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Changes in osmoregulatory metabolism of cotton genotypes during water deficit and recovery period

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ABSTRACT: The aim of this study was to evaluate the changes in osmoregulatory metabolism of cotton genotypes subjected to water deficit and recovery period. In a greenhouse, six cotton genotypes and two water managements were combined in a 6 x 2 factorial scheme, in a completely randomized design, evaluated after 14 and 22 days under water stress and recovery conditions, respectively. The water status, growth indicators and compatible solutes in the leaves were evaluated. The genotypes BRS Seridó, BRS Aroeira and BRS 7MH withstand better the stress condition because they had lower variations in their water potentials due to the osmotic adjustment and maintained their growth under water deficit conditions. The BRS 286 is the most sensitive genotype to the water deficit condition, since it showed greater variations in its water potential, which ultimately stopped its growth. Water replacement during the recovery period allowed growth resumption in most of the genotypes, mainly CNPA 5M and BRS 286. After the recovery period, variations in the concentrations of osmoregulators indicate the plasticity of cotton in regulating the concentrations of osmoregulators under favorable and unfavorable water conditions.

Key words: *Gossypium hirsutum* L., osmotic adjustment, compatible osmolytes, drought

Mudanças no metabolismo osmorregulador de genótipos de algodoeiro durante déficit hídrico e período de recuperação

RESUMO: O objetivo do presente estudo foi avaliar as mudanças no metabolismo osmorregulador de genótipos de algodoeiro submetidos ao déficit hídrico e ao período de recuperação. Em casa de vegetação, seis genótipos de algodoeiro e dois manejos hídricos foram combinados em esquema fatorial 6 x 2, em delineamento inteiramente casualizado, avaliados após 14 e 22 dias em condições de estresse hídrico e recuperação, respectivamente. Foram avaliados o status hídrico, indicadores de crescimento e solutos compatíveis em folhas. Os genótipos BRS Seridó, BRS Aroeira e BRS 7MH suportam melhor a condição de estresse por apresentarem menores variações nos seus potenciais hídricos devido ao ajustamento osmótico e por manterem seu crescimento em condições de deficit hídrico. O BRS 286 é o genótipo mais sensível à condição de deficit hídrico, pois apresentou maiores variações no seu potencial hídrico que repercutiu na paralização do seu crescimento. A reposição hídrica durante o período de recuperação permitiu a retomada do crescimento da maioria dos genótipos, principalmente CNPA 5M e BRS 286. Após o período de recuperação hídrica, as variações nas concentrações dos osmorreguladores indicam plasticidade do algodoeiro em regular os níveis de osmorreguladores em condições favoráveis e desfavoráveis de água.

Palavras-chave: *Gossypium hirsutum* L., ajustamento osmótico, osmólitos compatíveis, seca

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Introduction

Cotton (*Gossypium hirsutum* L.) cultivation encompasses an area of 956.7 thousand hectares. However, in the Northeast region there was a 13% reduction in its planted area in 2016 (CONAB, 2017), associated with the scarcity of water and irregular rainfalls.

This scenario causes water deficit in plants because it contributes to the loss of cell homeostasis and affects the processes of water absorption and accumulation in their tissues, with clear effects on growth and yield. These effects affect cell expansion and division, functioning of enzymes, mineral nutrition, stomatal conductance, photosynthesis and synthesis of compatible solutes (Flowers et al., 2014).

To stimulate defense mechanisms against drought, the use of a water deficit period followed by a subsequent water replacement constitutes a recovery period. In this case, morphophysiological losses can be minimized by the osmotic adjustment, through the accumulation of solutes in the cells (Snowden et al., 2013). The synthesis and accumulation of soluble sugars and amino acids (proline) in the cytosol reduce the osmotic potential and increase cell turgor potential, contributing to the osmotic adjustment under water deficit conditions, as reported for cotton plants (Parida et al., 2007; Joseph et al., 2015).

Thus, there is the need for scientific research on cotton genotypes adapted to water deficit, through physiological and biochemical analyses, to assist genetic breeding programs in the selection of materials for environments under both irrigated management and rainfed conditions, meeting the global demand.

Therefore, the present study aimed to evaluate the changes in osmoregulatory metabolism of cotton genotypes to water deficit, by means of growth indicators, physiological and biochemical descriptors, in addition to evaluating their recovery capacity after the water replacement period.

Material and Methods

The experiment was carried out in a greenhouse at Embrapa Cotton, in Campina Grande, PB State, Brazil, at geographic coordinates 7º 13' 50" S, 35º 52' 52" W, with 600 m altitude, under an Awi climate (rainy tropical) according to Köppen's classification.

Six cotton genotypes were evaluated: BRS 368 RF (genotype A), BRS Seridó (genotype B), CNPA 5M (genotype C), BRS 286 (genotype D), BRS Aroeira (genotype E) and BRS 7MH (genotype F), subjected to two water managements (without water deficit - field capacity; with water deficit - suspension of irrigation), combined in a 6 x 2 factorial scheme, in a completely randomized design with 3 repetitions, totaling 36 experimental units, composed of two plants per pot. For the recovery period, other 36 experimental units, also composed of two plants per pot, resulting from treatments with and without water deficit, were subjected to irrigation up to field capacity, using the same experimental design.

Cottonseeds were delinted and then the systemic fungicide Captan® was applied at dose of 0.22 g 100g^{-1} of seeds, which remained at rest for 24 h. For each genotype, four seeds were planted in polypropylene pot (7 L) and, after 15 days from emergence, thinning was performed, leaving only two seedlings per pot. The soil, classified as Oxisol, of sandy loam texture, was previously corrected and fertilized with organic matter at 2:1 proportion, according to the soil analysis.

Soil physical analysis showed the following characteristics - sand: 659 g kg^{-1}; silt: 101 g kg^{-1}; clay: 240 g kg^{-1}; degree of flocculation: 1000 kg dm^{-3}; bulk density: 1.38 kg dm^{-3}; particle density: 2.63 kg dm^{-3}; total porosity: 0.48 m^3 m^{-3}; field capacity at 0.01 MPa tension: 1.52 g kg^{-1}; permanent wilting point at 1.5 MPa tension: 75 g kg^{-1}.

At the beginning of the experiment, irrigation was performed daily for 20 days, applying a sufficient amount of water to maintain the soil moisture content at field capacity. At 20 days after emergence, 18 experimental units were subjected to daily irrigation, which corresponded to the treatment with no water deficit (NWD), while in other 18 experimental units, irrigation was completely suspended for 14 days, corresponding to treatment with water deficit (WD).

The irrigations performed daily, based on the evapotranspiration, were quantified by the weighing method (Souza et al., 2016). The weight at field capacity was determined by saturating the pots with water and subjecting them to drainage. When the drainage process stopped, the pots were weighed to obtain their weight under a condition of maximum water retention.

After 14 days under stress condition, three pots with two plants of each genotype, with and without water deficit, were removed and their plants were subjected to physiological and biochemical analyses of leaf tissue. After this period, irrigation was normally resumed for all plants of the remaining pots, simulating their water recovery process. All genotypes remained under daily irrigation for 22 days, until the plants recovered leaf turgor and, after this period, the leaves of plants from the WD and NWD treatments were collected again.

Two evaluations were performed: after 14 days under water deficit condition (35 days from emergence) and after 22 days in recovery period (65 days from emergence). The physiological variables analyzed were: leaf water potential (Ψw), relative water content (RWC), leaf area (LA), shoot fresh matter (FM) and shoot dry matter (DM). Additionally, the biochemical analyses were represented by: total free amino acids (TFAA), free proline (PRO) and total soluble sugars (TSS).

Leaf water potential (Ψw) was determined using the Scholander pressure bomb (Soil Moisture 3000), between 5 and 6 A.M., and expressed in MPa. Relative water content (RWC) was determined using the mathematical expression of Irigoyen et al. (1992). The total leaf area of the plant (cm^2) was measured using the 3100 Li-Cor planimeter. Fresh matter was determined on a high-precision digital scale and, after drying at 80 °C in an oven for 72 h, the dry matter (g) was obtained.

TSS concentration was measured by the phenol-sulfuric acid method described by Dubois et al. (1956), expressed in mg g^{-1} of fresh matter, with reading taken in a spectrophotometer at 490 ηm of absorbance. TFAA contents were determined according to the method described by Peoples et al. (1989), expressed in ηmol g^{-1} of fresh matter, with reading taken in a spectrophotometer at 570 ηm absorbance. Proline was determined according to the methodology described by Bates et al. (1973), expressed in µmol g^{-1} of fresh matter, with reading taken at 520 ηm.

The collected data were subjected to analysis of variance by F test and then the means were compared by Tukey test ($p \leq 0.05$), using the computer program Sisvar 5.6 (Ferreira, 2011).

Results and Discussion

There were reductions in the water potentials of all genotypes during the stress period, compared to irrigated plants. However, the genotypes BRS Seridó, CNPA 5M, BRS Aroeira and BRS 7MH had lower variations in the mean values of water potential under water deficit conditions, compared to their respective controls (Figure 1A), suggesting the participation of intraspecific mechanisms of mitigation of water deficit effects.

According to Zandalinas et al. (2018), plants cultivated under water deficit show reductions in leaf tissue water potential, which may contribute to the physiological, biochemical, molecular and morphological processes, resulting in changes in cell metabolism. However, the smaller variations in the mean values of water potential in some genotypes, under water deficit conditions, suggest that cotton has the ability to perform osmotic adjustment to ensure higher stability of its yield under stress conditions.

After the recovery period, it was observed that the genotypes BRS 368 RF, BRS 286 and BRS 7MH, from the treatment with water deficit, had RWC percentages similar to those found in their respective controls, suggesting a recovery in cell water status (Figure 1B). Under this condition, the genotype BRS 7MH stood out with a 16% increase in the RWC, compared to its irrigated control (Figure 1B). However, the RWC percentage decreased by 19, 8 and 18% in the genotypes BRS Seridó, CNPA 5M and BRS Aroeira, respectively, even after the recovery treatment, compared to their respective controls (Figure 1B).

For Efeoğlu et al. (2009), the dehydration process caused by water deficit is reversible after the resumption of water supply to the plant. Thus, the increase in the relative water content observed during the recovery period, especially in the genotypes BRS 368 RF, BRS 286 and BRS 7MH, suggests the maintenance of cell water status during the rehydration period.

It was observed that the genotype BRS 7MH increased 39% in its leaf area under water deficit conditions, compared to its irrigated control. The genotypes BRS 368 RF, CNPA 5M and BRS 286, in addition to having the lowest mean values of leaf area under the water deficit condition, also showed reductions of approximately 35, 54 and 70%, respectively, compared to their respective irrigated control groups (Figure 2A).

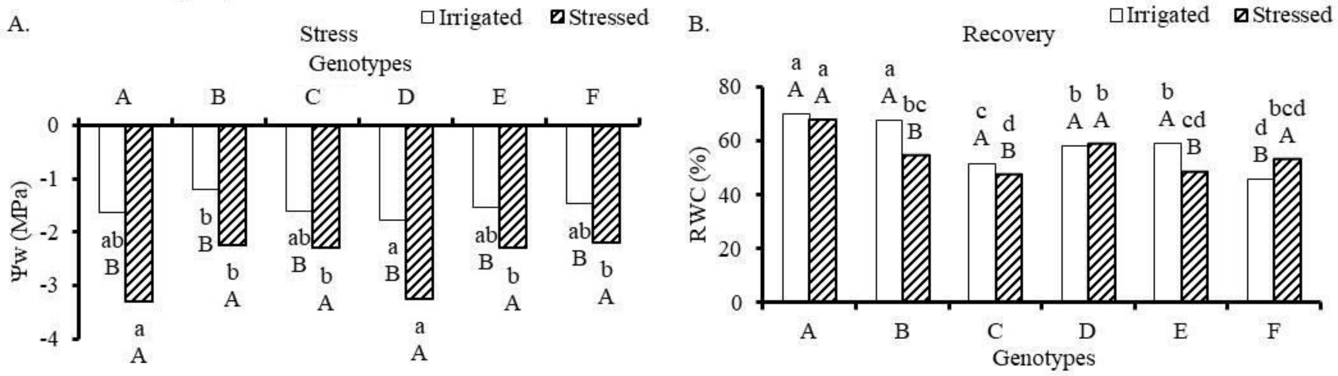
The increment and maintenance of leaf area, observed in the genotypes BRS Seridó, BRS Aroeira and BRS 7MH under water deficit conditions, may be related to the lower variation in the values of the water potential, compared to the control groups. Adequate water supply ensures the basal metabolism to perform photosynthesis and the consequent production of photoassimilates, which allows leaf tissue development (Erice et al., 2010).

After the recovery period, all genotypes, except BRS 368 RF, maintained leaf areas similar to those found in their respective controls (Figure 2B), especially the genotypes CNPA 5M and BRS 286, which recovered their leaf expansion after water reestablishment, suggesting high plasticity of cotton in resuming its growth after rehydration (Figure 2B). Leaf growth regulation under stress conditions and after water recovery is considered a mechanism of adaptation to drought, because the leaves are the most responsive organs to water replacement after the stress period (Erice et al., 2010).

Contrarily, the reductions of leaf area, observed in the genotypes BRS 368 RF, both in stress and recovery periods, and CNPA 5M and BRS 286, during the stress period, may be directly related to the reduction in leaf water potential. Erice et al. (2010) highlight that this effect leads to drying and/or falling of leaves.

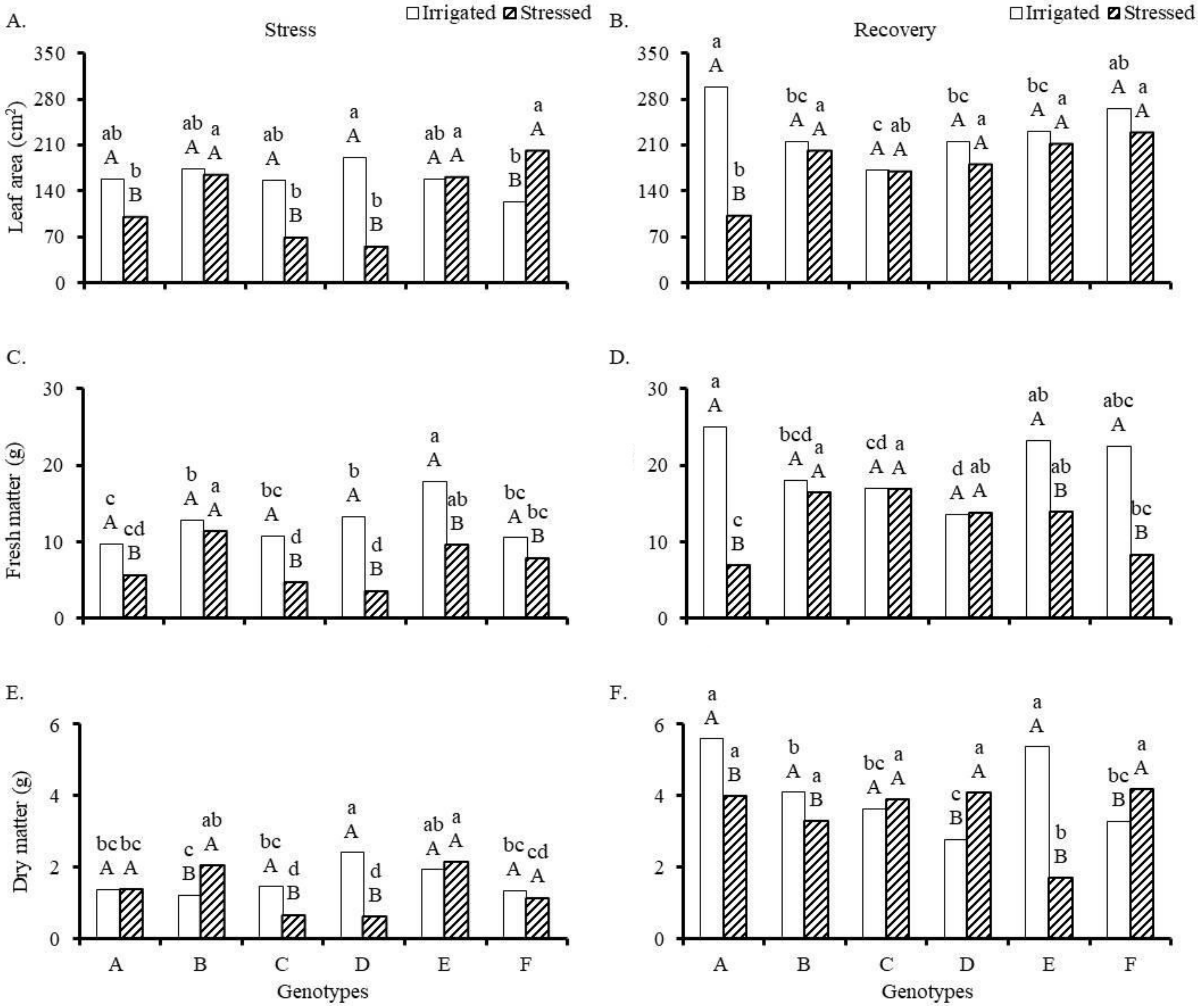
After being subjected to water deficit, all genotypes, except BRS Seridó, showed reductions in fresh matter (FM) (Figure 2C). After the recovery period, the genotypes BRS Seridó, CNPA 5M and BRS 286, from treatments with water deficit, did not differ significantly from their respective controls (Figure 2D).

During the periods of both stress and water recovery, the maintenance of the water status associated with the expansion in the leaf area of these genotypes, have possibly contributed to the maintenance of fresh matter production. Water plays an essential role in shoot fresh matter production, which



Means followed by uppercase letters compare water managements and means followed by lowercase letters compare genotypes by Tukey test ($p \leq 0.05$)

Figure 1. Water potential (Ψw) after 14 days under water deficit (A) and relative water content (RWC) after 22 days of water recovery (B) in cotton genotypes (A - BRS 368 RF; B - BRS Seridó; C - CNPA 5M; D - BRS 286; E - BRS Aroeira; F - BRS 7MH)



Means followed by uppercase letters compare water managements and means followed by lowercase letters compare genotypes by Tukey test ($p \leq 0.05$)

Figure 2. Leaf area (LA) (A and B), shoot fresh matter (FM) (C and D), shoot dry matter (DM) (E and F) after 14 days under water deficit and after 22 days of water recovery, respectively, in cotton genotypes (A - BRS 368 RF; B - BRS Seridó; C - CNPA 5M; D - BRS 286; E - BRS Aroeira; F - BRS 7MH)

guarantees better conditions to metabolize reserves into energy for plant development (Vieira et al., 2013).

The genotypes BRS 368 RF, CNPA 5M, BRS 286, BRS Aroeira and BRS 7MH, under water deficit conditions, had reductions of 41, 56, 72, 46 and 24% in the mean values of FM, respectively, compared to their controls (Figure 2C). Likewise, during the water recovery period, the shoot fresh matter of the genotypes BRS 368 RF, BRS Aroeira and BRS 7MH, from the treatment with water deficit, decreased by 72, 40 and 62%, respectively, compared to their groups under irrigated condition (Figure 2D).

Reduction in shoot fresh matter may be related to the reduction of leaf water potential and restriction of leaf area, especially in the genotypes BRS 368 RF and BRS 286 during the stress period. During the water replacement period, the lack of recovery of fresh matter in the genotype BRS Aroeira may be related to the reduction in its leaf RWC. For Díaz-López et al. (2012), protoplast dehydration decreases cell division and expansion rates, which may cause abscission and reduction of leaf surface, ultimately leading to reduction of fresh matter in the plant.

By evaluating shoot dry matter (DM), under water deficit conditions, it was possible to observe that the genotypes BRS 368 RF, BRS Aroeira and BRS 7MH did not differ from their controls and that BRS Seridó showed a 67% increase in DM, compared to its respective irrigated group (Figure 2E). After the water recovery period, there was a resumption in DM production, especially in the genotypes CNPA 5M, BRS 286 and BRS 7MH, with increments of 7, 47 and 27%, respectively (Figure 2F).

The increase or maintenance in DM production, observed in the genotypes BRS 368 RF, BRS Seridó, BRS Aroeira and BRS 7MH, subjected to water deficit, suggests that leaf area expansion played a fundamental role in the continuity of photosynthetic processes, ensuring the dry matter production in the leaves. Both osmotic adjustment and maintenance of photosynthesis are essential mechanisms for plants to maintain their biomass production under stress (Baghalian et al., 2011).

According to Avramova et al. (2015), the increase in DM production may be related to the resumption of gas exchanges, stomatal conductance, net photosynthesis and incorporation of carbon for the maintenance of leaf growth under favorable water conditions. The same was observed in the present study in the genotypes CNPA 5M, BRS 286 and BRS 7MH, after the rehydration period.

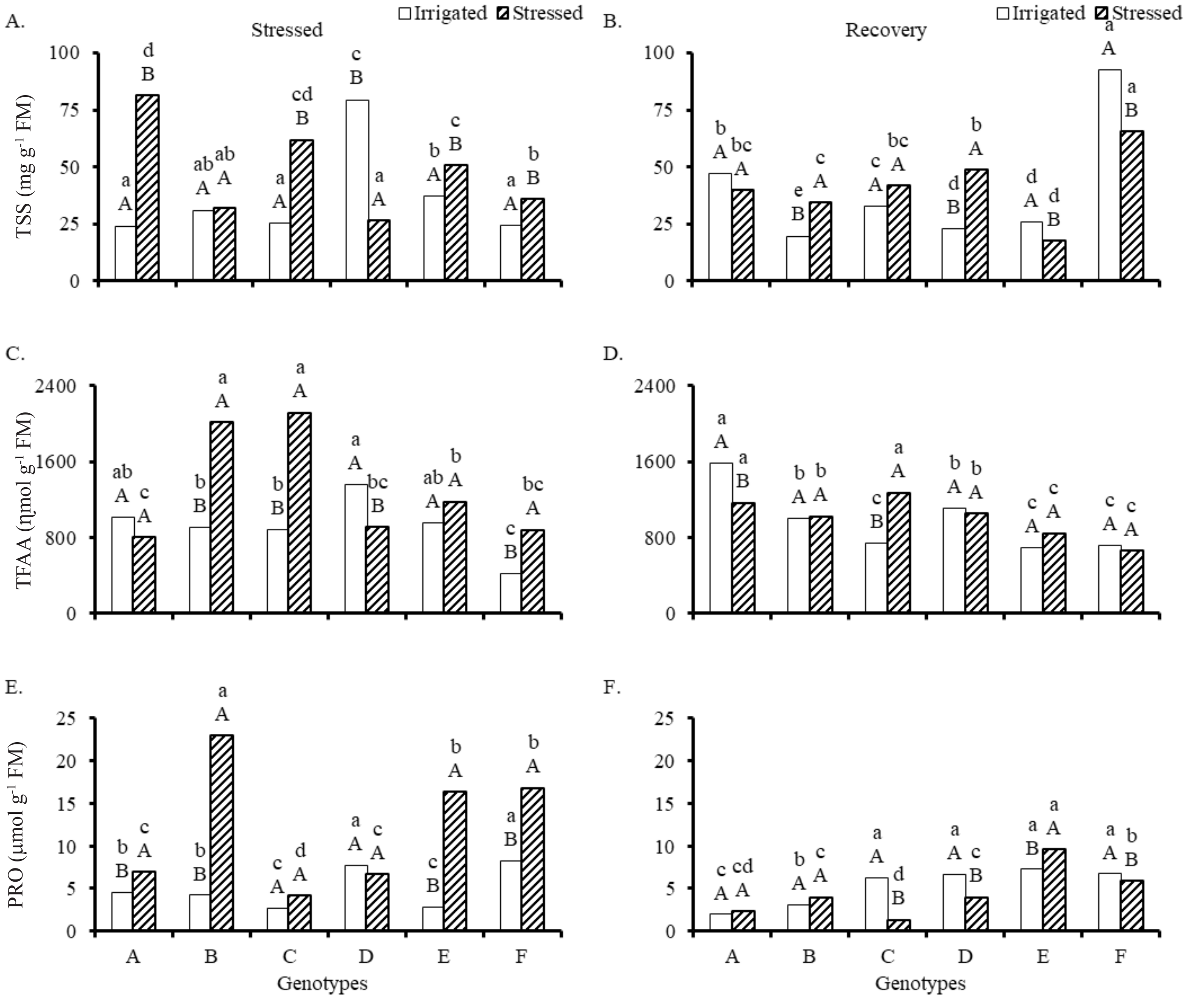
The genotypes CNPA 5M and BRS 286 showed reductions of 54 and 74% in shoot DM production, respectively, under water deficit conditions (Figure 2E). Additionally, during the recovery period, it was observed that the genotypes BRS 368 RF, BRS Seridó and BRS Aroeira, from the treatment with water deficit, also reduced their shoot dry matter production (28, 19 and 68%, respectively), compared to the respective control groups (Figure 2F).

A common adverse effect of water deficit on plants is the reduction in dry biomass production due to the reduction of leaf area, as observed in the genotypes CNPA 5M and BRS 286, during the stress period, and BRS 368 RF, during the recovery period. This reduction is related to the decrease in the efficiency of absorption of light, which is used for photosynthesis and biomass production (Manderscheid et al., 2014).

For total soluble sugars (TSS), it was observed that the genotypes BRS 368 RF, CNPA 5M, BRS Aroeira and BRS 7MH, subjected to water deficit, showed increases of 70, 58, 26 and 32% in TSS concentrations, respectively, compared to the their respective control groups (Figure 3A). Only the genotype BRS 286 showed reduction in TSS concentrations (67%), compared to its control (Figure 3A).

The increase in TSS levels, observed mainly in the genotypes CNPA 5M, BRS Aroeira and BRS 7MH subjected to water deficit, in which there was regulation of leaf water potential, suggests that the accumulation of carbohydrates plays an important role in cotton osmotic adjustment. Osmotic regulation mediated by soluble sugars ensures photosynthesis and adaptability of cotton plants under adverse cultivation conditions (Chen et al., 2011; Farooq et al., 2012).

After water recovery, the TSS levels in the genotypes BRS Seridó and BRS 286, from the treatment with water deficit, increased by 74 and 111%, respectively, compared to their



Means followed by uppercase letters compare water managements and means followed by lowercase letters compare genotypes by Tukey test ($p \leq 0.05$)

Figure 3. Total soluble sugars (TSS) (A and B), and total free amino acids (TFAA) (C and D) and free proline (PRO) (E and F), after 14 days of water deficit and 22 days of water recovery, respectively, for cotton genotypes (A - BRS 368 RF; B - BRS Seridó; C - CNPA 5M; D - BRS 286; E - BRS Aroeira; F - BRS 7MH)

controls (Figure 3B). The increase of TSS in the genotypes BRS Seridó and BRS 286, subjected to water deficit, may have contributed to their osmotic adjustment and RWC recovery during the recovery process. In the study of Parida et al. (2007), the increment of soluble sugars in cotton was related to the maintenance of plant growth during the recovery period and to the reduction in starch content under stress condition.

The genotypes BRS Aroeira and BRS 7MH, from the treatment with water deficit, showed reductions of 29 and 33% in the TSS concentrations, respectively, compared to their controls (Figure 3B). For these genotypes, TSS reduction after the recovery period indicates the plasticity of the crop in regulating the levels of the osmolytes used in its osmotic adjustment. Similar results were found by Parida et al. (2007) in cotton plants under stress and recovery conditions.

Regarding the total free amino acids (TFAA) of the cotton genotypes subjected to water deficit, it was observed that BRS Seridó, CNPA 5M and BRS 7MH were significantly superior to their respective irrigated control groups (123, 139 and 110%, respectively) (Figure 3C). By contrast, the genotype BRS 286 showed reduction of 33% in TFAA concentration, compared to the control group (Figure 3C).

The increase of TFAA concentrations in the genotypes BRS Seridó, CNPA 5M and BRS 7MH, under water deficit conditions, suggests an increase in their capacity to tolerate stress, justifying the lower variation in the mean values of their water potentials. For Ajithkumar & Panneerselvam (2014), the accumulation of amino acids in the plant can act as a compatible osmolyte, maintaining cell turgor with water potentials lower than that of the external environment.

After the water recovery period, it was observed that all genotypes, except BRS 368 RF and CNPA 5M, maintained their mean values of TFAA similar to those observed in the respective control groups (Figure 3D).

According to Parida et al. (2007), the increase in TFAA concentrations during the stress period and their reduction during the recovery period suggest the important role of these osmolytes in cotton osmotic adjustment, besides being an important indicator of drought-induced stress.

The genotypes BRS 368 RF, BRS Aroeira and BRS 7MH, under water deficit conditions, showed increments of 51, 491 and 104% in proline (PRO) concentrations compared to their respective controls (Figure 3E). The genotype BRS Seridó obtained the highest mean value among all treatments and genotypes evaluated (23.02 μmol g^{-1} FM), increasing proline concentration by 5 times compared to its irrigated control (Figure 3E).

The increase of proline contents in the above-mentioned cotton genotypes, under water deficit conditions, can be considered an indication of osmotic adjustment. It should be emphasized that the increase of this solute allows the plant to extract water from the soil, protecting cell integrity and, in addition, it participates in the constitution of nitrogen and carbon stocks readily used in the post-stress period (Ullah et al., 2017).

During the recovery period, it was observed that all genotypes, except BRS Aroeira, reduced their proline concentrations (Figure 3F), especially CNPA 5M, BRS 286 and BRS 7MH, which showed reductions of approximately 78, 40 and 12%, respectively, compared to their control treatments (Figure 3F).

In the present study, not only the accumulation of proline, but also the ability of the plant to catabolize this osmolyte seem to be important strategies during the periods of both stress and recovery. Fast catabolism of proline is considered useful for a better plant recovery from water deficit, because its rapid oxidation to glutamate provides extra energy during the recovery period (Sengupta et al., 2013).

CONCLUSIONS

1. The genotypes BRS Seridó, BRS Aroeira and BRS 7MH withstand better the stress condition due to lower variations in their water potentials promoted by the osmotic adjustment, maintaing their growth under water deficit conditions.

2. The BRS 286 shows greater variations in water potential imparing its growth, and so exibitting sensitivety to water deficit conditions.

3. Water replacement during the recovery period allowed the resumption of growth in most of the genotypes, especially CNPA 5M and BRS 286.

4. After the water recovery period, the variations in the concentrations of the osmoregulators indicate plasticity of cotton plants in regulating the levels of osmoregulators under favorable and unfavorable water conditions.

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