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Gas exchange, growth, and production of cotton genotypes under water deficit in phenological stages

Trocas gasosas, crescimento e produção de genótipos de algodoeiro sob déficit hídrico nas fases fenológicas

Lauriane A. dos A. Soares[1]*, Charles M. Felix[1], Geovani S. de Lima[2], Hans R. Gheyi[2], Luderlandio de A. Silva[2], Pedro D. Fernandes[2]

[1]Academic Unit of Agricultural Sciences, Center of Agrifood Science and Technology, Universidade Federal de Campina Grande, Pombal, PB, Brazil.[2]Post Graduate Program in Agricultural Engineering, Universidade Federal de Campina Grande, Campina Grande, PB, Brazil.

ABSTRACT - Cotton cultivation in the Brazilian Northeast region faces water scarcity problems caused by the irregularity of the rainy season, leading to losses in yield. In this context, the objective of the present study was to evaluate the gas exchange, growth, and production of colored-fiber cotton genotypes under water stress, varying the water deficit management strategies in the different phenological stages of the plant. The study was carried out in the experimental area of the Federal University of Campina Grande, located in the municipality of Pombal, Paraíba, Brazil. A randomized block design was used, in a 3 × 7 factorial scheme, corresponding to three colored cotton genotypes (‘BRS Rubi’, ‘BRS Jade’, and ‘BRS Safira’) and application of water deficit (40% of actual evapotranspiration - ETr) management strategies in seven phenological stages of the crop. The ‘BRS Jade’ genotype is the most suitable for cultivation under water deficit conditions with 40% of the actual evapotranspiration. Colored-fiber cotton cultivation under water deficit in the flowering stage caused a reduction in physiological variables and growth. Water deficit during the vegetative and yield formation stages promoted lower losses in the production of seed cotton and total seed weight of the genotypes ‘BRS Rubi’, ‘BRS Jade’, and ‘BRS Safira’.

Keywords: *Gossypium hirsutum* L.. Irrigation strategy. Physiology.

RESUMO - A cotonicultura da região Nordeste do Brasil enfrenta problemas de escassez hídrica ocasionada pela irregularidade do período chuvoso, proporcionando perdas no rendimento. Neste contexto, objetivou-se no presente estudo avaliar as trocas gasosas, o crescimento e a produção de genótipos de algodoeiro de fibra colorida sob déficit hídrico, variando as estratégias de manejo do déficit hídrico nas diferentes fases fenológicas das plantas. A pesquisa foi desenvolvida na área experimental da Universidade Federal de Campina Grande, localizado no município de Pombal, Paraíba. Foi utilizado o delineamento experimental em blocos ao acaso, em esquema fatorial 3 × 7, correspondendo a três genótipos de algodão colorido (‘BRS Rubi’, ‘BRS Jade’ e ‘BRS Safira’) e sete estratégias de manejo do déficit hídrico (40% da evapotranspiração real - ETr) em diferentes fases fenológicas. O genótipo ‘BRS Jade’ é o mais adequado para cultivo em condições de déficit hídrico com 40% da evapotranspiração real. O cultivo de algodoeiro de fibra colorida sob déficit hídrico na fase de floração ocasionou redução nas variáveis fisiológicas e no crescimento. O déficit hídrico durante a fase vegetativa e de formação da produção promoveu menores perdas na produção de algodão em caroço e massa de sementes totais dos genótipos ‘BRS Rubi’, ‘BRS Jade’ e ‘BRS Safira’ em relação às plantas cultivadas sem restrição hídrica.

Palavras-chave: *Gossypium hirsutum* L.. Estratégia de irrigação. Fisiologia.

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***Corresponding author:**
<lauriane.soares@pq.cnpq.br>

INTRODUCTION

Water scarcity is a global problem resulting in restrictions on agricultural production, a common characteristic in arid and semi-arid areas, causing reductions of more than 50% in crop yield worldwide (NDAMANI; WATANABE, 2015; BABOEV et al., 2017). Currently, cotton production areas are characterized by the occurrence of long periods of drought, causing considerable yield losses (CORDÃO SOBRINHO et al., 2015; LIMA et al., 2018).

Cotton cultivation in northeastern Brazil has stood out as one of the agricultural activities of great value for Brazilian agribusiness, with the production of 7,089,939 tons, accounting for 23.29% of the national production, of which the state of Paraíba produced a total of 3,596 tons of herbaceous cotton (IBGE, 2020). The areas are mostly cultivated under rainfed conditions, i.e., depending on rains that have irregular distribution, sometimes with prolonged periods of drought, and rains concentrated in a few months during the year, limiting crop development and production (NOBRE et al., 2012; SOARES et al., 2015).

In general, water deficit can affect the organs of developing plants, especially during the flowering and fruiting stages, which negatively affects the morphological characteristics of the plant and its production components (ZONTA et al., 2017; ARAÚJO et al., 2019; SOARES et al., 2020). Water deficit can inhibit cell and leaf expansion, stem elongation, and leaf area index (LIU et al., 2017). Leaf area and reduction in the production of new leaves are also associated with a decrease in leaf water potential and stomatal closure (QI; TORII, 2018).

Thus, maximizing water use efficiency at all scales is crucial in agricultural systems, especially during drought periods, when water for irrigation is scarce. Deficit irrigation is used to increase the water use efficiency of the crop, decreasing the water depth or the number and frequency of irrigation events (SAMPATHKUMAR et al., 2013). However, the efficiency of deficit irrigation depends mainly on the phenology of the crop and effects related to the moment, duration, physiological state of the crop, irrigation water quality, genotype, and stress severity (GARCÍA-TEJERO et al., 2012; CHAI et al., 2016).

In this context, genetic diversity and deficit irrigation can reduce water consumption in irrigation, increasing water use efficiency to ensure sustainable agricultural production under intermittent drought events (ABID et al., 2018; CORDÃO et al., 2018). Therefore, it is necessary to adopt appropriate agronomic strategies for agricultural production under water deficit in each developmental stage and recovery of plants in the phenological stages following water stress, as well as the consequences of the cumulative effect of water deficit in the phenological stages (ZONTA et al., 2015).

Thus, this study has the hypothesis that the identification of a cotton genotype tolerant to water deficit in a given stage through the effects on gas exchange, growth, and production will enable the increase of production capacity profitability. The effects of water deficit on plant physiological functions are dependent on genotype, stage, and period of exposure to stress. Therefore, the identification of the stage(s) in which the cotton crop is tolerant or sensitive is of paramount importance to ensure the sustainability of irrigated cultivation under semi-arid conditions and contribute to the generation of employment and income.

In this context, the objective of this study was to evaluate the gas exchange, growth, and production of colored cotton genotypes under water deficit in the different phenological stages.

MATERIAL AND METHODS

The experiment was carried out under field conditions at the Center of Science and Agri-Food Technology - CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraiba, Brazil, at the geographic coordinates 6º47'20" S latitude and 37º48'01" W longitude, at an altitude of 194 m. The meteorological data collected during the experimental period are presented in Figure 1.

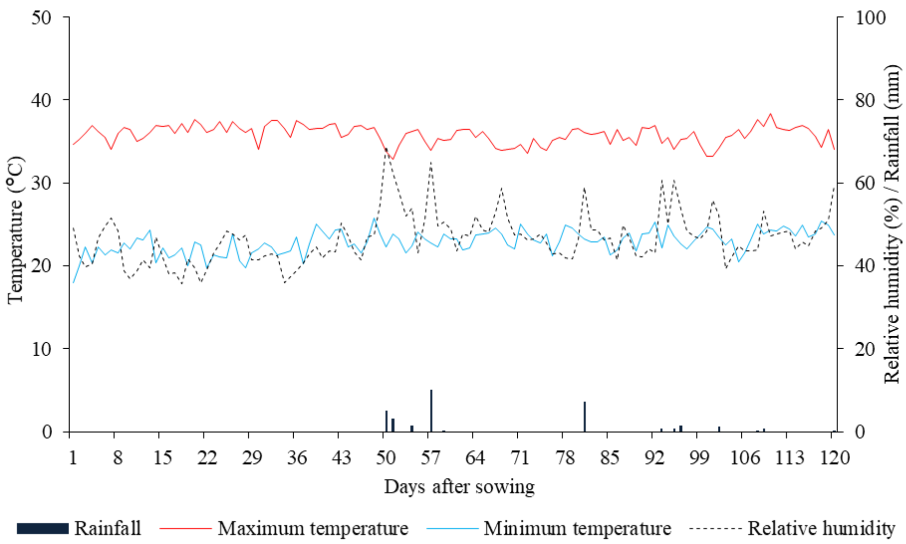


Figure 1. Climatic data of maximum and minimum temperature (ºC), rainfall (mm), and relative humidity of air (%) during the experimental period.

Three colored cotton genotypes (G) (G1 – 'BRS Rubi'; G2 – 'BRS Jade' and G3 – 'BRS Safira') and seven irrigation management strategies, under two water conditions, corresponding to irrigation with 100% of Actual Evapotranspiration - ETr (full irrigation) and irrigation with 40% of ETr (water deficit) were evaluated, as described in Table 1. The experimental design used was in randomized blocks, in a 3 × 7 factorial scheme, whose factors resulted in 21 treatments with three replicates and three plants per plot, totaling 189 experimental units. Irrigation strategies with water deficit can be characterized as alternative cultivation aiming at maximizing production and water use efficiency under conditions of water scarcity.

Table 1. Management strategies with water deficit in vegetative stage - A (20 - 60 days after sowing - DAS), flowering stage - B (61 - 75 DAS) and yield formation stage - C (76 - 120 DAS) of cotton genotypes.

Treatment (T)	Irrigation management strategies	Phenological stages		
		Vegetative (A)	Flowering (B)	Production (C)
		Actual evapotranspiration - ETr (%)		
T1	$A_1B_1C_1$	100	100	100
T2	$A_2B_1C_1$	40	100	100
T3	$A_1B_2C_1$	100	40	100
T4	$A_2B_2C_1$	40	40	100
T5	$A_1B_1C_2$	100	100	40
T6	$A_2B_1C_2$	40	100	40
T7	$A_1B_2C_2$	100	40	40

Index 1 (A_1, B_1, C_1): full irrigation (100% ETr); Index 2 (A_2, B_2, C_2): water deficit (40% ETr), in specified phenological stage(s).

The evaluated phenological stages of cotton were: vegetative - beginning with the appearance of the first pair of true leaves and end of stress with the opening of the 1st flower; flowering - from the appearance of the 1st flower extending up to the opening of the 1st boll; yield formation - from the opening of the 1st boll up to the final harvest of the bolls in each experimental unit.

The plants were grown in plastic containers (pots) with 20 L capacity (35 cm high × 31 cm upper diameter × 20 cm lower diameter), with a geotextile at the base to avoid loss of soil material and filled with a 3-cm layer of crushed stone number 0 and 24.5 kg of a *Neossolo Regolítico Eutrófico* (Psamment) of sandy loam texture (0-0.30 m depth) from an agricultural area in the municipality of Pombal-PB. Soil characteristics were determined according to Teixeira et al. (2017) before sowing and are described in Table 2. At the base of each pot, a transparent tube was connected to facilitate drainage, coupled to a 2.0 L container where the drainage water was collected to determine the water consumption of the plants through the difference between volume of water applied and drained.

Table 2. Physical-hydraulic and chemical attributes of the soil (0-0.30 m) used in the experiment.

Bulk density	Total porosity	Moisture (%)		Available water	Exchange Complex				pH_{sp}	EC_{se}
					Ca^{2+}	Mg^{2+}	Na^+	K^+		
kg dm^{-3}	%	0.33 atm[1]	15 atm[2]	%	---------------cmol$_c$ kg^{-1}--------------				-	dS m^{-1}
1.37	48.88	15.01	5.81	9.20	6.43	4.11	0.14	0.81	7.76	0.22

Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc; P - Mehlich-1 extractant; pH_{sp} - pH of saturated paste; and EC_{se} - electrical conductivity of saturation extract, index 1 and 2 correspond to soil moisture at field capacity (FC) and permanent wilting point (PWP).

Fertilization with N, P, and K was performed according to the fertilization recommendation of Novais, Neves, and Barros (1991), applying 100, 300, and 150 mg kg^{-1} of the soil of N, P_2O_5, and K_2O, respectively, using urea, monoammonium phosphate (MAP), and potassium chloride as sources, applied as top-dressing along with irrigation water, split into three portions and applied at 25, 45, and 75 DAS. The pots were arranged, under field conditions, in single rows at a spacing of 1 m between rows and 0.60 m between plants in the row. The supply of micronutrients started at 20 DAS, with weekly applications of a solution of 1.0 g L^{-1} of Dripsol micro® containing: N (15%), P_2O_5 (15%), K_2O (15%), Ca (1.4%), Mg (1.4%), S (2.7%), Zn (0.5%), B (0.05%), Fe (0.5%), Mn (0.05%), Cu (0.5%), and Mo (0.02%), applied to the leaves on adaxial and abaxial sides, using a backpack sprayer.

For the three cotton genotypes, sowing was performed using five seeds per pot at 2 cm depth and distributed equidistantly. After emergence, thinning was done, leaving the most vigorous plant. Soil moisture was maintained at the level corresponding to field capacity (FC) in all experimental units until the emergence of the first true leaf, when (after 20 days) the treatments began to be applied.

Irrigations were performed daily at 5 p.m., applying in each pot the volume of water corresponding to each treatment (40 or 100% ETr), determined by the water balance: volume applied minus the volume drained in the previous irrigation, plus a leaching fraction of 20% in plants irrigated with 100%

ETr, every seven days. The volume of water applied in each water deficit management strategy was determined based on the consumption of plants under 100% ETr, by the drainage lysimetry (BERNARDO et al., 2019). For the treatment with 40% ETr, the obtained value of ETr was multiplied by the percentage of evapotranspiration (0.40).

At 75 DAS, gas exchanges were determined based on the CO_2 assimilation rate - *A* (μmol CO_2 m^{-2} s^{-1}), stomatal conductance - *gs* (mol m^{-2} s^{-1}), intercellular CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}), transpiration - *E* (mmol H_2O m^{-2} s^{-1}), and these data were then used to calculate the instantaneous water use efficiency - *WUEi* (A/E) and instantaneous carboxylation efficiency - *CEi* (A/Ci). Gas exchange determinations were performed with an infrared gas analyzer - IRGA (Model LCpro - SD, from ADC BioScientific, UK). The measurements were performed between 7:00 and 10:00 a.m. on the third fully expanded and photosynthetically active leaf, counted from the apical bud, conducted under natural conditions of air temperature, CO_2 concentration, and using an artificial radiation source of 1,200 μmol m^{-2} s^{-1}, established through the curve of photosynthetic response to light (FERNANDES et al., 2021; ROQUE et al., 2022).

In the same period, the following growth variables were determined: number of leaves, plant height (PH), with a ruler graduated in millimeters, from the base of the plant to the apical meristem; stem diameter (SD), with a digital caliper 5 cm from the soil; and leaf area (LA), obtained by measuring the length of the leaves, using the methodology proposed by Grimes and Carter (1969), and calculated using Equation 1:

$$LA_{total} = \Sigma\ LA = \Sigma\ (0.4322x^{\ 2.3002}) \qquad (1)$$

Where:
LA - leaf area of each cotton leaf (cm^2);
x - leaf midrib length (cm); and
LA_{total} - total leaf area of the plant (cm^2)

In the evaluations performed at 75 DAS, only four irrigation management strategies were considered in the statistical analysis (T1- $A_1B_1C_1$, T2- $A_2B_1C_1$, T3- $A_1B_2C_1$, and T4- $A_2B_2C_1$), because in this evaluation, the deficit treatments (T5, T6, and T7) had not yet begun. At the end of the crop cycle, at 120 DAS, the plants were collected, separated into leaves, stems, and roots, placed in paper bags, and dried in a forced air circulation oven, maintained at 65 ºC until reaching constant weight. Subsequently, the material was weighed on a 0.001 g precision scale to obtain the biomass of the different parts, and the sum of leaves + stem resulted in shoot dry mass (SDM), thus allowing the calculation of root/shoot ratio (R/S). The production components were also quantified: seed cotton weight and total seed weight analyzed according to the methodology of Embrapa Cotton. The bolls were harvested per plant as they reached the harvest point, characterized by their opening along with drying of the bracts.

The data were subjected to the distribution normality test (Shapiro-Wilk test) and then evaluated by analysis of variance by the F test. In cases of significant effect, the Scott-Knott means comparison test was performed ($p \leq 0.05$) for water deficit management strategies, and the Tukey test ($p \leq 0.05$) was performed for the cotton genotypes (FERREIRA, 2019).

RESULTS AND DISCUSSION

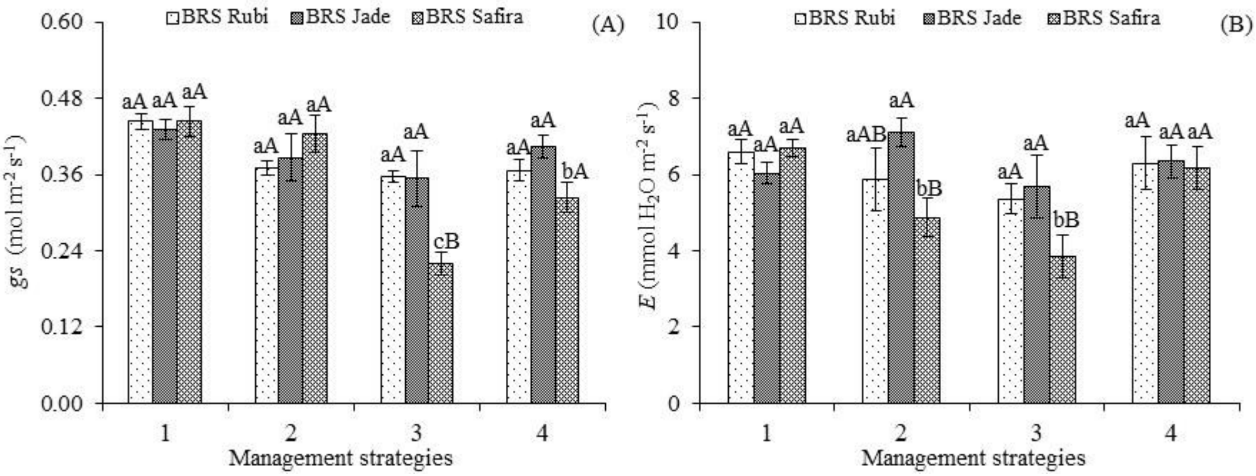
There were significant effects of the interaction between water deficit management strategies and genotypes on all physiological variables of cotton at 75 DAS (Table 3). All variables except CO_2 assimilation rate were affected by water deficit management strategies, and genotypes did not affect only transpiration and instantaneous carboxylation efficiency (Table 3).

Table 3. Summary of the analysis of variance for stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (*WUEi*), and instantaneous carboxylation efficiency (*CEi*) of the colored-fiber cotton genotypes (G), as a function of different water deficit management strategies (MS), at 75 days after sowing.

Sources of variation	DF	Mean squares					
		gs	*E*	*Ci*	*A*	*WUEi*	*CEi*
Management strategies (MS)	$3^{\#}$	0.026^{**}	3.889^{**}	5493.370^{**}	29.442^{ns}	9.592^{**}	0.203^{**}
Genotypes (G)	2	0.005^{ns}	2.565^{**}	990.527^{ns}	99.191^{ns}	1.881^{ns}	0.072^{*}
Interaction MS × G	6	0.006^{*}	1.501^{*}	2469.009^{**}	146.424^{**}	5.829^{**}	0.119^{**}
Block	2	0.003^{ns}	4.937^{ns}	97.444^{ns}	1.768^{ns}	5.673^{ns}	0.004^{ns}
Error	22	0.002	0.484	477.171^{ns}	41.249	1.513	0.021
CV (%)		13.46	11.78	19.35	16.90	18.44	35.48

^{ns}, *, **: not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively, by the F test. $^{\#}$Until the date of measurement (75 DAS), water deficit had been applied in only 4 out of the 7 management strategies.

The values of stomatal conductance of the genotypes ‘BRS Rubi’ and ‘BRS Jade’ were similar, as a function of water deficit strategies in the different phenological stages (Figure 2A). However, the stomatal conductance of the genotype ‘BRS Safira’ decreased significantly with a water deficit in the flowering stage (T3) and successively in the vegetative and flowering stages (T4) (Figure 2A). The stomatal conductance in the different phenological stages, regardless of genotypes, was classified as follows: T1 ($A_1B_1C_1$)>T2($A_2B_1C_1$) >T4($A_1B_2C_1$)>T3($A_2B_2C_1$). Compared to T1, the mean values of *gs* in the T2, T4, and T3 strategies decreased by 4.51, 27.06, and 50.37%, respectively. Differences between genotypes were observed in *gs* when the plants were subjected to water deficit in the vegetative/ flowering ($A_2B_2C_1$) and flowering ($A_1B_2C_1$) stages, with reductions in *gs* of 38.30 and 37.72% in 'BRS Safira', when compared to the genotypes 'BRS Rubi' and 'BRS Jade', respectively. In general, plants under low water availability in the soil close their stomata to reduce water loss and to maintain turgor in their tissues (HAWORTH et al., 2018; QI; TORII, 2018), being dependent on the degree of water saturation of stomatal cells, with possible inhibition in transpiration flow if water deficit is high in the plant (SUASSUNA et al., 2014). In addition, stomatal closure causes restriction of normal CO_2 flow towards the carboxylation site (LIMA et al., 2020).



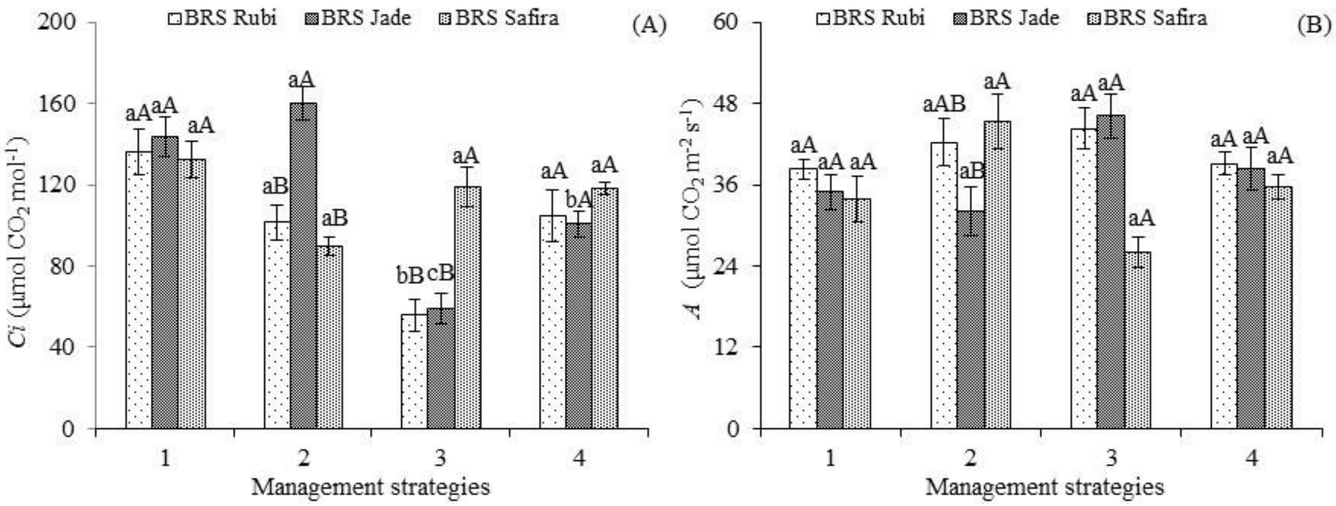
Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$) for the same genotype, and means followed by the same uppercase letter for the same strategy indicate that the genotypes do not differ by Tukey test, $p > 0.05$. Vertical bars represent the standard error of the mean (n = 3). Water deficit management strategies 1 - plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2 - water stress in the vegetative stage (20 - 60 DAS); 3 - water stress in the flowering stage (61 - 75 DAS); 4 - water stress in the vegetative and flowering stages (20 - 60 and 61 - 75 DAS).

Figure 2. Follow-up of the interaction between genotypes and water deficit management strategies for stomatal conductance - *gs* (A) and transpiration - *E* (B), of colored-fiber cotton genotypes, at 75 days after sowing.

Due to the partial closure of the stomata, there were reductions of 27.21 and 37.23% in the leaf transpiration of the genotype 'BRS Safira' for plants subjected to water deficit according to treatments T2 ($A_2B_1C_1$) and T3 ($A_1B_2C_1$), compared to plants under full irrigation (100% ETr - T1) (Figure 2B). Among the cotton genotypes, there was a significant difference between the water deficit management strategies only when plants were irrigated with 40% ETr in the vegetative and flowering stages (T2 and T3), with the highest leaf transpiration in the 'BRS Jade' genotype (Figure 2B). Stomatal closure reduced the water loss by the stomata in the genotype 'BRS Safira', which explains the lowest transpiration rate observed in the flowering stage under 40% ETr.

Stomatal closure is probably the most important factor in controlling carbon metabolism, but the relative role of stomatal limitation in photosynthesis depends on the intensity of water deficit. Under severe stress, photosynthesis can be more controlled by the capacity of chloroplast to fix CO_2 (such as RuBisCO activity) than by the increase in diffusive resistance (DEEBA et al., 2012). Lower water loss through the stomata allows survival under conditions of water scarcity. However, the mechanism of water saving through stomatal closure may compromise photosynthesis, limiting plant growth (NEGIN; MOSHELION, 2016; MUDO et al., 2020).

The internal CO_2 concentration (Figure 3A) varied, following a reverse trend observed for the other variables as a function of water deficit, i.e., in the genotypes 'BRS Rubi', and 'BRS Jade', in which stomatal conductance and transpiration increased, the values of *Ci* decreased, as a consequence of the CO_2 flow for the synthesis of organic compounds. The highest mean among genotypes was obtained in 'BRS Jade' under water deficit during the vegetative stage, with 160 μmol CO_2 mol^{-1} in the T2- $A_2B_1C_1$ strategy (Figure 3A). Although decreases in stomatal conductance simultaneously reduce CO_2 concentration, it is maintained or even increased in the substomatal chamber, indicating non-stomatal limitations to photosynthesis (MAFAKHERI et al., 2010).



Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, p > 0.05) for the same genotype, and means followed by the same uppercase letter for the same strategy indicate that the genotypes do not differ by Tukey test, p > 0.05. Bars represent the standard error of the mean (n = 3). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2- water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 - 60/ 61 - 75 DAS).

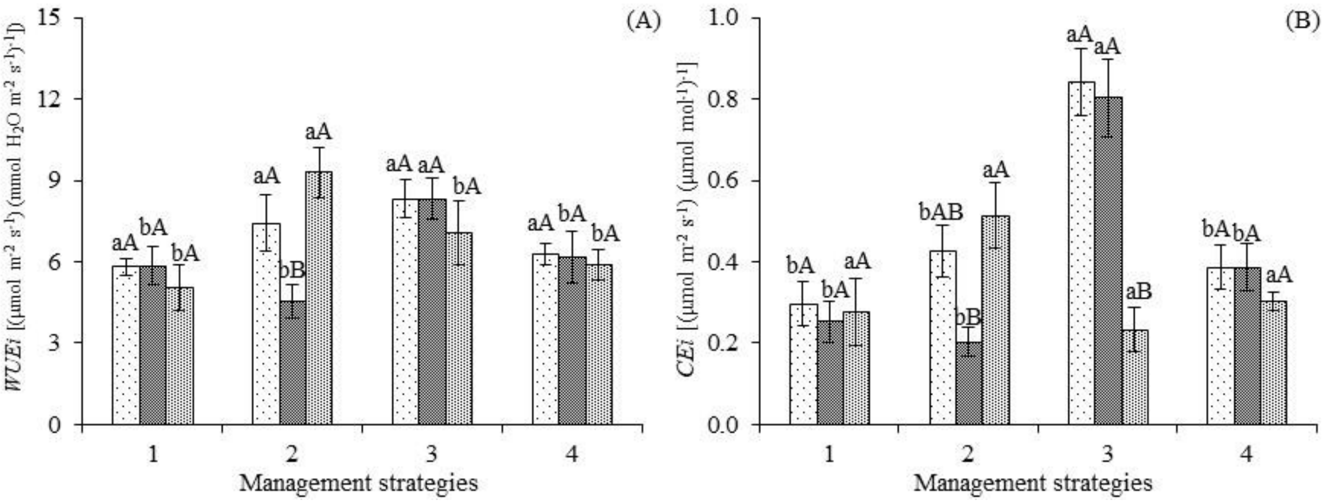
Figure 3. Follow-up of the interaction between genotypes and water deficit management strategies for internal CO_2 concentration - *Ci* (A) and CO_2 assimilation rate - *A* (B), of colored-fiber cotton genotypes, at 75 days after sowing.

The water deficit management strategies imposed on the CO_2 assimilation rate did not differ, with differences only among genotypes when water deficit was applied in the vegetative stage, T2 ($A_2B_1C_1$), where the lowest CO_2 assimilation rate was observed in the genotype 'BRS Jade' when irrigated with 40% ETr in the vegetative stage (Figure 3B). The reduction in the CO_2 assimilation rate verified in the genotype 'BRS Jade' under water deficit conditions may be related to the greater variation in the values of internal CO_2 concentration, compared to plants irrigated with 100% ETr. The decrease in *A* in cotton plants under water deficit conditions may be caused by the action of non-stomatal factors, such as the restriction of CO_2 diffusion in mesophyll cells and a decline in RuBisCO affinity with CO_2 (LUO, ZHANG; ZHANG, 2016), due to several coordinated events, such as stomatal closure and reduced activity of photosynthetic enzymes (DEEBA et al., 2012).

In the genotypes 'BRS Rubi' and 'BRS Safira' under water stress, the highest values of instantaneous water use efficiency, 8.32 and 9.29 [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$], were obtained when the water deficit was applied in the flowering stage, T3 ($A_1B_2C_1$), for 'BRS Rubi' and in the vegetative stage, T2 ($A_2B_1C_1$), for 'BRS Safira', resulting in the respective increments of 42.87 and 39.65% compared to plants irrigated with 100% ETr - T1 ($A_1B_1C_1$). However, among genotypes, *WUEi* was lower in 'BRS Jade', with an average of 4.55 [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] in the vegetative stage while in the genotypes 'BRS Rubi' and 'BRS Safira' higher means, 7.42 and 9.29 [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$], were observed in plants under water deficit in this same stage (Figures 4A). Similar results were reported by Sabir et al. (2020), in *Ziziphus jujuba,* observing a decrease in stomatal conductance caused an increase in the instantaneous water use efficiency under water stress. This stomatal response in plants subjected to water deficit may be related to the production of abscisic acid rather than water limitations, which may result in sustainable leaf water potential under water stress conditions (PAPACEK; CHRISTMANN; GRILL, 2019; YANG et al., 2019).

Plants of the genotypes 'BRS Rubi' and 'BRS Jade', when irrigated with 40% ETr in the flowering stage, according to the T3 treatment ($A_1B_2C_1$), showed increments in the instantaneous carboxylation efficiency of 183.20 and 217.13% compared to plants irrigated with 100% ETr (T1-$A_1B_1C_1$), with *CEi* of 0.84 and 0.80 [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$], respectively, surpassing the genotype 'BRS Safira', with 0.23 [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] under the T3 strategy ($A_1B_2C_1$) (Figures 4B). The highest *Ci* of the genotype 'BRS Safira' was due to the increase in CO_2 assimilation rate and reduction in the internal CO_2 concentration of the genotypes 'BRS Rubi' and 'BRS Jade' under water deficit in the flowering stage (Figure 3).

There was a significant effect of the interaction ($p \leq 0.01$) between the factors strategies of water deficit management and genotypes only on plant height (Table 4). Water deficit management strategies significantly influenced plant height, leaf area, and the number of leaves, while genotypes significantly affected leaf area and stem diameter ($p \leq 0.05$).



Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$) for the same genotype, and means followed by the same uppercase letter for the same strategy indicate that the genotypes do not differ by Tukey test, $p > 0.05$. Bars represent the standard error of the mean (n = 3). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2- water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 – 60/ 61 - 75 DAS).

Figure 4. Follow-up of the interaction between genotypes and water deficit management strategies for the instantaneous water use efficiency (*WUEi*) (A) and instantaneous carboxylation efficiency (*CEi*) (B), respectively, of colored-fiber cotton genotypes, at 75 days after sowing.

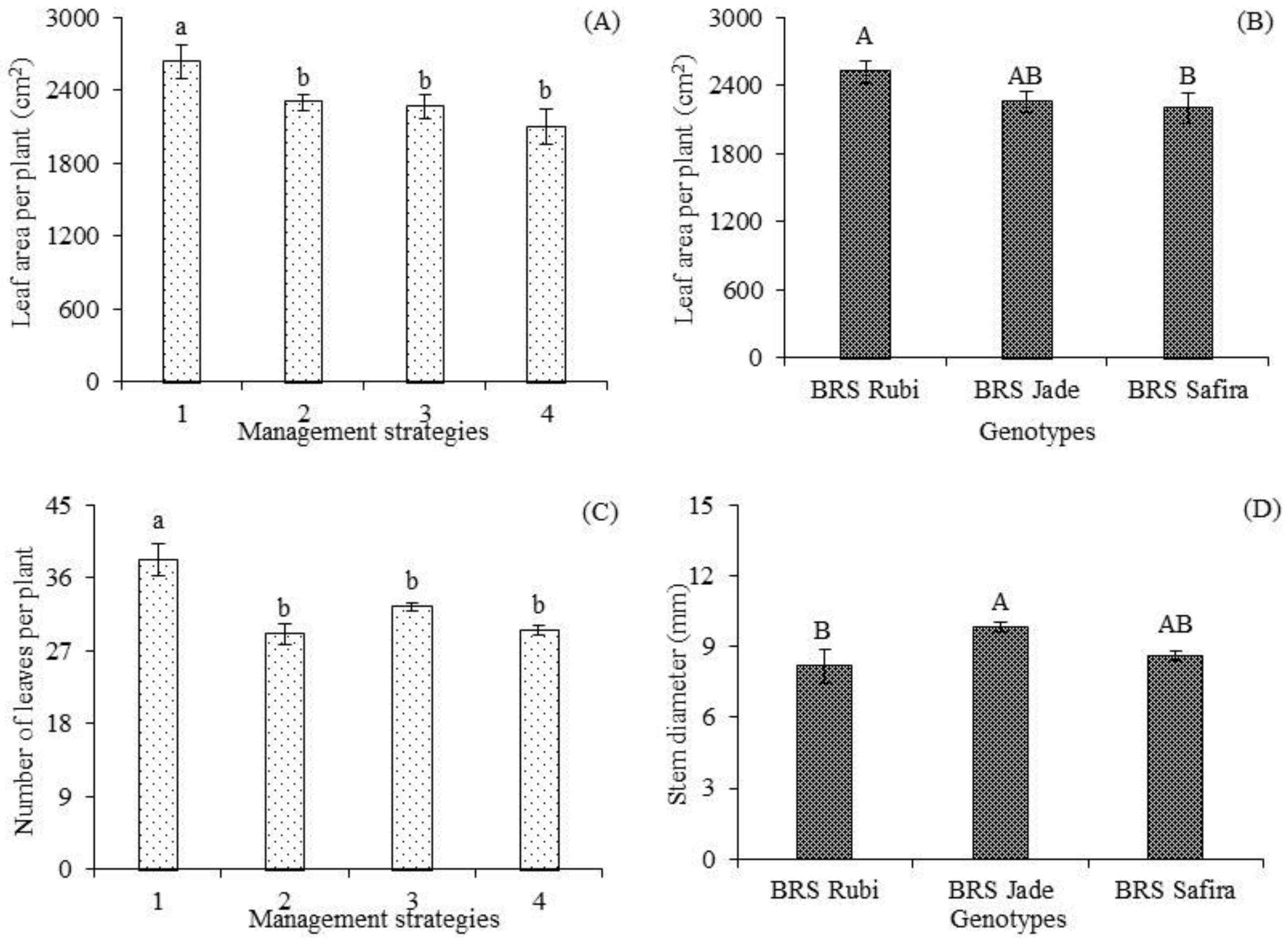
Table 4. Summary of the analysis of variance for leaf area (LA), number of leaves (NL), stem diameter (SD), and plant height (PH) of the colored-fiber cotton genotypes (G), as a function of different water deficit irrigation management strategies (MS), at 75 days after sowing.

Sources of variation	DF	Mean squares			
		LA	NL	SD	PH
Management strategies (MS)	3#	448392.619**	158.458**	2.645ns	282.577**
Genotypes (G)	2	345014.1308*	10.912ns	8.863*	27.779ns
Interaction MS × G	6	143923.865ns	22.299ns	1.974ns	49.149**
Block	2	405665.022ns	2.608ns	1.202ns	4.991ns
Error	22	79225.263	12.729	2.280ns	13.834
CV (%)		12.09	11.05	17.04	7.09

ns, **, *: not significant and significant at $p \leq 0.01$ and $p \leq 0.05$; respectively, by the 'F' test. # Up to the date of measurement (75 DAS) only 4 out of the 7 management strategies had been applied to water deficit.

At 75 DAS (final period of the flowering stage), the management strategies with a water deficit in the vegetative and flowering stage, either isolated or combined (T2-$A_2B_1C_1$, T3-$A_1B_2C_1$ and T4-$A_2B_2C_1$), resulted in reductions in LA of 12.58, 13.89, and 20.22%, respectively, in comparison to plants irrigated with 100% ETr (Figure 5A). It was observed that the reduction of LA is not mainly due to water deficit but also to the senescence and/or abscission of adult leaves (Figure 5C), verified by reductions of 23.95, 15.12, and 22.59% in the number of leaves in plants subjected to the T2, T3, and T4 strategies, respectively, compared to the management without water deficit. Reduction in LA can be considered an important strategy to protect and/or acclimate plants to water deficit, eventually reducing water losses through transpiration and maintaining a high water potential in cells (LIU et al., 2017).

The genotype 'BRS Rubi' obtained the highest mean leaf area (2521.75 cm^2), regardless of the water deficit management strategy, surpassing the genotype 'BRS Safira' by 12.50% (Figure 5B). The mean stem diameter of the genotype 'BRS Jade' was statistically higher than that of 'BRS Rubi', regardless of the strategies of water deficit management (Figure 5D). Thus, it can be observed that the genotypes 'BRS Rubi' and 'BRS Jade' showed higher growth in the leaf area; another important fact to point out in the leaf area is its direct relationship to the photosynthetic process through the interception of light energy. As the photosynthesis rate depends on leaf area, the faster the plant reaches a greater leaf area index and the longer it remains in photosynthetic activity, the higher the crop yield (SANQUETTA et al., 2014).



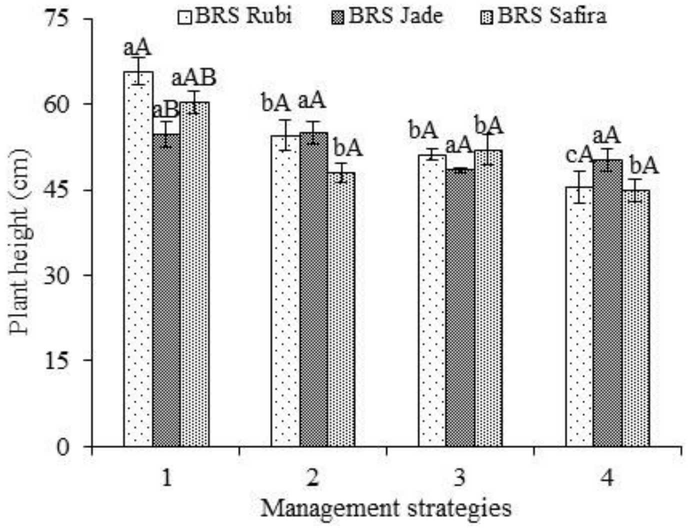
Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$), and means followed by the same uppercase letter indicate that the genotypes do not differ from each other by Tukey test, $p > 0.05$. Bars represent the standard error of the mean (n = 3 for strategies and n = 3 for genotypes). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2- water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 – 60/ 61 - 75 DAS).

Figure 5. Leaf area and number of leaves per plant as a function of water deficit management strategies (A and C) and leaf area per plant and stem diameter of colored-fiber cotton genotypes (B and D), respectively, at 75 days after sowing.

Among the cotton genotypes as a function of the different strategies of water deficit management at 75 DAS, compared to plants without stress, it is observed that water stress reduced the values of height by 17.08, 22.16, and 30.79% (BRS Rubi) and by 20.40, 13.66, and 25.39% (BRS Safira) in plants under the strategies $A_2B_1C_1$ (T2), $A_1B_2C_1$ (T3) and $A_2B_2C_1$ (T4), respectively (Figure 6). The maximum reduction in plant height induced by water deficit was observed in 'BRS Rubi', with a decrease of 20.22 cm when water stress was applied successively in the vegetative and flowering stages, although this genotype had the highest height under the 100% ETr condition (Figure 6). When plants of the genotype 'BRS Jade' were subjected to different strategies of water deficit management, these strategies did not cause a significant difference in plant height, which was already observed for the CO_2 assimilation rate, reinforcing the hypothesis of a resumption in plant growth after the application of water stress (Figure 6).

The reduction in growth under water deficit conditions may be associated with reductions in the physiological variables of plants, such as CO_2 assimilation rate, instantaneous water use efficiency and instantaneous carboxylation efficiency of cotton genotypes. In line with these results, previous studies have also reported a significant reduction in the growth and physiological traits of cotton related to water stress (SAHITO et al., 2015; BOZOROV et al., 2018).

The water deficit management strategies and colored-fiber cotton genotypes significantly influenced ($p \leq 0.01$) shoot dry mass, seed cotton weight, and total seed weight, in addition to root/shoot ratio was only influenced by the water deficit management strategies (Table 5). Regarding the interaction between the factors, there was no significant effect ($p > 0.05$) for the variables analyzed at 120 DAS (Table 5).



Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$) for the same genotype, and means followed by the same uppercase letter for the same strategy indicate that the genotypes do not differ by Tukey test, $p > 0.05$. Bars represent the standard error of the mean (n = 3). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2 - water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 – 60/ 61 - 75 DAS).

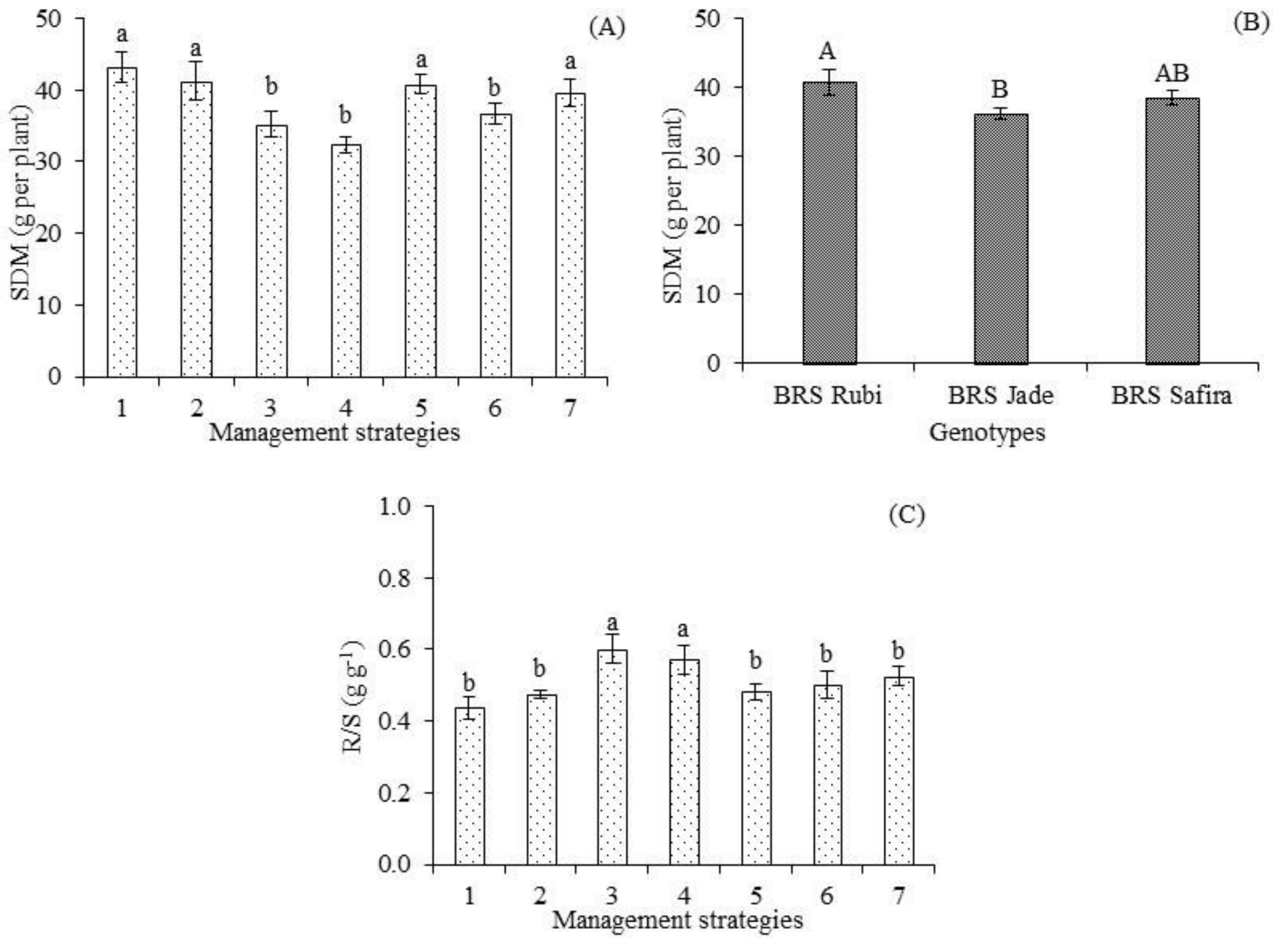
Figure 6. Follow-up of the interaction between genotypes and water deficit management strategies for the plant height of colored-fiber cotton genotypes, at 75 days after sowing.

Table 5. Summary of the analysis of variance for shoot dry mass (SDM), root/shoot ratio (R/S), seed cotton weight (SCW), and total seed weight (TSW) of the colored-fiber cotton genotypes (G), as a function of different water deficit management strategies (MS), at 120 days after sowing.

Sources of variation	DF	Mean squares			
		SDM	R/S	SCW	TSW
Management strategies (MS)	6	132.194^{**}	0.029^{**}	2587.236^{**}	1114.798^{**}
Genotypes (G)	2	111.320^{**}	0.008^{ns}	20045.643^{**}	5041.668^{**}
Interaction MS × G	12	38.907^{ns}	0.015^{ns}	371.886^{ns}	151.333^{ns}
Block	2	15.674^{ns}	0.005^{ns}	412.633^{ns}	175.943^{ns}
Error	40	24.106	0.007	251.744	99.749
CV (%)		12.77	16.47	11.70	10.84

^{ns}, *, **: not significant and significant at $p \leq 0.05$ and $p \leq 0.01$; respectively, by the F test.

The water deficit management strategies significantly influenced shoot dry mass (Figure 7A) at 120 DAS, when plants subjected to the strategy $A_1B_1C_1$ (T1) were statistically superior to those cultivated under the strategies $A_1B_2C_1$ (T3), $A_2B_2C_1$ (T4), and $A_2B_1C_2$ (T6), which showed reductions in SDM of 18.60, 25.07, and 15.11%, respectively. Differently from the results observed when water stress was applied according to the strategies $A_2B_1C_1$ (T2), $A_1B_1C_2$ (T5), and $A_1B_2C_2$ (T7), there were no significant differences in SDM during the crop cycle compared to the management with 100% ETr (Figure 7A). The shoot dry mass of plants of the genotype 'BRS Rubi' was statistically higher than that of 'BRS Jade', regardless of water deficit management strategies (Figure 7B). The decrease in shoot dry mass production of cotton plants under water deficit occurs due to the adaptive physiological mechanisms developed by plants, such as the acceleration of leaf senescence, so the restrictions observed in the phenological stages (T3, T4, and T6) of cotton are correlated with the reductions in leaf area and, on the other hand, to the surface exposed to water losses through transpiration (ANDRADE; ABREU, 2007).



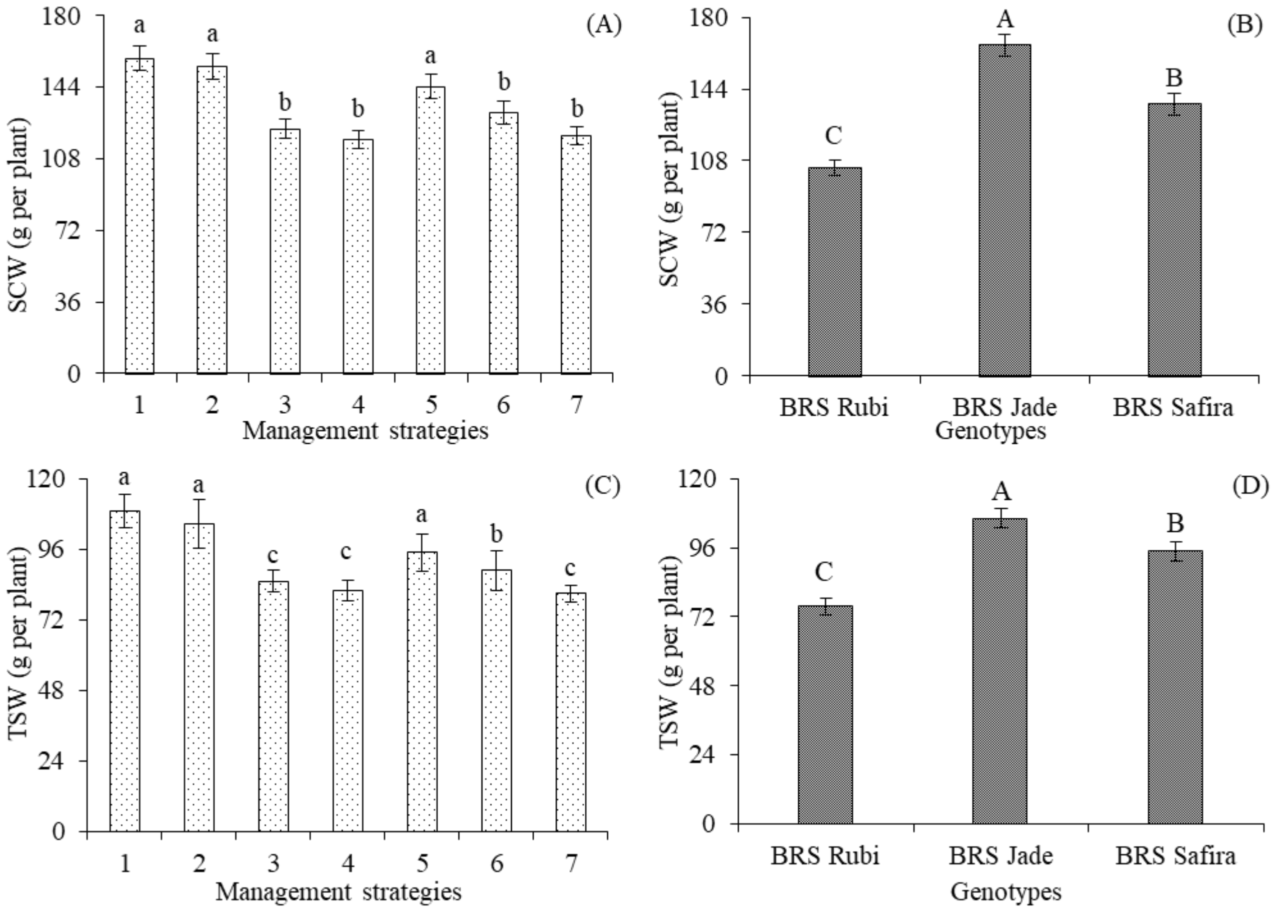
Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$), and means followed by the same uppercase letter indicate that the genotypes do not differ from each other by Tukey test, $p > 0.05$. Bars represent the standard error of the mean (n = 3 for strategies and n = 3 for genotypes). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2- water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 - 60/ 61 - 75 DAS); 5 - water stress in the yield formation stage (76 - 120 DAS); 6- water stress in the vegetative and yield formation stages (20 - 60/76 - 120 DAS); 7- water stress in the flowering and yield formation stages (61 – 75/76 - 120 DAS).

Figure 7. Shoot dry mass (SDM) – A and root/shoot ratio (R/S) – C of colored-fiber cotton genotypes as a function of water deficit management strategies and SDM as a function of genotypes (B), at 120 days after sowing.

For the root/shoot ratio (R/S), there were increments of 27.36 and 23.55% in plants subjected to the water deficit management strategies $A_1B_2C_1$ (T3) and $A_1B_2C_2$ (T4) compared to those cultivated under full irrigation throughout the cycle, respectively (Figure 7C). It is important to highlight that there were no significant differences among the T1, T2, T5, T6, and T7 treatments. Water deficit in the successive vegetative and flowering stages and the flowering stage reduced shoot dry mass accumulation and consequently increased the root/shoot ratio of cotton plants. Cotton response to water stress involves osmotic adjustment, the elasticity of the photochemical apparatus, and control of stomatal conductance; on the other hand, the nature of root growth and development is related to a greater uptake of water and nutrients, and the synthesis of plant hormones, organic acids, and amino acids (RAMAMOORTHY et al., 2017).

Regarding seed cotton weight - SCW (Figure 8A), plants under a deficit of 40% ETr in the vegetative stage ($A_2B_1C_1$ - T2) and yield formation stage ($A_1B_1C_2$ - T5) showed mean production of 154.46 and 144.46 g per plant, respectively, standing out as an indication of acclimatization to water stress, and the SCW values obtained were compatible with those of plants without water stress (158.69 g per plant). Plants irrigated with 40% ETr in the same stages also obtained the highest total seed weight (TSW), not differing from plants irrigated with 100% ETr throughout the cycle, with TSW of 104.65 and 94.78 g per plant in the strategies $A_2B_1C_1$ (T2) and $A_1B_1C_2$ (T5), respectively (Figure 8C). This may be related to the positive regulatory mechanism, which can compensate for the effect of water stress through the action of antioxidant enzymes, synthesis of organic solutes, and greater growth of the root system (NIU et al., 2018).

The results obtained for seed cotton weight and total seed weight are consequences of other variables already discussed, such as gas exchange and biomass accumulation, denoting greater resistance to water deficit during the vegetative and yield formation stages of cotton. Zonta et al. (2017), while evaluating cotton cultivars subjected to water deficit in different stages of the cycle, also found that water deficit imposed during the initial growth stages and after the opening of the first boll does not compromise cotton yield. According to Kumar; Sharma; Kumar (2008), tolerant plants usually stand out with higher dry mass accumulation and/or yield under water deficit than under other environmental conditions.



Means followed by the same lowercase letter indicate there is no significant difference between water deficit strategies (Scott-Knott, $p > 0.05$), and means followed by the same uppercase letter indicate that the genotypes do not differ from each other by Tukey test, $p > 0.05$. Bars represent the standard error of the mean (n = 3 for strategies and n = 3 for genotypes). Water deficit management strategies 1- plants under full irrigation throughout the cycle (1-120 days after sowing - DAS); 2- water stress in the vegetative stage (20 - 60 DAS); 3- water stress in the flowering stage (61 - 75 DAS); 4- water stress in the vegetative and flowering stages (20 - 60/ 61 - 75 DAS); 5 - water stress in the yield formation stage (76 - 120 DAS); 6- water stress in the vegetative and yield formation stages (20 - 60/76 - 120 DAS); 7- water stress in the flowering and yield formation stages (61 – 75/76 - 120 DAS).

Figure 8. Seed cotton weight (SCW) and total seed weight (TSW) as a function of water deficit management strategies (A and C) and colored-fiber cotton genotypes (B and D), respectively, at 120 days after sowing.

For seed cotton weight and total seed weight as a function of colored-fiber cotton genotypes (Figures 8B and D), it was verified that 'BRS Jade' stood out with the highest production, reaching values of 166.03 and 106.16 g per plant, respectively, which are 37.21 and 17.81% higher than the values of 'BRS Rubi' and 28.88 and 10.80% higher than the values of 'BRS Safira', respectively. It was found that the changes in gas exchange, explicitly related to the greater growth in stem diameter, contributed to higher SCW and TSW in 'BRS Jade', which is justified by the inherent genetic constitution of each genotype. According to information from Embrapa (2006), there are colored-fiber cotton genotypes with peculiar agronomic characteristics, which make them important for breeding programs. However, the genetic bases for the tolerance of crops is not fully understood due to the complexity of stress conditions and are influenced by multiple genes and environmental factors with small and varied effects (ABDELRAHEEM et al., 2019).

CONCLUSIONS

'BRS Jade' genotype is the most suitable for cultivation under water deficit conditions with 40% of the actual evapotranspiration. Colored-fiber cotton cultivation under water deficit in the flowering stage causes reductions in physiological variables and growth. Water deficit during the vegetative and formation phases of production promotes lower losses compared to other water deficit management strategies in seed cotton production and total seed weight regardless of cotton genotypes.

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