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Photosynthetic Efficiency and Water Status as Determinants for the Performance of Semiarid-Adapted Cotton Cultivars Under Drought in Greenhouse

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Abstract: Searching for crop cultivars that are resilient to environmental stresses is crucial for maintaining global agricultural production. Our study aimed to screen semiarid-adapted cotton cultivars performing well under water-restricted conditions. Trials were conducted in a greenhouse involving six cotton cultivars (FM 911, FM 912, FM 970, FM 974, FM 978, and FM 985) subjected to four water levels (100, 80, 60, and 40% field capacity—FC). At 20 days post-drought imposition, the growth, leaf succulence, osmotic potential, gas exchanges, photosynthetic pigments, and lipid peroxidation were measured. Cotton plants showed reduced growth and gas exchanges at 60% and 40%, displaying elevated sensibility at 40% FC. Under 60% FC, FM 970 and FM 985 exhibited a superior dry biomass, leaf area, and growth, indicating high drought tolerance. FM 911, FM 912, and FM 978 displayed higher rates of net photosynthesis, transpiration, stomatal conductance, and chlorophyll content under 60% and 40% FC, but also demonstrated an increased lipid peroxidation. Additionally, FM 911, FM 970, and FM 974 had the lowest osmotic potential values. Field capacity at 60% and 40% represent moderate and severe drought conditions for cotton. The superior performance of FM 970, FM 978, and FM 985 under drought is attributed to pigment accumulation and photosynthetic efficiency. Our findings suggest that a water-saving strategy with an 80% FC can enhance sustainable production and identify promising cotton cultivars for cultivation in water-scarce regions.

Keywords: *Gossypium hirsutum* L.; plant defense; photosynthetic performance; water restriction

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1. Introduction

Cotton is widely known in some countries as “white gold” due to its economic importance, as the fiber composes an essential raw material for the textile industry, with an annual economic impact of at least USD 600 billion, including cotton lint and by-products. Annually, about 25 million tons of cotton are produced worldwide, with the top ten cotton-producing countries being India, China, the United States, Pakistan, Brazil, Australia, Uzbekistan, Turkey, Turkmenistan, and Burkina Faso [1]. Projections suggest a modest increase in global production, reaching 65 million tons by 2040 [2].

The crop is adapted to cultivation in temperate, subtropical, and tropical climates worldwide, but future climate changes may challenge the plant's metabolism by impairing fruit production, photosynthesis, and respiration [3,4]. Regions experiencing rainfall fluctuations and high temperatures face prolonged droughts that can compromise crucial processes in plants, such as photosynthesis and cell expansion [5]. The situation becomes more critical during the cotton plant's reproductive period, which is a phase that is sensitive to water restriction [6]. This sensitivity results in economic losses, directly affecting fiber production and quality [7].

Exposure to prolonged water deficits can disrupt electron transport processes, leading to oxidative stress—characterized by the increased production of reactive oxygen species (ROS), which have the potential to oxidize cellular components, including proteins, lipids, and DNA, resulting in cell death [8]. Studies on water deficit commonly emphasize the high lipid peroxidation content as a robust indicator of oxidative damage induced by water scarcity in plants. This phenomenon has been reported in cotton [9,10], as well as in apple trees [11].

Throughout evolution, plants have developed biochemical and molecular responses to re-establish homeostasis, which is an essential aspect of the acclimation strategy. Indeed, the accumulation of organic solutes and proteins is crucial for osmotic adjustment [12], thus contributing to the regulation of stomatal opening and transpiration control, which are essential for efficient water use [13]. Additionally, plants possess a sophisticated defense system composed of enzymatic and non-enzymatic mechanisms, which play a crucial role in eliminating reactive oxygen species (ROS), thus contributing to maintaining cellular membrane integrity. The accumulation of proline, small heat shock proteins, abscisic acid, and various phytohormones also play a fundamental role in plant defense [14–16].

In recent decades, to meet the high demand for cotton production, new breeding cotton cultivars were constantly developed, often incorporating traits such as resistance to herbicides, diseases, and pests [3]. However, in many cases, the acquisition of tolerance to a specific biotic stress does not necessarily confer tolerance to abiotic stress. Therefore, evaluating the performance of improved cultivars under water deficit conditions is essential to maximize the crop's potential in semiarid regions. Identifying and characterizing cultivars with a high performance under water deficit conditions becomes essential for maintaining and/or expanding cotton cultivation in semiarid regions and transitional areas [17]. Screening cultivars can immediately assist producers in selecting suitable genetic materials for the climatic conditions of semiarid regions, contributing to the prospect of new drought-tolerant genotypes in cotton breeding programs.

Our investigative study was carried out to test the hypothesis that the photosynthetic efficiency of *Gossypium hirsutum* cultivars from semiarid regions is crucial for plant performance under drought conditions. The hypothesis was tested by subjecting six cotton cultivars to four water levels. Growth parameters and physiological indicators were examined to elucidate the relationship between the tolerance and susceptibility of the tested cultivars.

2. Materials and Methods

2.1. Experimental Area

The experiment was conducted in a greenhouse from 19 November 2022 to 9 January 2023, and later analyses were carried out at the Plant Propagation Laboratory, both situated at the Federal University of Piauí, on the Professora Cinobelina Elvas Campus

(UFPI/CPCE), located in Bom Jesus city, Piauí state, Brazil, under the geographical coordinates 9°04′46″ S and 44°19′38″ W (Figure S1).

2.2. Growing Conditions and Treatments

The experiment was conducted using a completely randomized design in a 6 × 4 factorial scheme, involving six cotton cultivars (FM 985 GLTP, FM 978 GLTP RM, FM 974 GL, FM 970 GLTP RM, FM 912 GLTP RM, and FM 911 GLTP) and four water levels (100, 80, 60, and 40% of field capacity—FC), with four replications.

Samples of a dystrophic yellow latosol were collected from the 0–20 cm layer and were subjected to chemical analysis (Table S1). The pH and fertilization were adjusted as necessary based on saturation by bases described in the soil analysis, following recommendations for cotton cultivation [18]. Then, the soil was used to fill pots with a capacity of 11 dm^{-3}.

Cotton cultivars were selected based on their adaptation and cultivation in regions of semiarid climate (Table S2). Sowing was carried out with five seeds per pot. At 7 days after sowing (DAS), thinning was carried out to ensure two plants per pot. Subsequently, a second thinning was carried out at 14 DAS to ensure only one plant per pot. The plants were irrigated daily to maintain soil moisture equivalent to 100% FC until the treatments were differentiated.

During the trials, the environmental conditions inside the greenhouse were as follows: maximum and minimum temperatures of 30.1 ± 1.8 °C and 25.3 ± 1.5 °C, respectively; relative air humidity of approximately 50.5 ± 4.0%; and a photoperiod of approximately 12 h.

2.3. Irrigation Management

The treatments were applied to plants at 30 DAS. Irrigation management was carried out using the pot-weighing method, based on field capacity, following soil saturation for 24 h. Pots were weighed daily using an electronic scale to monitor water loss through evapotranspiration and replenish the necessary amount of water to achieve pre-established soil moisture regimes, i.e., 100 (control), 80, 60, and 40% FC [19]. The irrigation regimes were maintained for 20 days until the plants reached the flowering stage—the time-point to harvest the plant material.

2.4. Dry Mass, Leaf Area, and Relative Drought Tolerance

During harvest, plants were divided into leaves, stems, and roots. Leaf area was determined using a leaf area meter (model LI-3100C area meter, LI-COR, Inc., Lincoln, NE, USA). Then, plant material was placed in paper bags and kept in a forced-air oven at 65 °C. Subsequently, after reaching a constant mass, the dry mass was determined using a precision balance. The relative stress tolerance index was calculated considering the total dry mass of stressed plants compared to the dry mass of control plants, following adaptations of the methodology in [19,20].

2.5. Leaf Succulence and Osmotic Potential

Leaf succulence was determined using a methodology adapted from [21] and calculated with the following formula:

$$\mathrm{LS}\left(\mathrm{g\ H_2O\ m^{-2}}\right) = \frac{(\mathrm{SFM} - \mathrm{SDM})}{\mathrm{LA}} \quad (1)$$

where SFM = fresh mass of the shoot; SDM = dry mass of the shoot; and LA = leaf area.

Osmotic potential (Ψs) was determined by obtaining clear leaf sap through syringe pressing, followed by an osmolarity reading on a micro-osmometer (Vapor Pressure Osmometer, model 5600, Wescor, UT, USA). Subsequently, Ψs values were obtained using the Van't Hoff equation:

$$\pi = -R \times T \times C \quad (2)$$

where R is the universal gas constant (0.00831 kg MPa mol^{-1} K^{-1}), T is the temperature in Kelvin, and C is the solute concentration [22].

2.6. Gas Exchanges

At 20 days after drought imposition, gas exchange parameters were measured from 08:00 h to 11:00 h on the first fully expanded leaf using an infrared gas analyzer (IRGA, Walz—GFS3000, Effeltrich, Germany) with a coupled fluorometer. The measurements were performed in individual plants, with four repetitions, with a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1} and an internal CO_2 concentration of 400 ppm. The analyzed parameters included net photosynthesis (A), transpiration (E), stomatal conductance (gs), and internal CO_2 concentration (Ci). Using the data, the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation efficiency (A/Ci), intrinsic water use efficiency (A/gs), and instantaneous water use efficiency (A/E) were calculated [18,19].

2.7. Quantification of Photosynthetic Pigments

The photosynthetic pigment extraction, including chlorophyll a (*Chl a*), b (*Chl b*), and total (*Chl* total), as well as carotenoids, was carried out using leaf disks in a dimethyl sulfoxide (DMSO) solution saturated with $CaCO_3$ in the dark for 72 h. Concentrations of pigments were determined based on readings at 665, 649, and 480 nm, applying the formulas proposed in [23].

2.8. Membrane Lipid Peroxidation

The assessment of lipid peroxidation in leaf tissue was conducted by measuring malondialdehyde (MDA) production, which is a by-product of lipid peroxidation. Briefly, 300 g of fresh leaf material was macerated in liquid nitrogen, following a reaction with thiobarbituric acid (Sigma-Aldrich, St. Louis, MI, USA), and absorbance readings were taken at 532 nm and 600 nm [24]. The MDA content was then calculated using the molar extinction coefficient (ε = 0.155 M^{-1} cm^{-1}).

2.9. Statistical Analysis

Data were assessed for normality using the Shapiro–Wilk test, homogeneity of variance was assessed using Bartlett's test, and independence of errors was assessed through a graphical analysis of residuals. Once these assumptions were met, an analysis of variance (ANOVA) was conducted via F-test ($p \leq 0.05$). Mean comparisons were performed using the Scott–Knott test ($p \leq 0.05$), and treatment grouping was conducted via cluster analysis. To evaluate the variation in characteristics across treatments and their relationships with the measured variables, Principal Component Analysis (PCA) was performed. All analyses were conducted using R version 4.3.1 software (R Core Team, 2023, Vienna, Austria).

3. Results

3.1. Biomass Accumulation, Leaf Area, and Relative Tolerance to Drought

The highest dry mass accumulation and leaf area were registered in cultivars growing at 100% FC (Figures 1 and S2). However, in some cases, similar or even higher growth occurred in plants under 80% FC treatments (Figure 1a). In all cases, water restriction significantly decreased the dry biomass of cotton plants, with effects intensified by reducing the water depth. The lowest biomass accumulation was observed in cultivars growing under the most severe water deficit condition (40% FC).

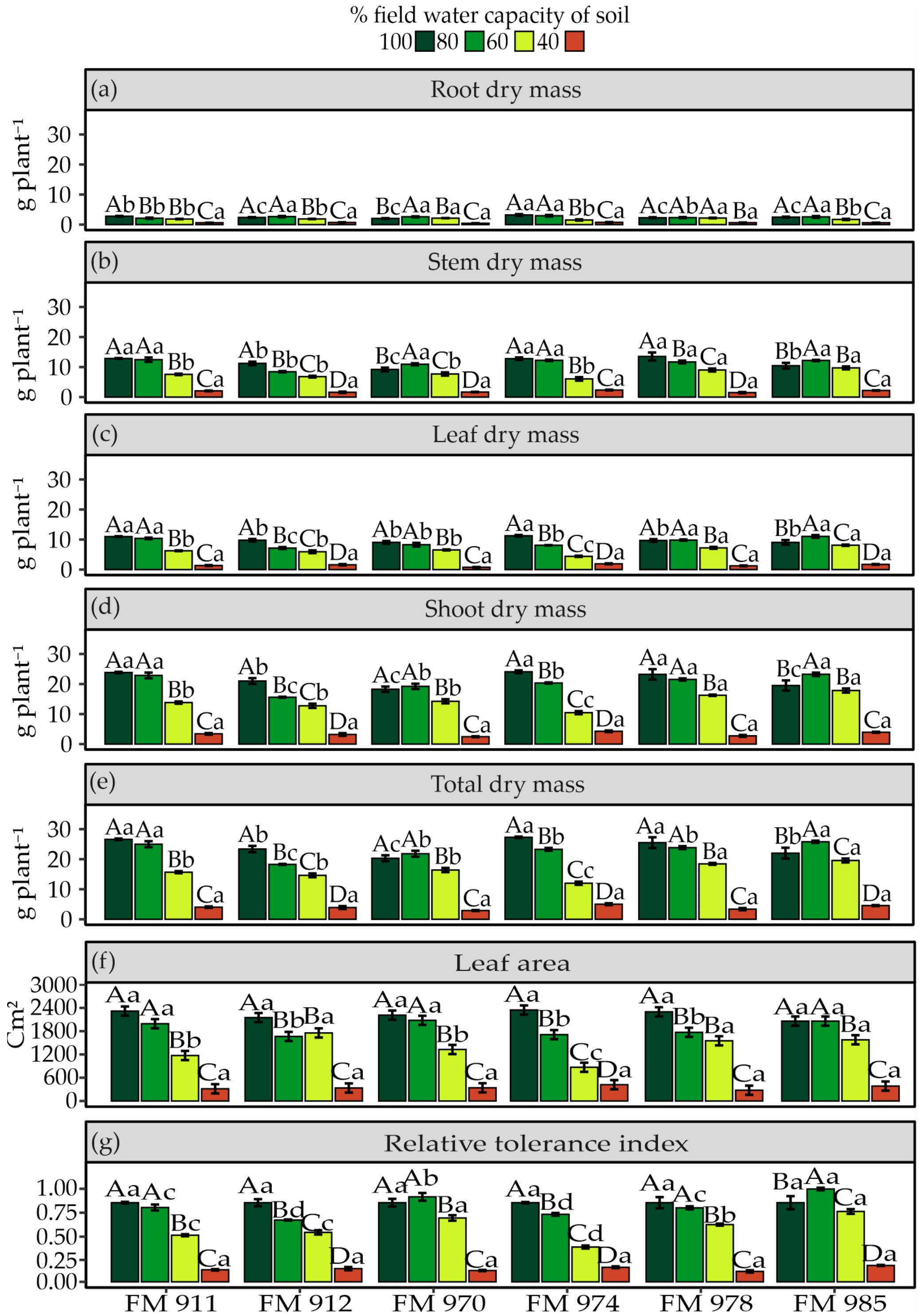

Figure 1. Dry mass of roots (**a**), stem (**b**), leaves (**c**), shoot (**d**), total dry mass (**e**), leaf area (**f**), and drought tolerance (**g**) of cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) grown under different water regimes (100, 80, 60, and 40% of field capacity—FC). The columns represent the mean of four repetitions ± standard error (Mean ± SE). Uppercase letters compare water regimes within the same cotton genotype. Lowercase letters compare cotton genotypes within the same water regime. Different uppercase and lowercase letters indicate a significant difference according to the Scott–Knot test ($p \leq 0.05$).

At 60% FC, cultivars FM 970 and FM 978 exhibited a higher root dry mass accumulation compared to the other studied cultivars. Additionally, cultivars FM 978 and FM 985 displayed superior values for stem, leaf, and total dry mass, as well as leaf area, in comparison to the other cotton cultivars. The lowest growth performance at 60% FC was recorded in FM 974 plants, which showed a lower dry mass and leaf area compared to other cultivars. On the other hand, at 40% FC, little or no significant differences were observed among cotton cultivars, with similar values of dry mass in root and aerial tissues and leaf area (Figures 1 and S2).

The relative tolerance index revealed that the 80% FC water level only restricted the performance of cultivars FM 912 and FM 974, but may stimulate the performance of cultivar FM 985 or keep it unchanged for cultivars FM 911, FM 970, and FM 978 (Figures 1g and S2).

Water levels at 60 and 40% FC drastically decreased the tolerance of cotton plants, with effects being intensified by the reduction in water availability. At 60% FC, the cultivars FM 970 and FM 985 exhibited the highest tolerance, while the lower tolerance indices were observed in cultivars FM 974, FM 911, and FM 912. At 40% FC, all cultivars showed similar tolerance indices, indicating a condition of severe water deficit for cotton (Figures 1g and S2).

3.2. Osmotic Potential, Leaf Succulence, and Lipid Peroxidation

Under well-irrigated conditions (100% FC), lower osmotic potential values were observed in FM 911 and FM 974 compared to other cotton cultivars (Figure 2a). Reductions in osmotic potential due to water restriction were observed only in FM 911, FM 970, and FM 974 plants, particularly at 40% FC compared to 100% FC. Moreover, these cultivars exhibited lower osmotic potential values under water deficit conditions.

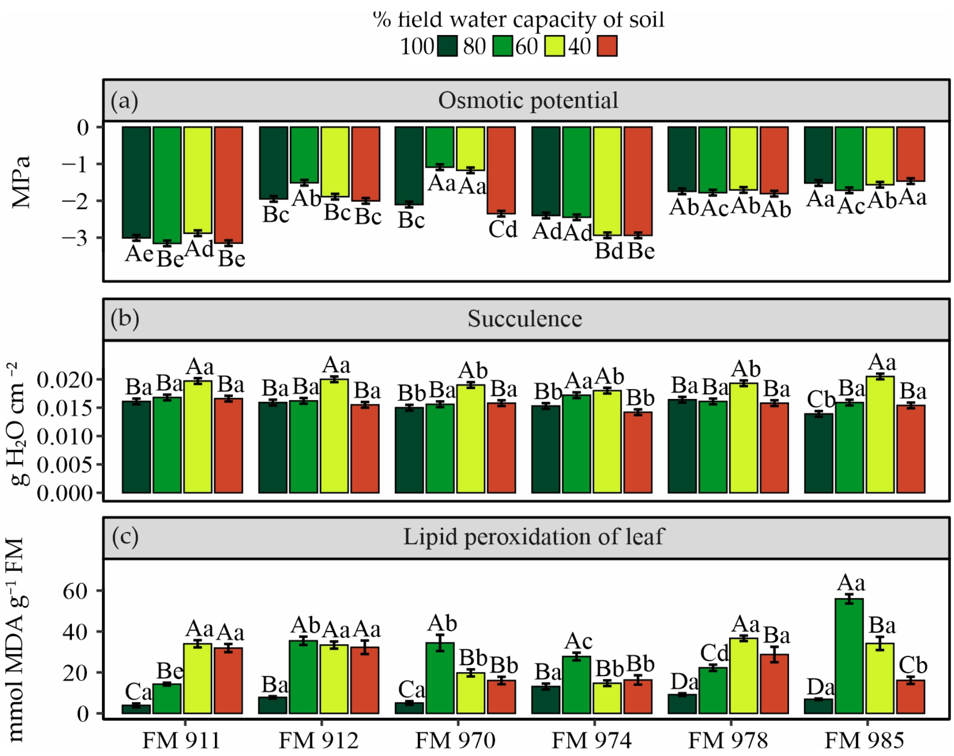

Figure 2. Osmotic potential (**a**), leaf succulence (**b**), and lipid peroxidation (**c**) of cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) under different water regimes (100, 80, 60, and 40% FC). The columns represent the mean of four repetitions ± standard error (Mean ± SE). Uppercase letters compare water regimes within the same cotton genotype. Lowercase letters compare cotton genotypes within the same water regime. Different uppercase and lowercase letters indicate a significant difference according to the Scott–Knot test ($p \leq 0.05$).

In general, all cultivars showed the highest leaf succulence at 60% FC compared to other water treatments (Figure 2b). Under the 60% FC water condition, the FM 911, FM 912, and FM 985 cultivars exhibited higher leaf succulence indices than other studied plants. Under severe water restriction (40% FC), all plants showed reduced leaf succulence values, with the most prominent effects observed in the FM 974 cultivar, whereas little or no changes were observed at 100 and 80% FC.

MDA levels increased in plants grown under lower water regimes (60 and 40% FC) compared to the well-irrigated control, except for the FM 974 cultivar, which showed similar values to the control (Figure 2c). At 60 and 40% FC, the highest MDA levels were recorded in the FM 911, FM 912, FM 978, and FM 985 cultivars compared to other studied plants.

3.3. Photosynthetic Efficiency

The cultivars exhibited distinct regulation in gas exchange and efficiency indices, a response dependent on the soil water level (Figure 3). Surprisingly, plants FM 911, FM 912, and FM 978 showed increases in CO_2 assimilation rates when the soil water level was reduced to 60% of field capacity (FC), compared to their well-irrigated controls at 100% FC (Figure 3a). However, the water level at 40% FC led to severe decreases in CO_2 assimilation rates for all cultivars compared to the control, except for the FM 985 cultivar, which maintained similar rates across all water regimes. At 60% FC, the highest assimilation rates were observed in cultivars FM 911, FM 912, and FM 978, while at 40% FC, the highest rates were recorded in FM 985 plants, followed by FM 970 plants.

The regulation of stomatal conductance and transpiration rates by the water treatments occurred in a practically identical manner to what was observed for CO_2 assimilation among the cotton cultivars (Figure 3b,c). In all cases, the reductions were more conspicuous in plants irrigated at 40% field capacity (FC), and plants FM 911, FM 912, and FM 978 at 60% FC exhibited stomatal conductance and transpiration rates higher than those of well-irrigated treatments.

Herein, a decrease in internal CO_2 concentration (Ci) was observed only in the cultivar FM 912 subjected to stressful conditions (60 and 40% FC), compared to control plants. For FM 912, FM 974, and FM 985, Ci reductions were observed when grown at 40% of FC, distinguishing them from the other cultivars (Figure 3d). On the contrary, the Rubisco carboxylation efficiency (A/Ci) was differentially regulated depending on the cotton cultivar. Notably, the cultivar FM 985 remained unaltered in relation to A/Ci, regardless of water stress conditions, whereas all other cultivars displayed reduced A/Ci values at 40% FC (Figure 3e).

Water regimes did not impact the intrinsic water use efficiency (A/gs), except for the cultivar FM 912, which showed lower A/gs values when subjected to 80% FC (Figure 3f). Regarding instantaneous water use efficiency (A/E), only the cultivars FM 970, FM 974, and FM 985 showed no differences, regardless of the irrigation level used. The cultivar FM 978 was the only one that exhibited lower A/E values under stressful conditions (60 and 40% FC) (Figure 3g).

3.4. Photosynthetic Pigments

Photosynthetic pigments are presented in Figure 4, revealing that the plants exhibited differential behavior depending on the water regime. In general, the main changes caused by drought were observed in *Chl b*, with a decrease in almost all cultivars regardless of the water level, except for cultivars FM 911 and FM 970. The most significant decreases in *Chl a* and carotenoid contents due to drought were observed in FM 985 and FM 970 plants, respectively, compared to well-irrigated treatments (100% FC). Interestingly, the cultivar FM 911 showed similar pigment levels when irrigated at 100%, 60%, and 40% FC, with increases in all pigments when cultivated at 80% FC compared to the control.

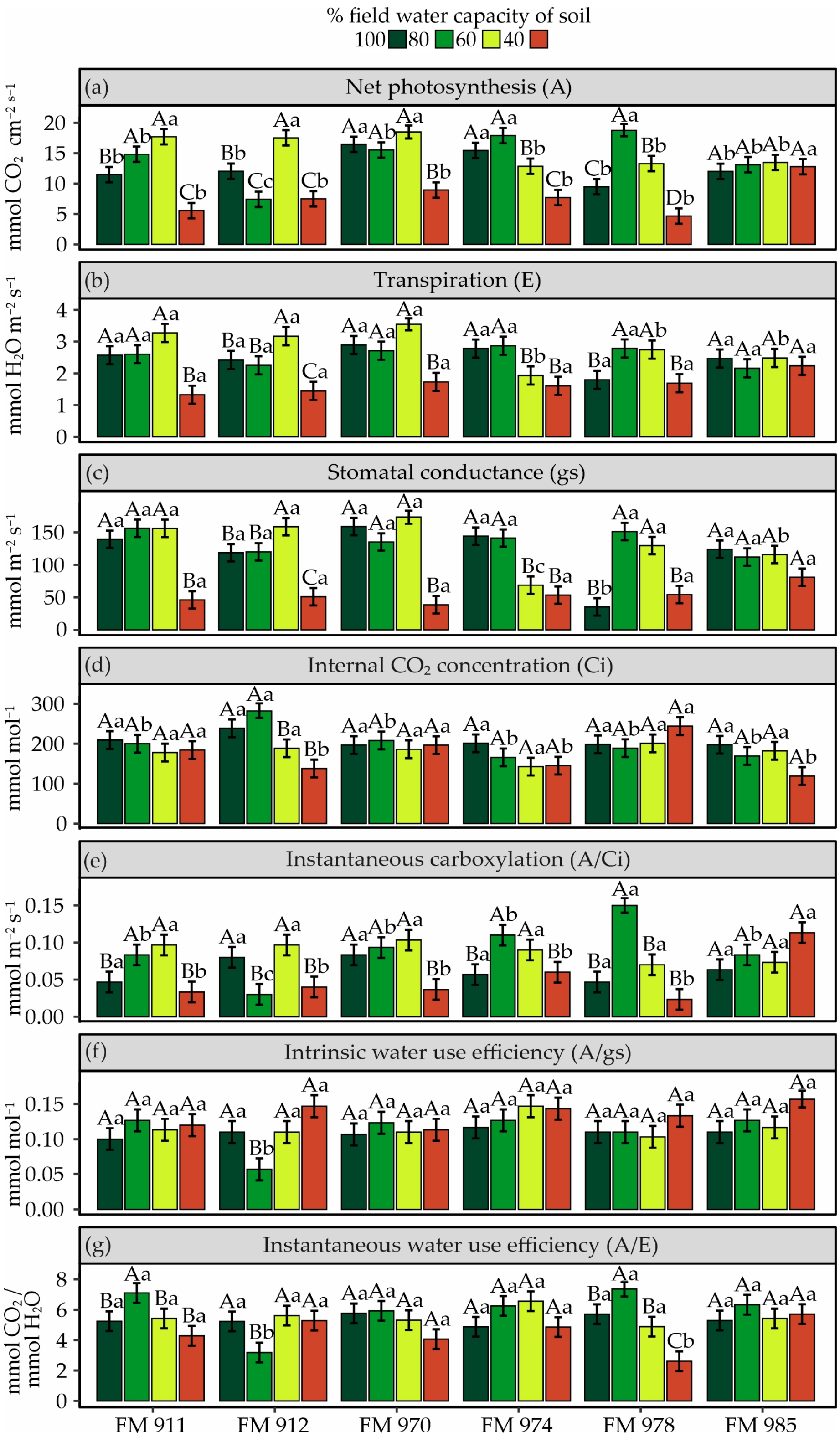

Figure 3. Net photosynthesis (A, **a**), transpiration (E, **b**), stomatal conductance (gs, **c**), internal CO_2 concentration (Ci, **d**), Rubisco carboxylation efficiency (A/Ci, **e**), intrinsic water use efficiency (A/gs, **f**), and instantaneous water use efficiency (A/E, **g**) of cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) under different water regimes (100, 80, 60, and 40% FC). The columns represent the mean of four repetitions ± standard error (Mean ± SE). Uppercase letters compare water regimes within the same cotton genotype. Lowercase letters compare cotton genotypes within the same water regime. Different uppercase and lowercase letters indicate a significant difference according to the Scott–Knot test ($p \leq 0.05$).

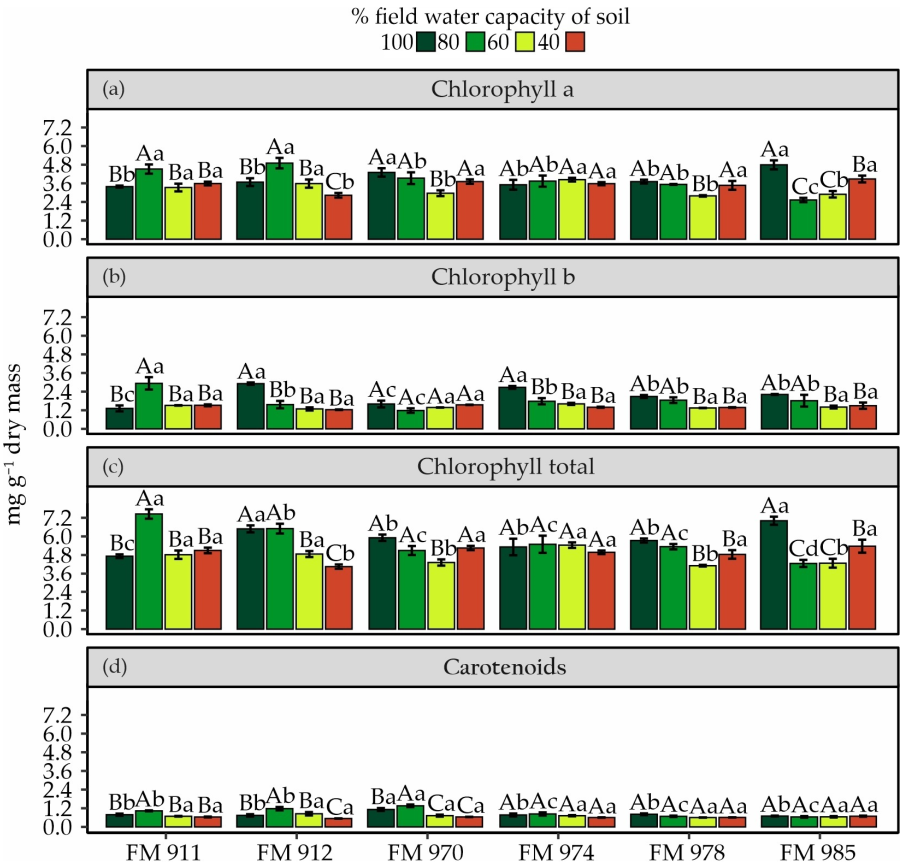

Figure 4. Chlorophyll a (*Chl a*, **a**), chlorophyll b (*Chl b*, **b**), chlorophyll total (*Chl total*, **c**), and carotenoids (**d**) levels of cotton cultivars (FM 911 GLTP, FM 912 GLTP RM, FM 970 GLTP RM, FM 974 GL, FM 978 GLTP RM, and FM 985 GLTP) under different water regimes (100, 80, 60, and 40% field capacity—FC). The columns represent the mean of four repetitions ± standard error (Mean ± SE). Uppercase letters compare water regimes within the same cotton genotype. Lowercase letters compare cotton genotypes within the same water regime. Different uppercase and lowercase letters indicate a significant difference according to the Scott–Knot test ($p \leq 0.05$).

3.5. Principal Component Analysis

To investigate the influence of water deficit on the evaluated parameters and the interrelation among all components, a multivariate analysis was conducted using Principal Component Analysis (PCA) (Figure 5). The data indicated that the assessed parameters were sufficient to identify correlations and the multiple effects of treatments with different water levels on cotton cultivars. The data explain 72.5% of the total distribution, with 57.61% of the data in PC1 and 14.89% in PC2. Three groups are observed, organized randomly based on the cultivar × irrigation level interaction.

The poor performance of cultivars subjected to 40% FC was evident, as indicated by the distinct separation of a group that showed little correlation with biometric parameters such as leaf area, stem dry mass, root mass, and overall aboveground and dry mass. This particular group of plants displayed heightened sensitivity to water deficit conditions, showcasing a negative correlation with relative stress tolerance. A second group comprised all cultivars irrigated with 100% FC, along with the cultivars FM 970, FM 912, and FM 911 at 80% FC. This group demonstrated a strong correlation with photosynthetic pigments (*Chl a*, *Chl b*, total *Chl*, and carotenoids), biomass accumulation (aboveground dry mass, root dry mass, leaf dry mass, stem dry mass, and total dry mass), leaf area, internal CO_2 concentration, and relative stress tolerance. The third group, encompassing all cultivars growing at 60% FC and the cultivars FM 985, FM 978, and FM 974 at 80% FC, exhibited correlations with gas exchange parameters (A, E, and gs), carboxylation efficiencies (A/Ci), water use efficiencies (A/gs and A/E), lipid peroxidation, leaf succulence, and osmotic potential.

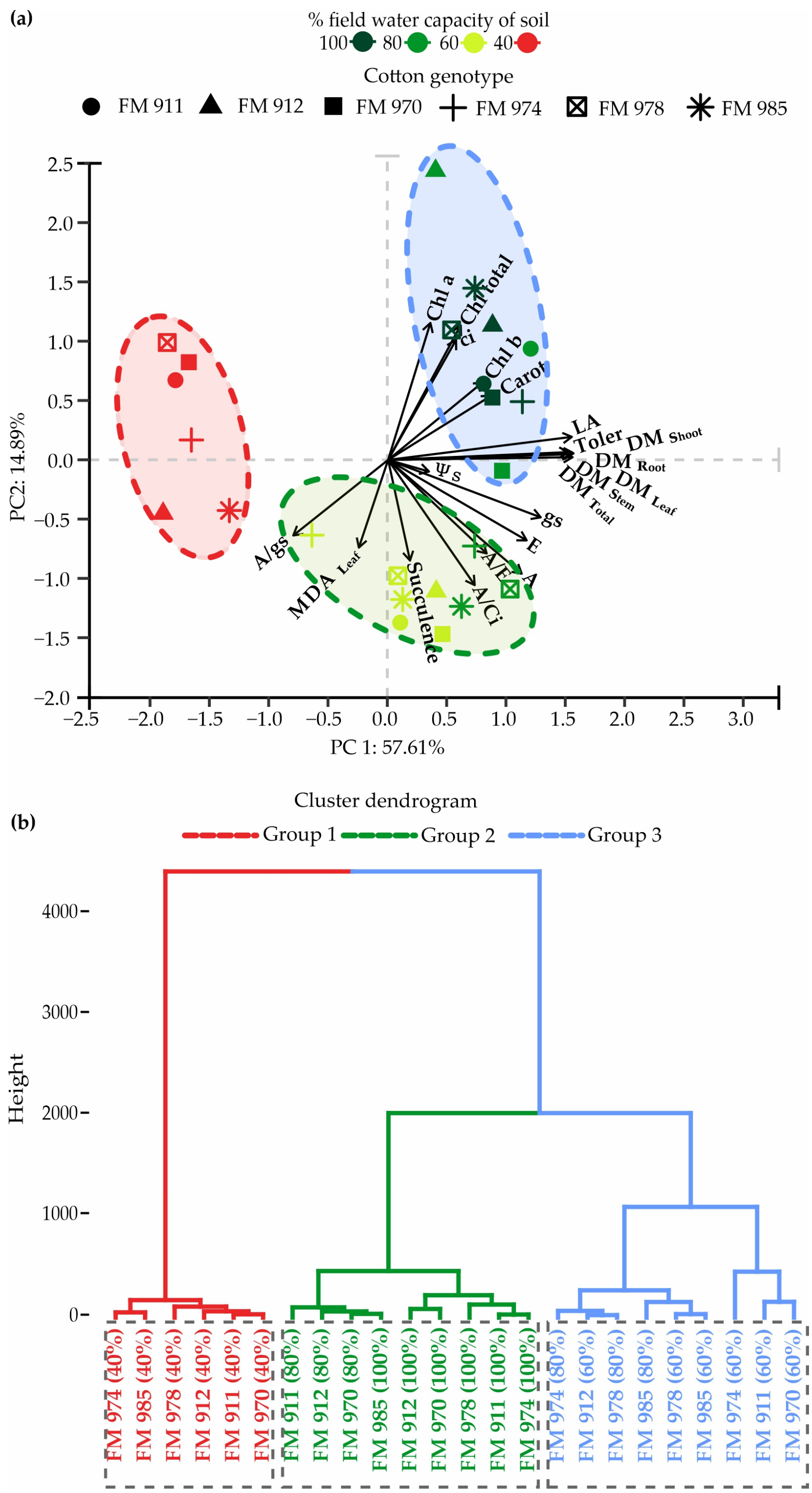

Figure 5. Principal Component Analysis (**a**) and cluster (**b**) for biomass and physiological attributes—total dry mass (DM_{Total}), stem dry mass (DM_{Stem}), leaf dry mass (DM_{Leaf}), root dry mass (DM_{Root}), shoot dry mass (DM_{Shoot}), leaf area (LA), relative stress tolerance (Toler), lipid peroxidation (MDA_{Leaf}), leaf succulence (succulence), osmotic potential (Ψs), chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl* total), carotenoids (Carot), internal CO_2 concentration (Ci), intrinsic water use efficiency (A/gs), Rubisco carboxylation efficiency (A/Ci), instantaneous water use efficiency (A/E), net photosynthesis (A), transpiration (E), and stomatal conductance (gs).

4. Discussion

4.1. Cotton Plants Exhibit a Tolerance Threshold to Water Deficit Depending on the Water Regime

In the current study, several cotton cultivars were exposed to distinct water regimes and exhibited remarkable physiological performance when grown under conditions of abundant water availability. This was evidenced by the vigorous growth observed in the control groups with 100% and 80% FC (Figure S2). Some cultivars showed a similar or even higher performance when grown in 80% FC, such as the FM 970, FM 974, and FM 985 cultivars, reflecting in better water use, management, and overall economy (Figure 5) [25]. Conversely, the FM 970, FM 974, and FM 985 cultivars displayed a reduced growth and leaf area under water deficit conditions (60% FC) compared to well-irrigated plants. Surprisingly, these cultivars demonstrated a higher biomass accumulation than plants subjected to severe drought (40% FC). These findings suggest that even under stressful conditions, plants under 60% FC were able to partially restore the growth performance (Figure 1) [26]. Indeed, plants subjected to moderate drought (60% FC) displayed a heightened leaf succulence, indicating an adaptive response in leaf tissues to dehydration. This noteworthy phenomenon, particularly observed in the FM 911, FM 912, and FM 985 cultivars (Figure 2b), suggests a possible isohydric response, which may contribute to the mitigation of water deficit effects [27,28].

Our findings also emphasize that a 40% field capacity condition should be recognized as severe drought stress for cotton (Figure S2). This corroborates with all six tested cultivars showing a lower growth performance, as evidenced by reduced dry biomass, leaf area, and decreased levels of relative stress tolerance (Figures 1 and 5). Through these results, it is possible to categorize the field capacities of 100 and 80% as control conditions, and 60 and 40% FC as moderate and severe drought, respectively, for cotton cultivation.

4.2. Photosynthetic Machinery Efficiency Appears to Be a Determinant for Better Cotton Performance Under Water Deficit Conditions

The observed increase in net photosynthesis across all cultivars under 60% FC can be attributed to the interception of solar radiation optimization and efficient distribution within the plant canopy (Figures 1 and 3a). This improvement is noteworthy, especially considering the reduction in growth and subsequent decrease in shading under these conditions. It is relevant to highlight the close relationship between A (photosynthesis rate) and gs (stomatal conductance).

Several studies have cited stomatal conductance as an indicator of CO_2 availability in mesophyll cells, directly influencing the net photosynthesis and constituting important indicators for drought sensibility [29]. In agreement, for some cotton cultivars, the decrease in stomatal closure was associated with reduced photosynthesis, as reported in plants grown at 40% FC. However, the cultivars FM 985 and FM 970 showed important responses to drought, showing little or no variation in net photosynthesis and stomatal conductance under limited water conditions. Conversely, the cultivar FM 911 demonstrated contradictory responses to water treatments, with a decrease at 100, 60, and 40% FC compared to 80% FC, suggesting high sensibility to water depth around the roots (Figures 1 and 5).

The correlation observed between the decline in A, gs, and E rates (Figure 3a–c), as well as the decrease in water use efficiency (A/Ci) (Figure 3e), suggests the involvement of stomatal factors rather than non-stomatal traits [30,31]. The data suggest that factors related to water status, low CO_2 concentration, high temperatures, and CO_2 fixation prompt plants to rapidly close stomata [32]. Consequently, the decrease in Ci due to low stomatal conductance indicates the limited availability of substrate for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and explains the decreased photosynthetic performance of FM 912, FM 974, and FM 985 cultivars under drought conditions (40 and 60% FC) compared to other cultivars studied (Figure 3d).

Photosynthetic pigments play a crucial role in the photosynthetic machinery; *Chl a*, present in the antenna complexes, maximizes the capture of available light and is directly linked to photosynthesis [33]. However, despite maintaining an elevated content of photosynthetic pigments, the FM 970, FM 974, and FM 978 cultivars showed decreases in A. This response was associated with decreased gs, demonstrating that photosynthesis impairment was probably due to stomatal limitations [34].

In most plant species, *Chl a* and carotenoids play crucial adaptive roles in plants subjected to dry conditions, stimulating PS I activity, cyclic phosphorylation, and preserving thylakoid membrane integrity [35]. These compounds play versatile roles in photosynthetic efficiency, encompassing photoprotection, energy transfer, and the regulation of the balance between light absorption and photosynthesis, also acting in ROS scavenging [26,33]. Herein, *Chl a* and carotenoid accumulation reinforces the idea of the importance of compounds having a greater tolerance, as exemplified by the FM 970, FM 974, and FM 978 cultivars. These cultivars displayed a good balance between A, E, and Ci. Thus, our findings evidence that the standout performance of the FM 970 cultivar under stressful conditions (60% FC) was closely related to carotenoid accumulation, which acts in the form of antioxidants and protectors of photosystems.

4.3. The Reduction in Oxidative Damage in Cotton Appears to Be More Relevant for Stress Tolerance than Osmotic Adjustment

Malondialdehyde (MDA) content serves as a crucial marker for lipid peroxidation primarily caused by ROS accumulation and subsequent oxidative stress [36]. In our study, MDA content increased in sensitive cultivars as water availability decreased (60 and 40% FC), as observed in FM 911 and FM 912 plants. In contrast, tolerant cultivars (FM 970, FM 974, and FM 985) showed a lower MDA content under the same water conditions (Figure 2c). This variance in lipid peroxidation may be attributed to enzymatic and non-enzymatic defense mechanisms, which act to scavenge the free radicals [37,38]. The lower lipid peroxidation reinforces the elevated performance and high drought tolerance of the FM 970 and FM 985 cultivars, suggesting an indicator of drought tolerance for cotton. On the contrary, the FM 911 and FM 912 cultivars exhibited greater sensitivity when soil moisture decreased.

Our data evidence that lipid peroxidation is poorly correlated with osmotic adjustment, as there was minimal alteration in osmotic potential and leaf succulence in the studied cultivars (Figure 2a,b). Our data reveal that cultivars FM 978 and FM 985 present other mechanisms for drought acclimation at 60% FC (Figure 1), once no significant decrease in the osmotic potential was registered (Figure 2a). On the other hand, osmotic adjustment in FM 912 plants grown under 60% FC may have contributed to a better leaf area and reduced biomass losses if compared to those grown under 80% FC (Figure 1f). Therefore, the activation of response mechanisms depends on the characteristics of each cultivar, and amino acid synthesis may be an intrinsic factor of each genotype triggering these responses [39]. Additionally, through the regulation of osmotic adjustment, plants can adapt to the environment by making better use of water and adjusting photosynthesis [40].

5. Conclusions

The data reveal that cotton cultivars display distinct responses under water restriction and exhibit high sensitivity to a 40% FC water deficit, representing a severe stress condition. The 80% FC water depth proves to be a viable strategy for cultivating FM 970, FM 974, and FM 985 cotton cultivars, showing a similar or superior performance compared to plants under 100% FC. The data suggest water saving properties, aligning with the objectives of sustainable development. The 60% FC water depth represents moderate water stress and, among the studied cultivars, FM 970, FM 978, and FM 985 plants exhibit greater stress tolerance. The superior performance of cotton cultivars under drought is associated with the accumulation of pigments and photosynthetic efficiency, resulting from lower oxidative damage to aerial tissues. The sensitivity of cultivars FM 911 and FM 912 was evident with a drastic reduction in biomass, a higher lipid peroxidation, and a lower photosynthetic performance.

Our findings suggest that a water-saving strategy with 80% FC is capable of maintaining sustainable cotton production, indicating promising cotton cultivars for cultivation in water-scarce regions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15020500/s1. Figure S1: Map of the Brazilian territory highlighting the location of Bom Jesus, PI; Figure S2: Phenotypical appearance of cotton cultivars; Table S1: Soil analysis results collected from the 0–20 cm layer for filling the pots; Table S2: Cotton cultivars grown in agricultural areas of the Brazilian Cerrado.

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Abbreviations

ROS	Reactive oxygen species
FC	Field capacity
DAS	Days after sowing
LS	Leaf succulence
SFM	Fresh mass of the shoot
SDM	Dry mass of the shoot
LA	Leaf area
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl total	Chlorophyll total
MDA	Malondialdehyde
ANOVA	Analysis of variance
PCA	Principal Component Analysis
PS	photosystem

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