murphy, 2017).

As water stress increases, mainly in the period between the emergence of the first flower buds and the opening of the flower, during periods of greater sensitivity to water deficit, the plant prioritizes the growth of older reproductive structures as a survival mechanism (Yeates, 2014). If water stress persists, when there are no small fruits to abort, boll size is reduced, seriously compromising cotton yield and production (Zonta et al., 2017).

Several studies have been conducted on water stress in cotton. Most effects of water deficiency on soil have been studied using records of cotton fiber growth, productivity, and quality being affected (Snowden, Ritchie, Cave, Keeling, & Rajan, 2013; Snowden, Ritchie, Simao, & Bordovsky, 2014; Zonta et al., 2017; Cordão, Araújo, Pereira, Zonta, & Ferreira, 2018; Lima et al., 2018). Knowing the phenological phases that are most sensitive to water deficit, as described in previous lines, researches are challenged with identifying technologies to use organic compounds in plants subjected to water stress that can reduce the deleterious effects of drought.

Pyruvate is one of the products that can be used because, in the metabolism of plants, it is present in the processes of respiration and ATP production, which are vital for plant development. The exogenous application of pyruvate may favor the energy mechanisms of cotton, which do not require activation of the processes involved in glycolysis, as pyruvate molecules are available, reinforcing the substrate for ATP production. With this supplementation, part of the energy spent in glycolysis for the production of pyruvic acid is used in adaptation processes to water stress, which motivated the hypothesis of this research. In glycolysis, pyruvate is crucial for the metabolism of the Krebs cycle and the respiratory chain, resulting in the production of ATP molecules (Kerbauy, 2008; Taiz et al., 2017).

In the literature, studies have found that the exogenous application of silicon and salicylic acid in several oilseeds, including cotton, under water stress has a beneficial effect on plants (Gama et al., 2017; Silva et al., 2017). With pyruvate, the first work found in the literature was carried out in China, when Shen et al. (2017) incubated Arabidopsis leaves for a period of 2.5 h in pyruvate at concentrations of 10, 100, and 1000 μM and found that at a concentration of 100 μM, pyruvate increased the anionic current in guard cells, inducing stomatal closure. In Brazil, the research group of the present study carried out in-depth studies on cultivated species. In the first study, Barbosa et al. (2021) evaluated the effects of exogenous pyruvate in two peanut cultivars subjected to water restriction and found that a concentration of 50,000 μM pyruvate mitigated the effects of water stress in the cultivar IAC Caiapó, a water-demanding genotype, and restored antioxidant enzymes in cv. BR1, which is drought tolerant.

Therefore, the application of pyruvate as a water deficit attenuator could be an advancement in agriculture, especially in regions where water is the limiting factor. Thus, the objective of this study was to evaluate pyruvate supplementation as a way to mitigate the effects of water stress in colored cotton.

Material and methods

An experiment was conducted in a greenhouse, from April to August 2019, in the experimental area of the Academic Unit of Agricultural Engineering of the Federal University of Campina Grande (UFCG), at the geographical coordinates 07°15′18″ S, 35°52′28″ W and an average altitude of 550 m. According to the Köppen climate classification adapted to Brazil, the climate of this region is of the Csa type, which represents sub-humid mesothermal with a hot and dry period (4 to 5 months) in addition to a rainy period from autumn to winter. The average temperature during the experimental period was 23°C, and the average relative humidity was 76%.

The treatments consisted of two forms of pyruvate supplementation at 100 mM (SP1- via seed and foliar and SP2 - via foliar), three conditions of irrigation management of the plants (VE- 10 days of water restriction in the vegetative phase; FL- 7 days of water restriction at flowering and VE/FL- 10; 7 days of water restriction in the vegetative and flowering phases, respectively), and additional treatment (total irrigation throughout the crop cycle and without pyruvate supplementation). The experimental design used was a randomized block in a 2 x 3 + 1 factorial scheme. Combined, the factors resulted in seven treatments with three replications for 21 experimental units, consisting of two plants, totaling 42 plants.

The seeds of the cotton BRS Jade were provided by Embrapa Algodão. BRS Jade was grown in plastic pots, functioning as a lysimeter, which received a 3 cm layer of gravel and a geotextile blanket at the base of the container. In each lysimeter, a 4 mm diameter transparent hose was connected, coupled to 1.0 L volumetric capacity collectors to collect the drained water. Then, 19 dm^{-3} of soil was placed, and its physical and chemical attributes, determined in the laboratory, according to the methodology described by Teixeira, Donagemma, Fontana, and Teixeira (2017), are shown in Table 1.

Table 1. Chemical and physical attributes of the soil, determined in the laboratory of irrigation and salinity (LIS), Campina Grande, Paraíba State, Brazil, 2019.

Chemical Attributes		Physical Attributes		
		Sand	79.14%	Loamy Sand
pH	5.58	Silt	18.29%	
P	115 $cmol_c\ dm^{-3}$	Clay	2.67%	
K^+	0.26 $cmol_c\ dm^{-3}$	Soil density	1.54 $g\ cm^{-3}$	
Ca^{+2}	2.98 $cmol_c\ dm^{-3}$	Particle density	2.71 $g\ cm^{-3}$	
Na^+	1.33 $cmol_c\ dm^{-3}$	Porosity	47.17%	
Mg^{+2}	1.17 $cmol_c\ dm^{-3}$			
Al^{+3}	0.03 $cmol_c\ dm^{-3}$	Matric potentials (-kPa)	Soil moisture (%)	
$H^+ + Al^{+3}$	4.74 $cmol_c\ dm^{-3}$			
SB	5.74 $cmol_c\ dm^{-3}$	10	14.17	
OM	1.62%	33	11.42	
CEC	10.48 $cmol_c\ dm^{-3}$	100	9.41	
ECEC	5.77 $cmol_c\ dm^{-3}$	500	4.60	
V	54.77%	1000	4.56	
M	0.55%	1500	4.40	
		Available water	10.31	

pH: hydrogen potential; OM: organic matter; EMBRAPA method; extraction: water (pH); Mehlich (P, K, Na); KCl 1N (Ca, Mg, and Al); calcium acetate pH 7.0 (H + Al). CEC - cation exchange capacity at pH 7 e ECEC - cation exchange capacity is effective.

With soil moisture values at tensions of -10, -33, -100, -500, -1000, and -1500 kPa, the soil water retention curve (WCR) was fitted using the Van Genuchten (1980) model (Figure 1).

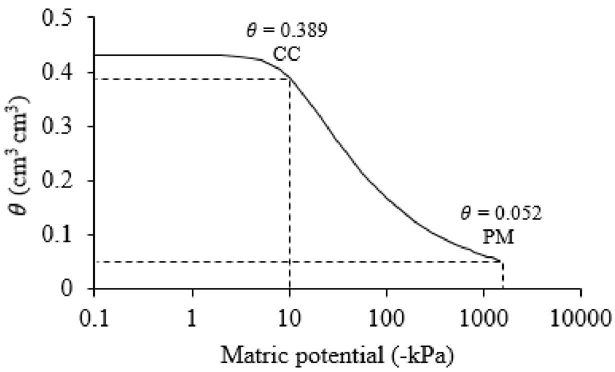

Figure 1. Soil water retention curve fitted according to the Van Genuchten model.

Before sowing, soil moisture was maintained at a level equivalent to that of the maximum retention water capacity in all experimental units and was determined using the capillary saturation method followed by drainage. Five seeds were sown at a depth of 1.5 cm and distributed equidistantly on the surface of the pot. Ten days after emergence (DAE), thinning was performed, leaving two plants per pot, which were the plants with the best vigor.

Calcium pyruvate, composed of 25% calcium and 75% pyruvate, was purchased from American Pharm Supplements LLC.

The pyruvate solution for pre-soaking seeds was prepared by dissolving calcium pyruvate in distilled water at 30°C. Then, the seeds were immersed in 50 mL of the solution for 12h and placed in a box protected using aluminum foil to maintain the solution at the desired temperature. For foliar use in the phenological phases (VE, FL, and VE/FL), pyruvate solution was prepared with distilled water at room temperature minutes before application. With the aid of a sprayer, 50 mL of the solution was applied to each plant, starting at 5:00 pm. The plants were protected using plastic during spraying, and the soil was covered with a blanket to avoid solution drift.

Irrigation was conducted daily at 5:00 pm and applied to each pot, with the volume of water corresponding to the demand of the plant submitted to treatment. The volume applied in each irrigation event was estimated using water balance based on Equation 1. A leaching fraction of 10% was used weekly to remove excess salt from the substrate.

$$VI = Vp - Vd \quad (1)$$

where:
VI: volume of water to be applied in the irrigation event (mL);
Vp: volume of water applied to the plants the previous day (mL);
Vd: drained volume, quantified the next morning (mL).

In the vegetative phase, water restriction started when the plants had three definitive leaves, lasting for ten consecutive days, and the last five days with the application of calcium pyruvate. In the flowering phase, water restriction occurred at the opening of the first flower and was subjected to seven days of water suppression, with the application of calcium pyruvate in the last three days. The cumulative stress (vegetative and flowering) consisted of the same number of days in each isolated phase.

At the end of the water deficit in each phase, soil samples were taken with the aid of a mini-auger, and the soil moisture content was determined using the standard greenhouse method. The samples were collected at 25 and 50 DAE. They were then placed in aluminum cans and weighed to obtain the wet mass. Next, they were dried in an oven at 105°C for 72h to obtain the dry mass, from which the soil moisture content was determined in relation to the soil matric potential (Table 2).

Table 2. Soil moisture and matric potential for treatments in the phase vegetative, bloom, and vegetative plus bloom. Campina Grande, Paraíba State, Brazil, 2019.

Irrigation management (MI)	Supplementation whit pyruvate			
	Seed and foliar		Foliar	
	Soil moisture ($cm^3 cm^3$)	Matric potential (-kPa)	Soil moisture ($cm^3 cm^3$)	Matric potential (-kPa)
VE (25 DAE)	0.054	1330	0.064	930
FL (50 DAE)	0.050	1620	0.050	1620
VE/FL (50 DAE)	0.050	1620	0.051	1550
	Control			
	Soil moisture ($cm^3 cm^3$)		Matric potential (-kPa)	
25 DAE	0.263		34.0	
50 DAE	0.259		35.0	

VE-water deficiency in the vegetative phase; FL-water deficiency in the bloom phase and VE/FL-water deficiency in the vegetative plus bloom phase.

At the end of the collection of soil samples, in all treatments, soil moisture was restored to a level equivalent to that of the maximum retention water capacity. Figure 2 shows photographs of the cotton plants under the conditions of irrigation management and exogenous supplementation with calcium pyruvate in the phenological phases.

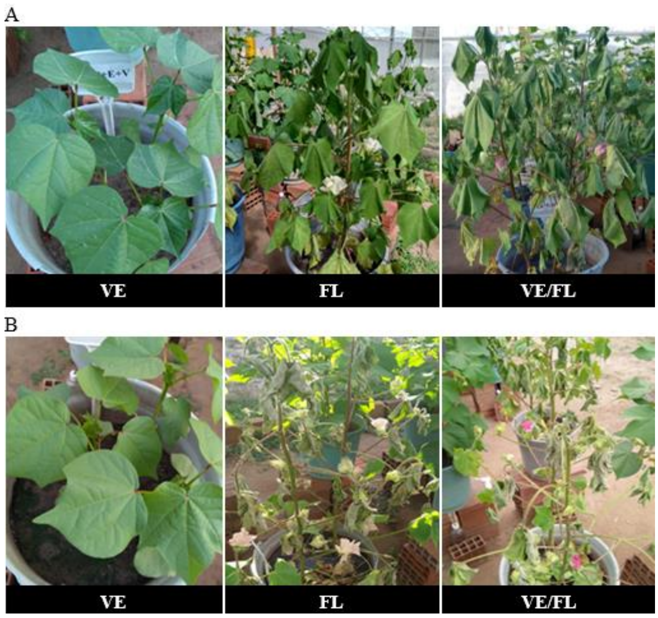

Figure 2. "BRS Jade" colored cotton plants under water stress in the phases vegetative (VE), flowering (FL), and vegetative plus flowering (VE/FL), with pyruvate supplementation via seed and foliar (A) and only via foliar (B). Campina Grande, Paraíba State, Brazil, 2019.

Fertilization with nitrogen, phosphorus, and potassium was performed according to the recommendations of Novais, Neves, and Barros (1991), with the equivalent of 100 mg N, 300 mg P_2O_5, and 150 mg K_2O per dm^3 of soil being applied to each pot, using urea, monoammonium phosphate, and potassium chloride, respectively. Fertilization with micronutrients was not carried out, as the Ca and Mg contents in the soil were at the average fertility levels.

Physiological parameters were evaluated at 50 DAE, when the plants were in full bloom, between 7:00 and 10:00 am. Stomatal conductance (*gs*) (mol m^{-2} s^{-1}), transpiration (*E*) (mmol of H_2O m^{-2} s^{-1}), internal CO_2 concentration (*Ci*) (µmol mol^{-1}), and CO_2 assimilation rate (*A*) (µmol m^{-2} s^{-1}) were evaluated using a portable infrared gas exchange device (Infra-Red Gas Analyzer-IRGA, from ADC BioScientific Ltd, model LC-Pro). Air temperature and CO_2 concentration assessments were performed under ambient conditions, and the luminosity was adjusted to 1200 µmol m^{-2} s^{-1} of radiation using an artificial source that came with the equipment.

In the morning, at the end of each phase of water stress (25 and 50 DAE), the third leaf from the apex to the base of the plant, completely expanded, of each treatment was collected. They were then wrapped in aluminum foil, protected in a thermal box with ice, and stored in a freezer at -80°C to determine the activity of the enzymes of the antioxidant complex.

For protein extraction, the procedures described by Pereira et al. (2015) were quantified using the Bradford (1976) method. The activity of antioxidative enzymes was estimated according to the methodologies described by Bulbovas, Rinaldi, Delitti, and Domingos (2005) for superoxide dismutase (SOD), Azevedo, Alas, Smith, and Lea (1998) for catalase (CAT), and Nakano and Asada (1981) for ascorbate peroxidase (APX). The boll harvest was started when 90% of the bolls were open. The number of bolls (NB), boll mass (MB), and cottonseed mass (MC) per plant were evaluated using a precision scale. Water productivity (WP) was calculated as the ratio of cotton seed mass (g) to the total volume of water applied during the crop cycle (L), as adapted from Geerts and Raes (2009).

Before the analysis of variance, the data were subjected to a normality test (Shapiro-Wilk). In some variables (Table 3) it was necessary to transform the data and the two models 'log x and √x' were chosen when they fully met the normality condition. With the positive results obtained in these tests, analysis of variance and 'F' test were performed. Then, the Tukey mean test was applied ($p \leq 0.05$) using Sisvar software (Ferreira, 2019).

Results and discussion

The studied factors influenced the gas exchange parameters, antioxidant enzymes, and production variables (Table 3). The effect was interactive, which means that the effect of pyruvate depended on the condition of irrigation management (MI) in most analyses, except for the variable water productivity (WP), in which the effect of pyruvate and management of irrigation were isolated. In cases of significant interactions, we discuss what happened in plants without addressing the isolated effect.

Interaction data are shown in Table 4. When comparing the forms of pyruvate (SP) supplementation under irrigation management (MI) conditions at 50 DAE, the highest means of *gs*, *E*, and *A* were recorded in plants that received pyruvate (SP1- seed and foliar and SP2- only via foliar) and were under water restriction in the vegetative phase (VE) compared to the other phenological phases studied. Comparing the factorial and additional treatments revealed that the imposition of cotton plants to water restriction reduced stomatal conductance (*gs*), transpiration (*E*), and net photosynthesis (*A*) by 44, 56, and 27%, respectively, and increased internal carbon (*Ci*) damming by 99.3%, regardless of the phenological phase.

The reduction in carbon stored in the cell observed in the VE water management condition indicates a continuous flow in the metabolization of the carbon entering the cell. The highest photosynthetic rate occurred owing to an increase in stomatal conductance, transpiration, and a reduction in stored carbon.

In addition, we found that plants under FL and VE/FL conditions (Table 4) were not efficient in metabolizing internal carbon. Consequently, they were stored in the cell, reflecting a low photosynthetic rate compared to the VE condition. The decrease in photosynthesis, under irrigation management conditions FL and VE/FL, resulted from the reduction of stomatal conductance (*gs*), with consequences on the loss of water in transpiration (*E*), proves that stomatal factors act on the gas exchange of cotton plants when subjected to water stress. This is because physiological parameters are dependent on stomatal opening and the availability of water in the soil (Yi et al., 2016; Wang et al., 2018).

Stomatal closure occurs to restrict water loss by transpiration and is considered an adaptive strategy for plants. Open stomata allow the absorption and exit of carbon dioxide, and when closed, they save water and reduce the risk of dehydration. Luo, Zhang, and Zhang (2016) and Loka and Oosterhuis (2014), when evaluating the effects of water stress on cotton plants, also observed that photosynthesis was significantly reduced as a function of water stress.

Table 3. Summary of analysis of variance for stomatal conductance (*gs*), transpiration (E), internal carbon concentration (*Ci*), CO_2 assimilation rate (*A*), superoxide dismutase (SOD) activity, catalase (CAT), ascorbate peroxidase (APX), boll number (NB), boll mass per plant (MB), seed cotton mass (MC), and water productivity (WP) of colored cotton plants cv. BRS Jade supplemented with pyruvate under different irrigation management (MI) conditions. Campina Grande, Paraíba State, Brazil, 2019.

Sources of variation	DF	Mean square			
		Gas exchange (50 DAE)			
		gs	*E*	*Ci*	*A*
Blocks	2	0.0001 ns	0.001 ns	1638.9 ns	0.054 ns
Pyruvate Supplement (SP)	1	0.0005 **	0.001 ns	13014.2 **	2.501 *
Irrigation management (MI)	2	0.0312 **	7.861 **	108251.2 **	340.651 **
SP x MI	2	0.0005 **	0.006 *	11534.1 **	2.680 **
Factorial x additional	1	0.0044 **	3.985 **	58372.5 **	7.281 **
Treatments	6	0.0113 **	3.287 **	51826.2 **	116.074 **
Residue	12	0.00004	0.002	1387.3	0.305
CV (%)	-	11.04	3.51	13.26	11.65
		Antioxidant enzymes (25 and 50 DAE)			
		SOD	CAT	APX[1]	
Blocks	2	0.010 ns	0.0001 ns	0.003 ns	
Pyruvate Supplement (SP)	1	1.673 **	0.0001 ns	0.132 **	
Irrigation management (MI)	2	17.430 **	0.0510 **	0.002 ns	
SP x MI	2	4.196 **	0.0020 **	0.148 **	
Factorial x additional	1	5.436 **	0.000 ns	0.457 **	
Treatments	6	8.394 **	0.0177 **	0.148 **	
Residue	12	0.007	0.0002	0.002	
CV (%)	-	4.74	12.55	1.37	
		Production components and water productivity (120 DAE)			
		NB[2]	MB[2]	MC	WP[2]
Blocks	2	0.359 *	2.147 ns	285.31 *	0.037 *
Pyruvate Supplement (SP)	1	0.259 ns	2.363 *	219.08 ns	0.236 **
Irrigation management (MI)	2	3.893 **	53.13 **	3109.22 **	0.521 **
SP x MI	2	0.260 *	3.366 *	241.06 *	0.018 ns
Factorial x additional	1	1.266 **	19.872 **	563.01 **	0.041 *
Treatments	6	1.639 **	13.949 **	1247.11 **	0.226 **
Residue	12	0.058	0.702	59.34	0.007
CV (%)	-	9.10	12.47	22.91	12.57

CV: coefficient of variation; DF: degrees of freedom; **, *, and ns significant at $p \leq 0.01$, $p \leq 0.05$, not significant, respectively, by the F test. [1]Transformed data and log of × [2] transformed data at √x

Table 4. Mean values of the interaction between pyruvate whit supplement (SP) and irrigation management (MI) for stomatal conductance (*gs*), transpiration (*E*), internal carbon concentration (*Ci*), and CO_2 assimilation rate (*A*) of cotton plants at 50 days after the emergency. Campina Grande, Paraíba State, Brazil, 2019.

Irrigation management (MI)	*gs*		*E*		*Ci*		*A*	
	mol m^{-2} s^{-1}		mmol de H_2O m^{-2} s^{-1}		µmol mol^{-1}		µmol m^{-2} s^{-1}	
	Pyruvate supplementation forms							
	SP_1	SP_2	SP_1	SP_2	SP_1	SP_2	SP_1	SP_2
VE	0.12 bA	0.15 aA	2.28 aA	2.26 aA	124.20 aB	171.60 aB	12.06 bA	14.35 aA
FL	0.01 aB	0.01 aB	0.27 aC	0.32 aB	447.33 aA	338.30 bA	0.33 aB	0.23 aB
VE/FL	0.01 aB	0.01 aB	0.36 aB	0.27 bB	416.00 aA	316.33 bA	0.01 aB	0.06 aB
LSD 1	0.01		0.07		66.23		0.933	
LSD 2	0.01		0.09		81.07		1.203	
	Factorial x additional							
Factorial	0.05 b		0.96 b		302.33 a		4.507 b	
Additional	0.09 a		2.21 a		151.67 b		6.19 a	

LSD, least significant difference; SP1, seed and foliar; SP2, via foliar; VE, FL, and VE/FL, water restriction in the vegetative, flowering, and vegetative plus flowering phases, respectively. LSD 1: for MI condition within the form of pyruvate supplementation (SP); LSD 2: for forms of pyruvate supplementation under MI conditions. For each gas exchange parameter, equal lowercase letters between forms of pyruvate supplementation in each MI condition and equal capital letters between MI conditions in the same pyruvate condition did not differ from each other ($p \leq 0.05$).

Plants under irrigation management of FL and VE/FL conditions do not recover their photosynthetic capacity, which could be due to damage to the photosynthetic apparatus. Ennahli and Earl (2005), in an experiment with cotton, by gradually lowering soil moisture from 75 to 5% in eight days, and then raising the soil moisture to 75% in two days, identified losses in photosynthetic II efficiency, attributed to the loss of photosynthetic function of chloroplasts. Working with water stress in cotton Luo et al. (2016) state that the ability of plants to recover photosynthetic activity depends on the degree of severity of stress.

In plants that received pyruvate supplementation via foliar (SP2), when water stress occurred in the vegetative phase (VE), there was a reduction in the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX). Pyruvate applied to the seed and foliar treatments (SP1) did not affect enzyme activity (Table 5).

In the FL irrigation management conditions, there was no significant difference between the forms of supplementation for SOD and APX, whereas, for CAT, the lowest value was observed in SP2. For VE/FL, SOD and CAT activities were reduced when plants were supplemented with pyruvate via seeds and foliar (SP1). For APX activity, there was no difference in APX activity between the forms of supplementation (Table 5).

The increase in SOD and APX activity due to the application of factorial treatments about the additional one was 1.45 U SOD min.$^{-1}$ mg^{-1} protein and 0.42 µmol ASC min.$^{-1}$ mg^{-1} protein for APX (from the transformed data), corresponding to 941.8 µmol ASC min.$^{-1}$ mg^{-1} protein in real values. CAT activity did not differ between treatments, suggesting that antioxidant enzymes were mobilized to protect plant tissues from oxidative damage under water deficit conditions (Table 5).

Table 5. Mean interaction splitting values for superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activity in colored cotton plants at 25 and 50 DAE. Campina Grande, PB, 2019.

Irrigation management (MI)	SOD		CAT		APX[1]	
	U SOD min.$^{-1}$ mg^{-1} protein		µmol H_2O_2 min.$^{-1}$ mg^{-1} protein		µmol ASC min.$^{-1}$ mg^{-1} protein	
	Pyruvate supplementation forms					
	SP_1	SP_2	SP_1	SP_2	SP_1	SP_2
VE	1.341 aB	0.766 bB	0.003 aC	0.001 aB	3.382 aA	2.854 bB
FL	0.893 aC	0.775 aB	0.182 aA	0.154 bA	3.093 aB	3.167 aA
VE/FL	2.628 bA	5.151 aA	0.132 bB	0.177 aA	3.180 aB	3.118 aA
LSD 1	0.144		0.024		0.074	
LSD 2	0.177		0.029		0.091	
	Factorial x additional					
Factorial	1.925 a		0.108 a		3.132 a	
Additional	0.471 b		0.109 a		2.711 b	

LSD, least significant difference; SP1, seed and foliar; SP2, via foliar; VE, FL, and VE/FL, water restriction in the vegetative, flowering, and vegetative plus flowering phases, respectively. LSD 1: for MI condition within the form of pyruvate supplementation (SP); LSD 2: for forms of pyruvate supplementation under MI conditions. For SOD, CAT, and APX, equal lowercase letters between forms of pyruvate supplementation in each MI condition and equal capital letters between MI conditions in the same pyruvate condition did not differ from each other ($p \leq 0.05$). Data transformed at Log *x*.

In general, one can understand the data in which the beneficial effect of pyruvate is evident as a result of the supplementation of an energy factor, pyruvic acid, which in the Krebs cycle will be transformed into ATP. In cellular metabolism, pyruvate originates from the oxidation of glucose molecules in the phenomenon called glycolysis, in which two molecules of pyruvate are formed (Shen et al., 2017; Taiz et al., 2017; Barbosa et al., 2021). With supplementation, a fact considered in the hypothesis of this work, pyruvate molecules are available to the cells for the production of ATP, and the energy that would be spent on glycolysis is available for other mechanisms of plant adaptation to water stress.

To alleviate water loss by regulating transpiration in response to water deficit, stomatal closure of plants occurred under FL and VE/FL conditions, limiting the availability of CO_2 in chloroplasts. According to Yi et al. (2016), this limitation causes an imbalance between photochemical activity and the need for electrons for photosynthesis, leading plants to be exposed to excess energy and reducing the electron chain. Therefore, the production of reactive oxygen species occurs in cells, impairing plant metabolism (Foyer, 2018).

To avoid or reduce the oxidative damage caused by water deficiency, plants have a complex antioxidant defense system capable of neutralizing the toxicity of reactive oxygen species of plant cells (ROS) (Khan et al., 2018). Plants eliminate these reactive substances through the antioxidant action of enzymes (SOD, CAT, APX were analyzed in this work), involved in the cellular detoxification mechanism.

Yi et al. (2016), when studying the rapid recovery of photosynthetic rate after soil water deficit, followed by rehydration in cotton plants, found that water deficit significantly increased SOD and APX activity and reduced CAT activity in plants with mild and moderate water deficits.

Barbosa et al. (2021), when evaluating the mitigating effect of pyruvate (100 and 50,000 µM) on water stress in peanuts, which also have C3 metabolism, identified that the application of exogenous pyruvate at 50,000 µM contributed to restoring the action of antioxidant enzymes in BR 1 (drought tolerant), based on SOD (45%), CAT (129%), and APX (60%) in stressed plants.

Although pyruvate still has little known function, when applied exogenously in the plant, the increase in superoxide dismutase activity in cotton found in this study may be related to the reduction of oxidative stress generated by water limitation, as it becomes critical for the plants. In studies related to water deficit, with exogenous application of other substances in bean *Vigna unguiculata*, Dutra et al. (2017) applied doses of salicylic acid and identified an increase in SOD activity. In a study by Meward, Desoky, and Rady (2018), positive changes were found in biochemical and physiological aspects using the application of the MET amino acid.

Table 6 shows that, regardless of the form of pyruvate supplementation, plants that underwent water restriction only in the vegetative phase (VE) produced more bolls (NB), cottonseed mass (MB), and boll mass (MC) in relation to FL and VE/FL irrigation management conditions.

With the irrigation management condition in the flowering phase (FL), there was a reduction of 25 and 44% for NB, 25 and 53% for MB, and 31 and 74% for MC. and When submitted to the MI condition in the vegetative and flowering phase (VE/FL), such reductions were 32 and 41% in NB, 43 and 49% for MB, and 60-73% in MC, respectively, when the plants received pyruvate, SP1, and SP2 when compared with additional treatment (full irrigation without pyruvate supplementation) (Table 6). Therefore, it was observed that the smallest reductions in cotton yield components under FL and VE/FL irrigation management conditions occurred when plants were supplemented with pyruvate via seed and foliar (SP1).

Water productivity was significant in isolation of the water conditions and forms of pyruvate supplementation. Plants under the VE management condition had higher WP (0.988 g L^{-1}) in relation to the FL condition (0.312 g L^{-1}) and VE/FL (0.206 g L^{-1}), with increases of 216.6 and 379.6%, respectively. Supplementation with pyruvate via seed and foliar was 0.824 g L^{-1}, and supplementation via foliar supplementation was 0.590 g L^{-1}. The increase in WP for SP1 compared to SP2 was 94% (using data without transformation).

Regarding the factorial x additional treatment (Table 6), it was verified that the application of water restriction in the phenological phases of the crop was responsible for the reduction in the number of bolls (4.1), boll mass (35.1 g), cotton mass in seed (14.8 g), and water productivity (0.19 g L^{-1}) in relation to the additional treatment. These reductions were obtained through the real averages of the treatments, being NB: 6.55 and 10.65 g; MB: 40.70 and 75.86 g; MC: 31.51 and 46.31; WP: 0.43 and 0.62 g L^{-1}, respectively for factorial and additional treatment.

Table 6. Mean values of the interaction for a number of bolls per plant (NB), cottonseed mass (MB), boll mass (MC), and mean data of the isolated factors for water productivity (WP) of colored cotton plants, at 120 DAE. Campina Grande, Paraíba State, Brazil, 2019.

Irrigation management (MI)	NB^2		MB^2		MC	
	Pyruvate supplementation forms					
	SP_1	SP_2	SP_1	SP_2	SP_1	SP_2
VE	3.39 aA	3.59 aA	9.02 aA	9.46 aA	54.87 aA	60.87 aA
FL	2.44 aB	1.82 bB	6.51 aB	4.01 bB	31.67 aB	11.68 bB
VE/FL	2.21 aB	1.91 aB	4.91 aB	4.38 aB	18.47 aB	12.18 aB
LSD 1	0.431		1.489		13.698	
LSD 2	0.527		1.823		16.767	
	Factorial x additional					
Factorial	2.56 b		6.38 b		31.51 b	
Additional	3.26 a		8.71 a		46.31 a	

Irrigation management (IM)	WP^2
VE	0.994 a
FL	0.558 b
VE/FL	0.453 b
LSD	0.311
Supplementation forms	
Seed and foliar	0.824 a
Foliar	0.590 b
LSD	0.097
Factorial x additional	
Factorial	0.657 b
Additional	0.784 a

LSD, least significant difference; SP1, seed and foliar; SP2, via foliar; VE, FL, and VE/FL, water restriction in the vegetative, flowering, and vegetative plus flowering phases, respectively. LSD 1: for MI condition within the form of pyruvate supplementation (SP); LSD 2: for forms of pyruvate supplementation under MI conditions. For production components, lowercase letters between forms of pyruvate supplementation in each MI condition and capital letters between MI conditions in the same pyruvate condition did not differ from each other ($p \leq 0.05$). For WP, the same lowercase letters do not differ within the MI and supplementation forms. ^{2} Data transformed at $\sqrt{x}$.

Therefore, it can be seen that water suppression in the vegetative phase, together with pyruvate supplementation, is not harmful to the cotton plant. However, the imposition of water suppression in the FL and VE/FL phases affected cotton production. As observed in this study, under FL and VE/FL irrigation management conditions, soil moisture reached an average matric potential of -1600 kPa (see Table 2).

These results show that the BRS Jade cotton plants were able to extract water at a more negative potential than the wilting point. It should be noted that plants when reaching the soil water content corresponding to the wilting point, spend more energy preserving water in their tissues (Carmo-Silva et al., 2012).

Under such conditions, flower bud drop, flower abortion, and/or apple drop occur as survival mechanisms. According to Yeates (2014), water deficit reduces the carbohydrate supply due to the lower photosynthetic rate, thus causing abortion because the need for carbohydrate accumulation is reached faster than in a non-stressed plant. Such information corroborates the results obtained in this study, in which the reduction in the photosynthetic rate affected fruit development.

The effect of water deficit on different phenological phases of cotton has been reported by several authors, such as Almeida et al. (2017), who found reductions in the number of bolls and productivity. Zonta et al. (2017) and Maniçoba et al. (2021) observed reductions in yield when water deficit was applied during the opening of the first flower and the peak of flowering due to the high demand at this stage of crop development.

In areas where water is a limiting factor, such as in semi-arid regions, maximizing water productivity is more economically profitable for the producer than maximizing crop productivity (Geerts & Raes, 2009). Therefore, the producer can use plants grown under VE irrigation management conditions since the water productivity was approximately 1.0 g L^{-1}. This better WP was presumably due to stomatal closure as a defense mechanism because, with the reduction in stomatal conductance, there is a reduction in transpiration, which possibly contributed to the increase in water productivity.

Conclusion

Water restriction in the vegetative phase does not cause losses in BRS Jade cotton when supplemented with pyruvate. However, in the flowering and vegetative plus flowering phases, it reduces gas exchange and production components and increases the activity of antioxidant enzymes compared to plants under full irrigation. Supplementation with pyruvate via seed plus foliar was better for BRS Jade cotton under water restriction.

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