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Physiological aspects in cotton cultivars in response to application leaf gibberellic acid

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The cotton plant is a species of the *Malvaceae* family and is relevant to the Brazilian and world economy, mainly because of the textile fiber. However, there was an increase in cotton production because it is necessary for the use of inputs or stimulants, such as the use of gibberellic acid, which has contributed in improving the physiological processes of plants. The study aimed to evaluate the effects of gibberellic acid doses and foliar application on the physiological aspects of different cotton cultivars. The experiment was conducted under field conditions in 5 × 3 factorial scheme, corresponding to five doses of gibberellic acid (0, 0.01, 0.02, 0.04 and 0.06 mg L^{-1}) and three cultivars upland cotton (BRS 8H, BRS Rubi and BRS Safira) in the design of randomized blocks, three replications and 25 plants per plot. The photosynthetic pigments, which are represented by the contents of chlorophyll *a* and *b*, total, carotenoid and relative water content in the sheet were determined. In BRS 8H the chlorophyll levels were high, 287.914 µmol m^{-2} to 468.796 µmol m^{-2} being the treatments without and sprayed with 0.06 mg L^{-1} GA_3, with 62.82% increase. The application of 0.06 mg L^{-1} GA_3 generally promotes increased levels of photosynthetic pigments and relative water content in cotton leaves. The cotton BRS 8H was the culture that best meets the application of gibberellic acid.

Key words: Genetic material, *Gossypium hirsutum* L., photosynthetic pigments phytohormone.

INTRODUCTION

The cotton (*Gossypium hirsutum* L.) is a species of the *Malvaceae* family originating from the Mesoamerican region (D'eeckenbrugge and Lacape, 2014). The cotton crop has been cultivated for thousands of years and has great relevance in the Brazilian and world economy, mainly because of textile fiber (Carvalho et al., 2015). On the international scene, according to Carvalho et al. (2015), Brazil ranks fifth in world's cotton ranking, with

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Table 1. Soil chemical characterization as fertility before the experiment

pH	Ca^{2+}	Mg^{2+}	Na^+	K^+	BS	$H^+ + Al^{3+}$	CEC	V	P	O.M
1:2.5				$mmol_c\ dm^{-3}$				%	$mg\ dm^{-3}$	$g\ kg^{-1}$
7.5	51.6	6.9	0.9	2.9	62.3	0.0	62.3	100	126.2	15.3

BS = Base sums; O.M. = Organic Matter; CEC = Cation Exchange Capacity.

production of about 4.404,600 t in an area of 1.121,600 ha during the 2013 to 2014 season (Conab, 2015). Brazil comes after China, India, the United States and Pakistan in the world's ranking. However, that there is an increase in cotton production, it is necessary for the use of inputs or stimulants, such as the use of growth regulators which is an agronomic technique interfered for plant growth and increasing production in various cultures (Campos et al., 2009; Ferrari et al., 2008). Among the growth regulators, the gibberellic acid (GA_3) which is a plant growth hormone ($C_{19}H_{22}O_6$) widely used in the improvement of the regulation of physiological processes of plants, including cotton was used (Onanuga et al., 2012). It is known that the application of gibberellic acid is important in many metabolic processes of plants, works in stimulated seed germination, elongation and cell division, leaf expansion, flowering and fruit development, and stimulate the secondary metabolism species (Ahmad Dar et al., 2015). Recently, research has turned to elucidate the role of GA_3 in the preservation and stimulation process in the production of photosynthetic pigments in plants. The results have shown that the application of low concentrations phytoregulator has increased the carotenoid and chlorophyll content in leaves (Ali et al., 2012; Jaleel et al., 2009).

In addition, gibberellic acid is often employed when plants are under stress, especially those related to water and salt (Ali et al., 2012; El-Tohamy et al., 2015), since under such conditions, leaf water content is reduced and it in turn, severely affects the growth and development of the plant. Thus, the exogenous application of GA_3 as mitigating, has enabled the plants retain a larger amount of water in the leaves, favoring the growth process, mainly related to elongation and cell division, even under stress conditions (Kaya et al., 2006; Taiz and Zeiger, 2013). However, so that the plants can respond to the stimulus applying GA_3, studies are needed to elucidate the application form and the correct dose of phytohormone for each species. Since, according to Carvalho et al. (2016), the answer depends on several factors, such as plant species and variety within the same crop species. This is proven by Alia-Tejacal et al. (2011) and Onanuga et al. (2012) who found that there are differences in the requirement of gibberellic acid-flower native cultivars (*Euphorbia pulcherrima* Willd. Ex Klotz) and cotton varieties. Due to the economic importance of the cotton crop to the national scene and the lack of information on the effect of GA_3 on cotton cultivars in parameters related to the physiology of the species. The study aimed to evaluate the physiologically effects of gibberellic acid doses and foliar application on cotton cultivars.

MATERIALS AND METHODS

The experiment was conducted under field conditions between March and June 2012, at the National Center for Research on Cotton, Brazilian Agricultural Research Corporation - Embrapa Cotton, situated in Campina Grande city, Paraiba, Brazil. The municipality is geo-referenced by the coordinates: latitude 7°13'1 "South and 35°52'31" West and at an altitude of 551 m. The climate of the region is related with hot and humid climate with autumn-winter rain, according to Köppen climate classification. The rainy season is between the months of April and July, and the monthly rainfall in the experimental period was 12.1; 5.0; 58.3; 213.1 mm respectively in the months of March, April, May and June (AESA, 2016). The experiment was conducted in a 5 × 3 factorial design, the design of randomized blocks, with three replications and 25 plants per plot. The factors corresponded to five doses of gibberellic acid (0, 0.01, 0.02, 0.04 and 0.06 mg L^{-1}) and three varieties of herbaceous cotton (BRS 8H, BRS Rubi and BRS Safira). The plants were grown in 5 rows of 5 plants each, spaced at 0.80 m between rows and 0.50 m between plants, which corresponds to 25,000 plants per ha. However, for evaluation purposes, only the three central rows of block, totaling 15 plants per plot were considered. This choice was made in order to avoid border errors which are not controllable in this case. The soil of the experimental area was classified as Entisol, dystrophic, of sandy loam texture (Santos et al., 2013). Before the experiment, samples were collected from the soil at a depth of 0-20 cm, which were homogenized, transformed into a sample and put in a dry shade. After these procedures, the sample was taken to the Soil and Water Analysis Laboratory to perform analysis of the chemical, following the methodology contained in Donagema et al. (2011) as indicated in Table 1.

During the experiment, the driving period made daily collections of the maximum temperature, average, minimum and relative humidity of experimental area through a weather station located at Embrapa Cotton, as is verified the results in Figure 1. Fertilizing plants of cotton cultivars, followed the recommendations suggested by the Soil Analysis Laboratory at Embrapa Cotton, which indicated the application of 20 kg ha^{-1} of N and 30 kg ha^{-1} of P_2O_5, provided in form of urea (45% N) and superphosphate (18% P_2O_5), respectively. Fertilization in foundation and coverage was performed 15 days after emergence (DAE), applying the phosphate fertilizer directly into furrows in a half moon shape, the depth of 30 cm. As for nitrogen fertilization, this was partitioned into three equal applications, the first fertilization performed at 15 DAE, the second at 30 DAE and third at 45 DAE. Because of the irregularity in precipitation, the plants were irrigated daily in accordance with the water demand of the culture, i.e 6.5 mm day^{-1}, as determined for upland cotton (Azevedo et al., 1993). The water used for irrigation of the plants was analyzed for its chemical composition in the Soil

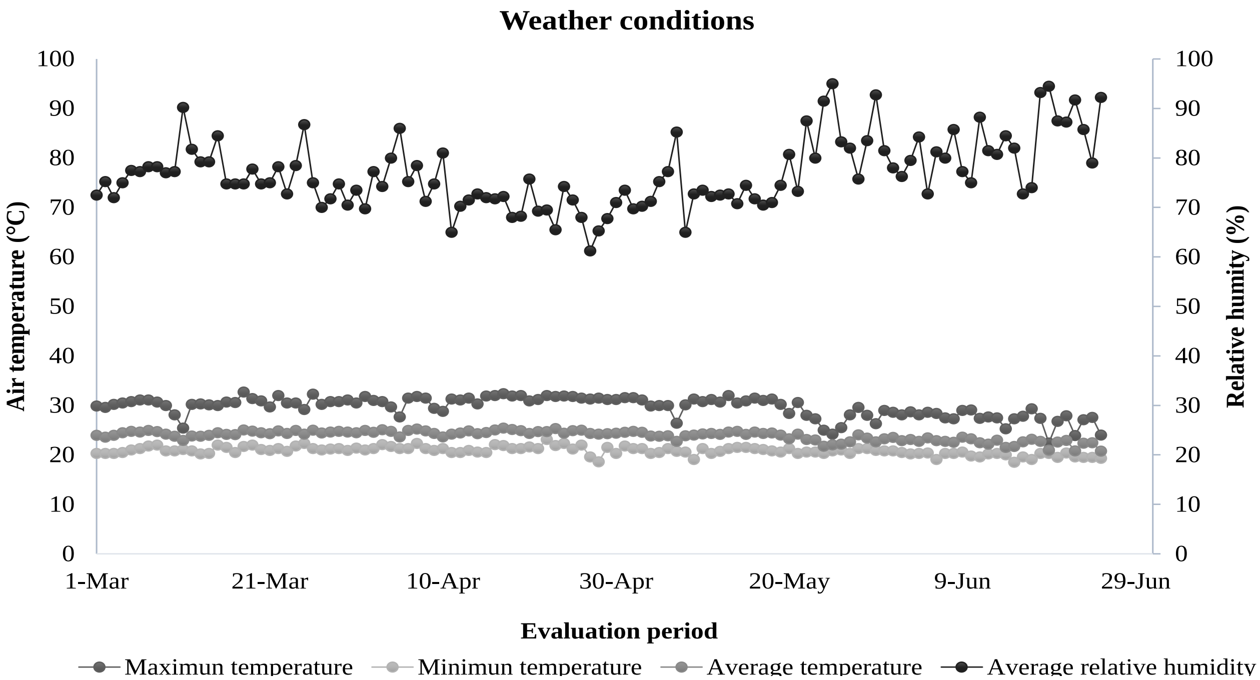

Figure 1. Values of maximum temperatures, average and minimum relative humidity recorded during the experiment period of driving.

Analysis Laboratory and Water Embrapa Cotton, following the methodology described by Richards (1954), with the following characteristics: pH = 7.7 (alkaline) , Cl = 266.25 mg L^{-1} (moderate); $CaCO_3$ = 92.50 mg L^{-1} (high level); Ca^{2+} = 29,00 mg L^{-1}; Mg^{2+} = 30.60 mg L^{-1}, Na^{+} = 98.90 mg L^{-1} (low level), ECiw (Electrical conductivity of irrigation water) = 730 µS cm^{-1} and SAR (Sodium adsorption ratio) = 3 mmol L^{-1}, classified as C_2S_1 (water with average risk of salinization and low risk of sodium), according to Ayres and Westcott (1999).

The gibberellic acid doses were applied at 20, 40 and 60 DAE, and the application through foliar sprays directed on abaxial faces and adaxial of cotton leaves. To better absorption efficiency of gibberellic acid in the leaf surface was used surfactant in the spray solution (Carvalho Júnior et al., 2016). Spraying the plant growth regulator, it was made with the aid of a prior compression manual spray with high molecular weight polyethylene tank with volume capacity of 3 L and pump type piston diameter of 34 mm nozzle. At 90 days after the application of GA_3 solutions, which corresponds reproductive cotton stage, the third pair of fully expanded leaves were collected, counting from the apex to the base of the plant, for the determination of the photosynthetic pigments, which are represented by contents of chlorophyll a (CL*a*), b (CL*b*), total (CL*t*), carotenoids (CAR) and the chlorophyll *a*/*b* (Cl*a*/CL*b*). For this, the leaves were collected and immediately placed in aluminum envelopes, stored in boxes with thermal insulation containing dry ice and transported to the laboratory. Then the middle part circular fractions were taken without the midrib of the leaf tissue with size of 113 mm^2. Fractions were macerated tissue and placed in test tubes coated aluminum foil, which was added 5 ml of dimethylsulfoxide (DMSO). The tubes were left in a dark environment at room temperature of 25°C for a period of 48 h. After this time the solution containing DMSO + fraction of the plant tissue was filtered through a "filter paper" during the 5 min period. With the solution extracted absorbance readings were performed in a spectrophotometer at respective wavelengths of 480, 649 and 665 nm (Wellburn, 1994).

For quantification of photosynthetic pigments, the following equations were used according to the proposed by Wellburn (1994):

Chlorophyll *a* (µmol m^{-2}) = $12.19 \times A_{665} - 3.45 \times A_{649}$
Chlorophyll *b* (µmol m^{-2}) = $21.99 \times A_{649} - 5.32 \times A_{665}$
Total chlorophyll (µmol m^{-2}) = chlorophyll *a* + chlorophyll *b*
Carotenoids (µmol m^{-2}) = ($1000 \times A_{480}$ – 2.14 × chlorophyll *a* – 70.16 × chlorophyll *b*)/220

In the same period of evaluation of photosynthetic pigments content, was measured relative water content in the leaf (RWC), using the methodology proposed by Weatherley (1950) and as is seen in the following equation:

RWC (%) = [(Mf - Ms)]/(Mt-Ms)] × 100

Where, Mf = Fresh pasta sheet; Ms = dry weight of leaf and Mt = Mass turgid leaf.

The data were submitted test by analysis of variance F to 5% probability to check the effects of the interaction doses of gibberellic acid × cotton cultivars on the variables analyzed. The average regarding cotton cultivars were compared by Tukey test at 5% probability and the average regarding the gibberellic acid doses by polynomial regression ($p<0.05$). For data analysis, we used the statistical software SISVAR 5.3 (Ferreira, 2014).

RESULTS AND DISCUSSION

As noted in the summary of the mean square values of variance analysis, interaction cotton cultivars × gibberellic acid doses led to significant effects on variables related to the content of photosynthetic pigments and relative water content in leaves of cotton cultivars, with coefficient

Table 2. Summary variance analysis for chlorophyll *a* (CL*a*), chlorophyll *b* (CL*b*), carotenoid (CAR), chlorophyll (CL*t*), relative water content (CRA) and chlorophyll *a*/*b* (CL*a*/CL*b*) in three cotton cultivars treated with gibberellic acid doses.

		Mean square					
S. V	**DF**	**CL*a***	**CL*b***	**CL*t***	**CL*a*/CL*b***	**CAR**	**RWC**
Blocks	2	50.86^{ns}	43.46^{ns}	75.35^{ns}	0.08^{ns}	40.15^{ns}	5.42^{ns}
Cultivars (C)	2	9064.06**	71.46^{ns}	8144.02**	4.62**	699.08**	0.82^{ns}
Gibberellic acid (G)	4	13696.36**	466.41**	17212.30**	2.14**	3669.55**	58.81^{ns}
C × G	8	8828.15**	607.32**	12600.80**	0.84**	1865.67**	121.87**
Residue	28	152.22	86.75	204.92	0.11	50.03	25.39
C.V (%)		3.86	17.64	3.86	5.45	3.94	7.30
Mean		319.60	50.80	371.24	6.15	191.55	69.04

S.V. = Source of variation; C.V = coefficient of variation; D.F. = degree of freedom; * = Significant at 5% probability by the F test; ** = Significant at 1% probability by the F test; ns = not significant.

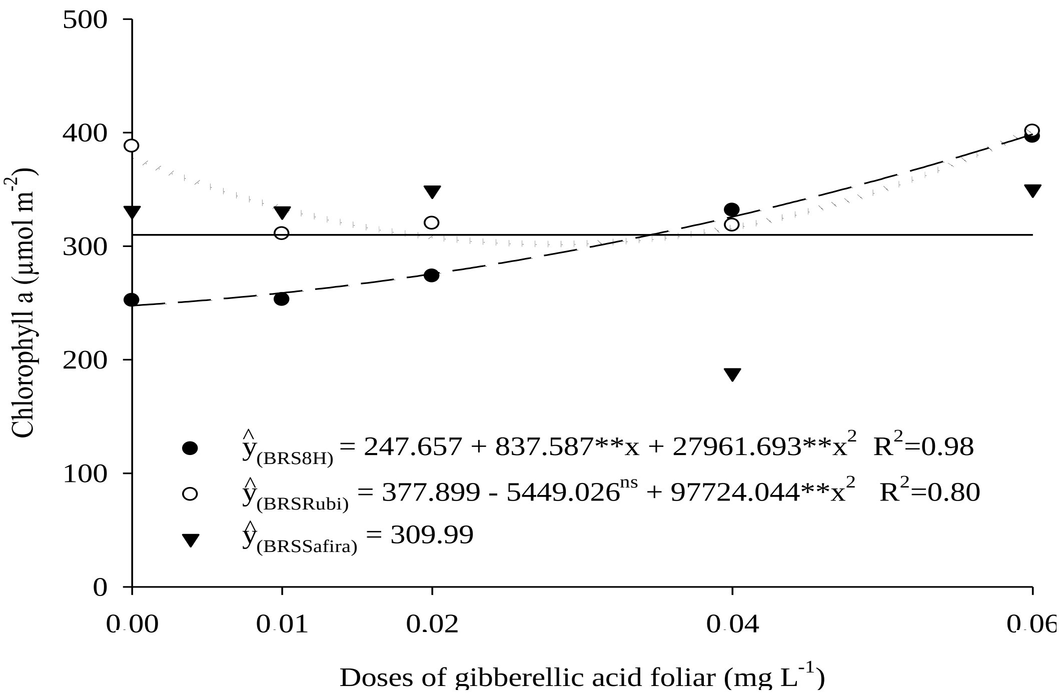

Figure 2. Leaf chlorophyll *a* content in the BRS-8H cotton cultivars (- -), BRS-Rubi (-) and BRS-Safira (•••) due to the application of increasing doses of gibberellic acid foliar.

relatively low variation, ranging from 17.64 to 3.86%, depending on the variable analyzed (Table 2).

The foliar concentration of chlorophyll *a* in cotton cultivars increased with the rise of gibberellic acid doses, except BRS Safira, not set to any regression model with increasing doses of GA_3 and was represented by the average level of 309.09 µmol m^{-2} (Figure 2). In cultivating cotton cv. BRS 8H, it was found that the application of 0.06 mg L^{-1} GA_3 provided increase in leaf chlorophyll *a*, it is found to confront the contents of 247.657 µmol m^{-2} (0 mg L^{-1}) and 398.574 µmol m^{-2} (0.06 mg L^{-1}) in which the highest dose of the plant growth regulator in increased 60.93% pigment content in the leaves. In cotton cv. BRS Rubi, there is a reduction in CL*a* content to the estimated dose of 0.0278 mg L^{-1} GA_3 above this dose increased the chlorophyll content of the cotton sheets, until the 402.763 µmol m^{-2} content when applied dose of 0.06 mg L^{-1}. In soybean (*Glycine max* L.), Campos et al. (2008) reported that leaf application of gibberellic acid inhibits degradation or even increases the chlorophyll content in the leaves. The gibberellic acid is often applied in harvest treatments and post-harvest in order to keep the fruits with greenish longer and hence promote the delay harvest, and increase the sale period of fruits (Modesto et al., 2006).

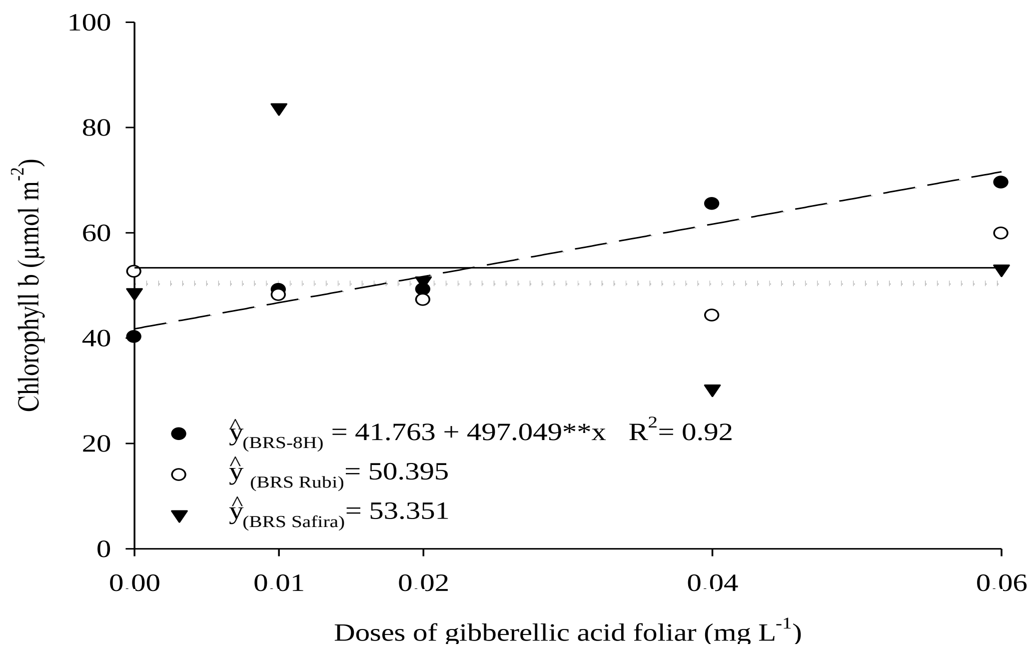

Figure 3. Foliar content of chlorophyll *b* in BRS-8H cotton cultivars (- -), BRS-Rubi (-) and BRS-Safira (•••) due to the application of increasing doses of gibberellic acid foliar.

These differences are also dependent on the genetic material as found Alia-Tejacal et al. (2011) evaluated five-flower native cultivars, plant for ornamental purposes originating in Mexico, found differences in chlorophyll content between cultivars. The foliar content of chlorophyll *b* in cultivars BRS Rubi and BRS Safira did not fit with any regression model with increased doses of gibberellic acid, with average levels of 50.395 and 53.351 µmol m^{-2}, respectively (Figure 3). In cv. BRS 8H, the CL*b* content increased linearly 497.049 µmol m^{-2} per unit increase in the dose of plant growth regulator applied. This is evidenced by comparing the plants without and sprayed with a dose of 0.06 mg L^{-1}, in which the highest dose promoted 71.4% gains over the plants was not applied GA_3.

Campos et al. (2009) found that the application of gibberellic acid in soybean, increased the chlorophyll content up to 105 days after sowing. This is due mainly because of plant growth regulators influence in maintaining the integrity of the photosynthetic apparatus, interfering with chlorophyll synthesis (Synková et al., 1997). Onanuga et al. (2012) found that the production of chlorophyll *b* in cotton produced in China are variable depending on the cultivar, this response being attributed to the interaction of gene expression of each material with the culture environment. The maintenance and increases chlorophyll *b* content in the plant species sheets is of great importance to all photosynthetic process, since chlorophyll *b* is considering an accessory pigment, aiding in the absorption of light and the radiant energy transfer to the centers reaction that are located on the membranes of the thylakoids (Taiz and Zieger, 2013).

The total chlorophyll content in cotton cultivars of leaves increased with the rise of gibberellic acid doses, except BRS Safira, not set to any regression model, with average content of chlorophyll 359.719 µmol m^{-2}, as seen in Figure 4. To BRS 8H in the absence of GA_3, CL*t* content was 287.914 µmol m^{-2}, and increased to 468.796 µmol m^{-2} to apply 0.06 mg L^{-1} plant growth regulator, this increase corresponds to an increase of 62.82% in the foliar chlorophyll. In cv. BRS Rubi, the total chlorophyll content decreased to the estimated dose of 0.0277 mg L^{-1} GA_3, doses above notes to increase the levels of total chlorophyll pigments and applied at the maximum dose (0.06 mg L^{-1}) there is CL*t* content of 461.745 µmol m^{-2}.

Onanuga et al. (2012) found significant differences in the production of chlorophyll pigments (total chlorophyll) between cotton varieties tested under application of a solution containing hormones, which contained 40 µg L^{-1}gibberellic acid. Similarly, the application of 5 µg L^{-1} GA_3 in leaves of the vinca-of-Madagascar (*Catharanthus roseus*) stimulated increased leaf chlorophyll concentration (Jaleel et al., 2009). Furthermore, the response is variable and depends on the interaction between the genotype and environmental conditions imposed cultivation (Ferrari et al., 2008), given that each of cotton cultivar responded differently.

It can be seen in Figure 5, the cotton cultivars BRS 8H and BRS Rubi showed reductions in chlorophyll *a*/*b* to respective gibberellic acid doses of 0.033 and 0.030 mg L^{-1}. Lifting these doses appears in the list of chlorophyll pigments with 5.72 values in cv. RBS 8H and 7.23 in cv.

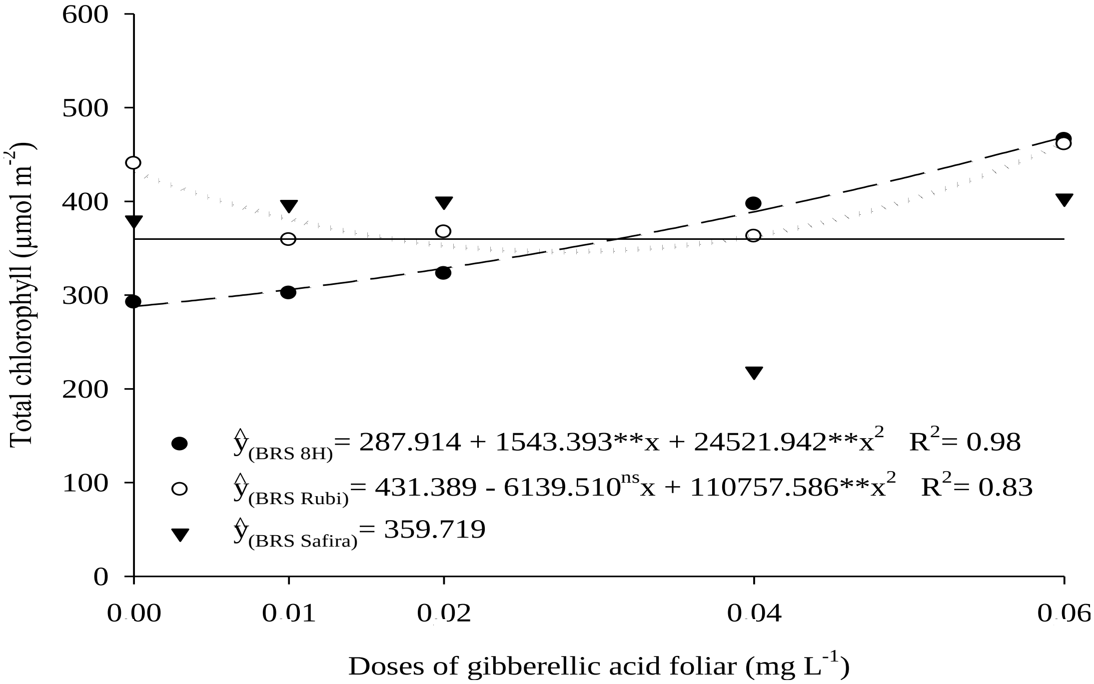

Figure 4. Foliar content of total chlorophyll in the BRS-8H cultivars (- -), BRS-Rubi (-) and BRS-Safira cotton (•••) due to the increasing doses application of gibberellic acid foliar.

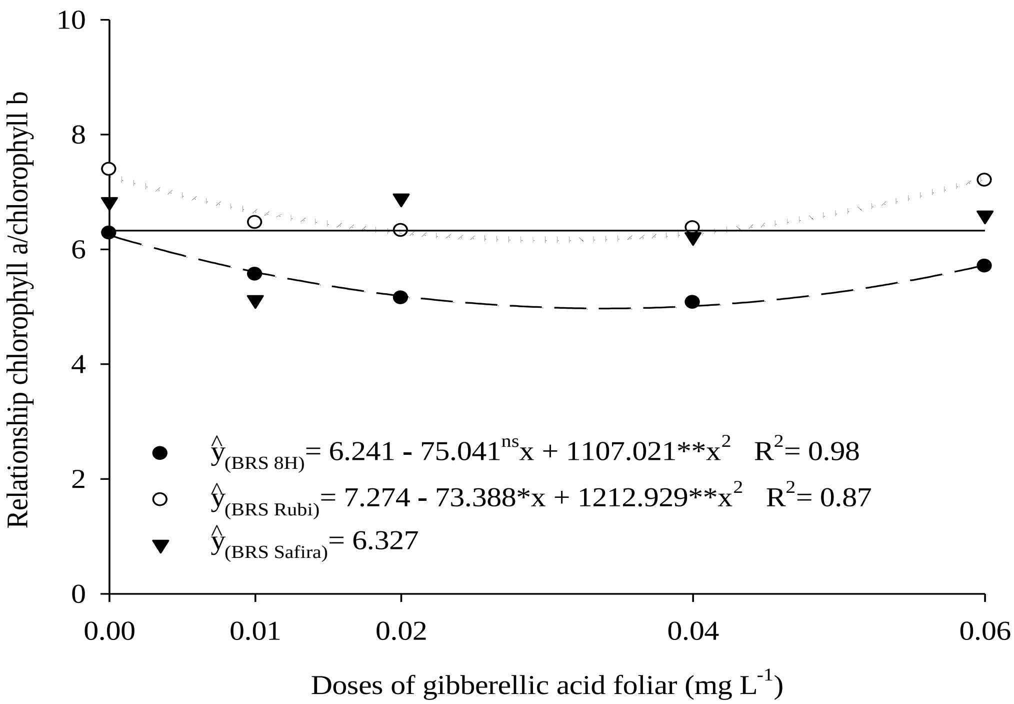

Figure 5. Relationship chlorophyll *a*/chlorophyll *b* in leaves of cotton cultivars BRS 8H (- -), BRS-Rubi (-) and BRS-Safira (•••) due to the application of increasing doses of gibberellic acid foliar.

BRS Rubi the maximum applied dose of 0.06 mg L^{-1}, however, these values are lower than the treatments without applying the plant growth regulator. As noted in the other variables related to chlorophyll levels, the

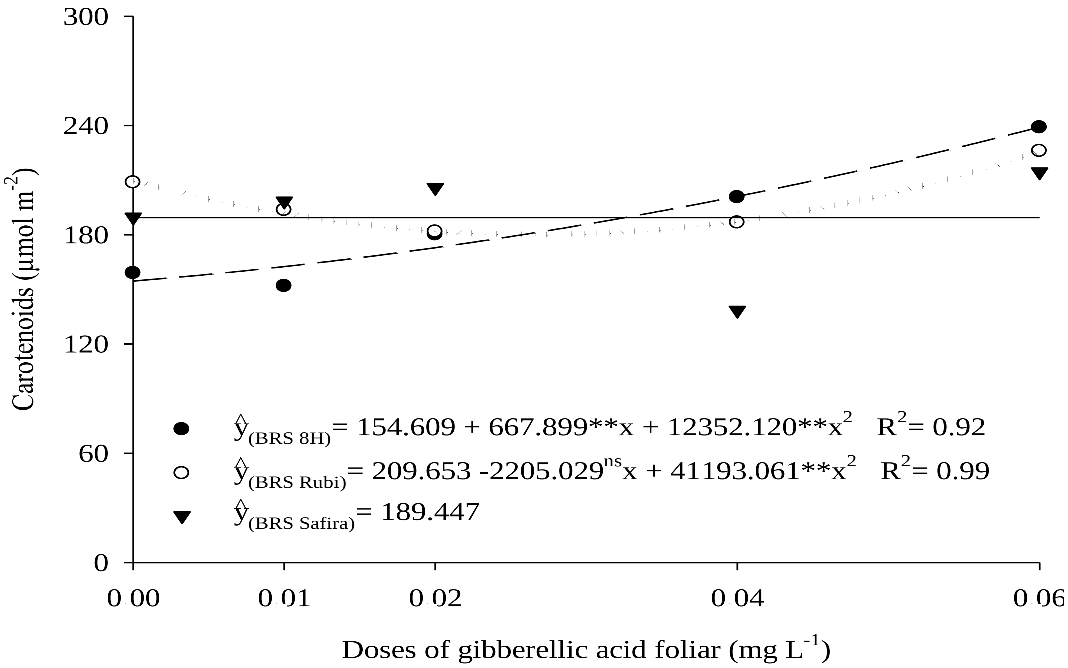

Figure 6. Foliar content of carotenoids in cotton cultivars BRS 8H (- -), BRS Rubi (-) and BRS Safira (•••) due to the foliar application of increasing doses of gibberellic acid foliar.

relationship CL*a*/*b* cv. BRS Safira, not set to no regression model with increased doses and GA_3, was represented by the average value of 6.32. Unlike the results observed in this study, Fioreze and Rodrigues (2012) evaluating the effect of applying foliar biostimulant auxin base + gibberellin + cytokinin in wheat plants (*Triticum* spp.), found no response in chlorophyll *a*/*b* flag leaf. The chlorophyll *a*/*b* is an important variable since the reduction ratio value is assigned a lot of times and it leads to increased leaf chlorophyll *b*; this response may be considered as an adaptive characteristic of the ambient conditions, since pigment that absorbs energy at a different wavelength of chlorophyll maximizes energy capture used in the photochemical step in photosynthesis and then transfer to the reaction centers - photosystems (Taiz and Zeiger, 2013). The leaf carotenoid levels responded differently for each cotton cultivar according to gibberellic acid levels (Figure 6). BRS 8H observed increase in levels of CAR 154.60 to 239.15 µmol m^{-2} between plants without spraying.

It was sprayed with 0.06 mg L^{-1} which corresponds to a larger percentage, 54.68%, in the sprayed treatment with the highest dose biostimulant. In BRS Rubi, the leaf levels of carotenoid decreased to 0.026 mg L^{-1} dose and from that dose the CAR levels became high, reaching a maximum value of 225.64 µmol m^{-2} at a dose of 0.06 mg L^{-1}. Meanwhile, the carotenoid content in cv. BRS Safira presented an average of 189.47 µmol m^{-2} with the increase of doses for plant growth regulator without adjusting any regression model. The increase in the dose of gibberellic acid promoted growth in the concentration of carotenoid per gibberellic acid and carotenoids to possess the same precursor, geranylgeranyl pyrophosphate (GGPP, C20). Therefore, the application of exogenous plant growth regulator may be applied to the need of the plant in relation to the concentration of gibberellic acid and the most of it diverted to the precursor for the synthesis of carotenoids (Castro et al., 2012). This trend of results was observed by Jaleel et al. (2009) in plant vinca-of-madagascar. After applying gibberellic acid foliar, they found that the leaf carotenoid content was high when applied to 5 µM L^{-1} GA_3. Carotenoids are extremely important for the plants, as they play a significant role in protecting the photosynthetic apparatus against photobleaching of photosystems (Taiz and Zeiger, 2013).

BRS 8H and BRS Safira cotton cultivars showed distinct trends cv. BRS Rubi relative water content in leaves in response to GA_3 doses (Figure 7), demonstrating that the response to plant growth regulator varies within the cultivars of the same species. In BRS 8H and Safira, the CRAs increased linearly from 65.90 to 78.90% and 62.74 to 77.82%, respectively, between treatments without application and application 0.06 mg L^{-1} biostimulant. Inverse response was observed in cv. BRS Rubi, where increasing doses GA_3 linearly reduced water on the leaves. From the results, it was found that when gibberellic acid was not applied, the sheets had an CRA 74.58%, reducing the value of 62.04% at the applied dose of 0.06 mg L^{-1} GA_3. Many authors have observed

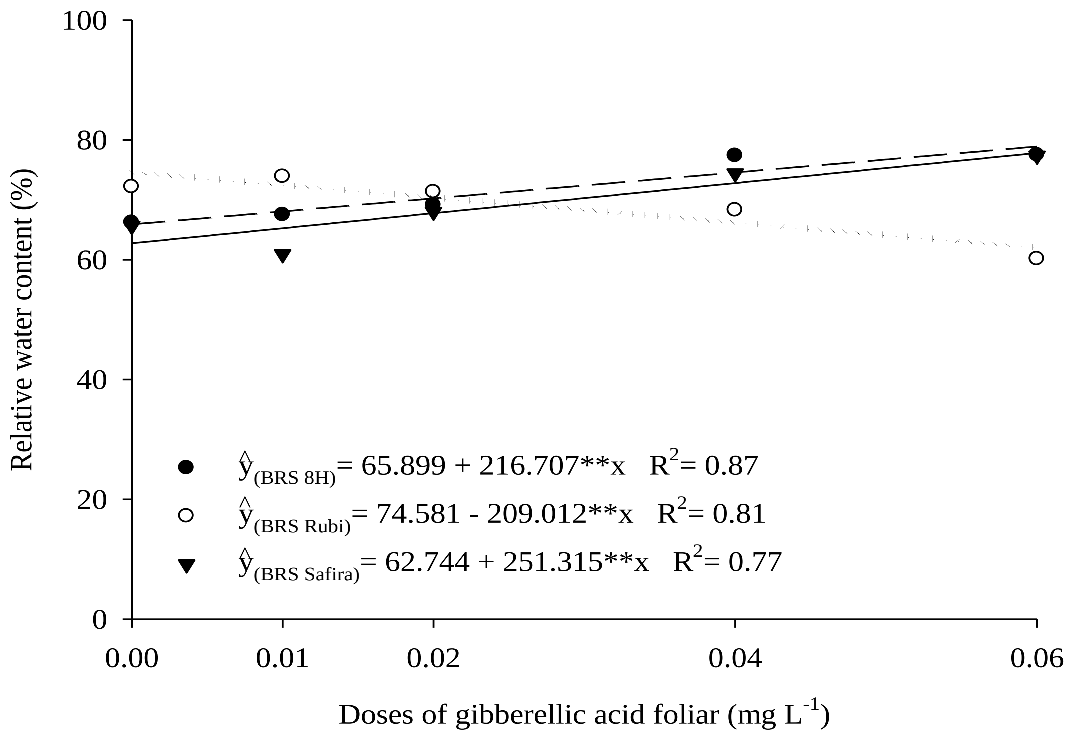

Figure 7. Relative water content in leaves of BRS 8H (- -), BRS-Rubi (-) and BRS-Safira cotton (•••) according to the foliar application of increasing doses of gibberellic acid foliar.

the benefits of gibberellic acid's foliar application in the relative water content of various crops, however, the correct dose to be applied depends on the plant species (Carvalho Júnior et al., 2016). This is verified by Ali et al. (2012) who found that the application of GA_3 increased the relative water content in the leaves of hibiscus (*Hibiscus sabdariffa* L.) under salt stress. In sweet potato plants (*Ipomoea batatus* L.), the exogenous application of gibberellic acid at a concentration of 10 ml L^{-1}, as well as improving the biometric and production parameters, promoted the increase in CRA in the leaves (El-Tohamy et al., 2015).

Conclusions

The application of 0.06 mg L^{-1}gibberellic acid, in general, promotes increased levels of photosynthetic pigments and relative water content in cotton leaves. However, cultivars respond differently to the application of plant growth regulator.

The BRS 8H cotton was among the evaluated materials with the plant variety that best meets the application foliar gibberellic acid. Despite the satisfactory results obtained in this work, although more studies are needed to clarify the effects of gibberellic acid on the physiological responses of cotton cultivars, especially those with colored fiber.

Conflict of Interests

The authors have not declared any conflict of interests.

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