# Physiological traits for drought phenotyping in cotton

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**ABSTRACT.** The objective of this study was to identify physiological traits that could distinguish between cotton genotypes that were tolerant or sensitive to water deficits. The experiment was conducted in a completely randomized design through a factorial combination to analyze four genotypes (BRS 187 8H and ACALA SJ-4 - water deficit tolerant; CNPA 7H and SU-0450/8909 - water deficit sensitive) and two water regimes (watered/always irrigated and stressed/with a water deficit imposed at flowering). Irrigation was suspended for the plants in the water deficit treatment groups when their first flowers appeared. Leaf water potential ($\psi$pd) was monitored until the plants reached -3.0 MPa predawn, at which point leaf samples were collected for analysis. The plants were re-irrigated and monitored for a recovery to 50% of leaf water potential. The maximum photochemical efficiency (*Fv/Fm*), chlorophyll content (SPAD index), relative water content (RWC), disruption of the cell membrane via membrane leakage, carbon isotope composition ($\delta^{13}$C), seed cotton yield and fiber quality were evaluated. The trends in membrane leakage and carbon isotope composition were different between the tolerant and sensitive genotypes under a water deficit, which makes these physiological traits suitable for screening for tolerance to water deficits in cotton.

**Keywords:** abiotic stress, *Gossypium*, water deficit.

**RESUMO. Traços fisiológicos para fenotipagem de algodoeiro sob seca.** Objetivou-se identificar variáveis fisiológicas para distinguir genótipos de algodoeiro tolerantes e sensíveis ao déficit hídrico. O experimento foi conduzido no delineamento inteiramente casualizado em arranjo fatorial, sendo testados quatro genótipos (BRS 187 8H e ACALA SJ-4 – tolerante ao déficit hídrico; CNPA 7H e SU-0450/8909 - sensíveis ao déficit hídrico) e dois regimes hídricos (controle – sempre irrigado e com déficit hídrico imposto na emissão da primeira flor. Na emissão da primeira flor, a irrigação foi suspensa para o grupo a ser submetido ao déficit hídrico. O potencial hídrico foliar foi monitorado na antemanhã até que as plantas dos cultivares em estudo atingissem -3,0 MPa, ponto no qual coletaram-se amostras foliares para análises fisiológicas. Após, irrigou-se as plantas até a obtenção de valor superior a 50% do potencial hídrico foliar (-1,50 MPa). Avaliaram-se a eficiência fotoquímica máxima (*Fv/Fm*), o conteúdo de clorofila via índice SPAD, o conteúdo relativo de água (CRA), o extravasamento de eletrólitos, a composição isotópica do carbono ($\delta^{13}$C), o rendimento de algodão em caroço por planta e a qualidade da fibra. A disruptura de membrana via extravasamento de eletrólitos e a $\delta^{13}$C apresentaram-se como potenciais indicadores fisiológicos visando a seleção de genótipos de algodoeiro tolerantes ao déficit hídrico.

**Palavras-chave:** estresse abiótico, *Gossypium*, déficit hídrico.

## Introduction

Drought is one of the major ecological factors limiting crop production and food quality globally, especially in the arid and semi-arid areas of the world. Recent evaluations have shown that approximately 64% of the world's soils are located in desert or in areas with limited water availability and that 57% of the potentially arable area is located in soils for dry-land crops (FAO, 2000).

Globally, reductions in yield in all arable regions are periodically noted due to the effects of drought, and the tendency for climate changes may increase this phenomenon (LE HOUEROU, 1996).

It is known that the quantity and quality of the fiber produced in cotton plant crops are directly related to water availability during the different phenological phases of development. Genetically equivalent cotton plant populations, when submitted

to water deficits, show reductions in yield of up to 50% if compared to those that have been irrigated, especially when the stress factor is imposed during the period between flowering and fructification (ARAÚJO et al., 2003). This finding shows the impact of this factor on the physiology and the development of the cotton plant. Thus, the tolerance of cotton genotypes to water deficits has been the target of various studies on both the physiological and the molecular levels (LEVI et al., 2009; LUBBERS et al., 2007; SARANGA et al., 2004).

Some researchers have shown that the cotton plant is characterized by genetic variability in its tolerance of water deficits and high temperatures (QUISENBERRY et al., 1982). However, the majority of cultivars from modern cotton plants have been developed under irrigated conditions and have undergone intensive screening to increase the yield and quality of the fiber produced, as well as screening to obtain the most adequate genotypes for mechanized harvesting and processing, which are often improved under irrigation conditions. Thus, the screening process under this condition has unintentionally led to a narrowing of genetic variability for stress factors (ROSENOW et al., 1983), increasing demand for the supply of water via irrigation. Currently, water availability for use in agriculture is increasingly limited and expensive, creating apriority impetus for research projects that can identify genotypes with more efficient use of water and/or greater tolerance to water-deficit conditions. Thus, identifying physiological traits that can be assessed in a fast and/or non-destructive manner and can characterize accessions in germplasm banks may assist cotton breeding programs in obtaining cultivars that are more tolerant to water deficits or that may even be used in the initial phases of a breeding program.

Although photosynthesis rates have been used to distinguish water deficit tolerant and sensitive genotypes in various species, including cotton (LEVI et al., 2009; SARANGA et al., 2004), procedures for measuring gas exchanges in photosynthetic analysis are laborious and impractical for use in a breeding program (EARL; TOLLENAAR, 1999). In studies with the barley crop, it was noted that the use of indirect and quick methods for determining photosynthetic activity, as well as the measuring of chlorophyll fluorescence, can identify genotypes that are tolerant and sensitive to water deficits and take less time when compared to that techniques for measuring gas exchanges (RONG-HUA et al., 2006). Other physiological traits, such as relative water content (RWC)

(COLOM; VAZZANA, 2003), rupture of the cellular membrane via electrolyte leakages (MATA; LAMATTINA, 2001) and carbon isotope composition ($\delta^{13}C$), can be used as indirect indicators for water usage efficiency (FARQUHAR; RICHARDS 1984; SARANGA et al., 2004) and demonstrating high correlation to water deficit tolerance (HALLIWEL; GUTTERIDGE, 1984; SILVA et al., 2007).

Each species can trigger distinct response mechanisms to water deficits, which makes the efficiency of these variables in distinguishing tolerant and sensitive genotypes dependent on the species studied (COLOM; VAZZANA, 2003; HALLIWEL; GUTTERIDGE, 1984; O'NEILL et al., 2006; RONG-HUA et al., 2006; SILVA et al., 2007). Therefore, this study aimed to identify physiological traits related to the tolerance of water deficits that could distinguish sensitive and tolerant cotton plant genotypes.

## Material and methods

The experiment was conducted at a greenhouse at Embrapa Algodão, located in Campina Grande city, Paraíba, Brazil. The photosynthetic photon flux density (PPFD) was monitored by a Hobbes sensor, and the data were stored in a datalogger. At noon, the PPFD averaged 900 μmol m$^{-2}$ s$^{-1}$. The experiment was conducted in a completely randomized design in a factorial combination that analyzed four genotypes (BRS 187 8H and ACALA SJ-4 - water deficit tolerant; CNPA 7H and SU-0450/8909 - water deficit sensitive) and two water regimes (watered/always irrigated and stressed/with a water deficit imposed at flowering). The experiment consisted of a factorial combination of four cultivars (BRS 187 8H, Acala SJ-4, SU-0450/8909 and CNPA 7H) in a completely randomized trial with four replications. There were four cotton genotypes (*Gossypium hirsutum* L.r. latifolium Hutch), two that were considered tolerant (BRS 187 8H and ACALA SJ-4) and two that were sensitive to water stress (CNPA 7H and SU-0450/8909), according to preliminary test results (unpublished data). The experimental unit consisted of a large polyethylene pot with a capacity of 30 L of substrate, filled with a mixture of washed sand and peat (1:1, v v$^{-1}$). The seeds were obtained from Embrapa Cotton's Active Germplasm Bank, and two seeds were planted per pot, leaving one plant per pot after thinning on the twelfth day after sowing. After emergence, every 2 weeks, 0.5 L of half-strength "Hoagland" solution (HOAGLAND; ARNON, 1950) was applied to supply nutrients. The plants

were irrigated regularly with water potential maintained at the substrate capacity until the pre-flowering stage (44 days after emergence). At first flower emission, when plants were 45 days old, plants of each cultivar were separated in two groups; one continued to receive regular irrigation (control plants), and water was withheld from the other (water deficit-stressed plants). For control plants, leaf predawn water potential ($\Psi$pd), as measured periodically with a Scholander-type pressure chamber, was always above -0.2 MPa. The water deficit was allowed to progress until $\Psi$pd reached about -3.0 MPa at predawn, when leaves samples were collected for analysis. Subsequently, the plants were re-irrigated, and the recovery of at least 50% of $\psi$wf (-1.50 MPa) was monitored for further physiological analysis.

After the suspension of irrigation, leaf water potential at predawn (between 4:30 and 5:00 a.m.) was determined every three days using an Oregon Corvallis pressure chamber, 97330 (pms Instrument CO). A leaf from the upper portion of the middle third of each plant with four plants per treatment were used; leaves were always collected from the same position on the plant on each measurement date. Measurements of water potential were made as indicated by Marur (1999) by using leaves opposite to those used for the evaluation of chlorophyll fluorescence *a* and from the SPAD index.

The photochemical efficiency of photosystem II (PSII) was determined at the ambient temperature in leaves adapted to darkness for 30 minutes using the ratio between variable fluorescence and maximum fluorescence ($F_v/F_m$) measured with a fluorometer (PEA Hansatech, Norfolk, United Kingdom) following a protocol proposed by Levi et al. (2009). All the above measurements were carried out at ambient (400 $\pm$ 10 $\mu$mol mol$^{-1}$) $CO_2$, 25 $\pm$ 2°C air temperature and 70 $\pm$ 2% relative humidity.

The leaf chlorophyll content (SPAD index) was estimated non-destructively for four leaves per treatment, using the SPAD-502 portable chlorophyll meter (Minolta Camera Co., Osaka, Japan), following a protocol proposed by Levi et al. (2009). This index was used due to the strong relationship between the read values from this chlorophyll meter and leaf chlorophyll content, as demonstrated by Torres Neto et al. (2005). The average of five measurements taken from different plants in each plot was recorded.

After the readings from the SPAD and *Fv/Fm*, leaf discs were collected from these same leaves with a punch. It was determined for one leaf per plant with four plants per treatment, when the plants

reached -3.0 MPa (maximum stress) and -1.50 MPa after re-irrigation. The leaf discs (113 mm$^{-2}$) were collected and then washed briefly three times in deionized water to remove solutes released during cutting of the discs. Ten discs of each leaf were then placed in Petri dishes and immersed in 10 mL of deionized water. The plates were sealed and stored at 25°C for 2 hours. After incubation, the conductivity in the medium (Xi) was determined using a bench conductivity meter (W12D, BEL ENGINEERING, Italy). Then the samples were subjected to a temperature of 80°C for 90 minutes, and the conductivity was measured again (Xt). Electrolyte leakage was expressed as the percentage of conductivity for the total conductivity after treatment for 90 minutes at 80°C [(Xi/Xt) x 100] (SCOTTI CAMPOS; THU PHAM THI, 1997).

Following the same procedure described above, after the readings from the SPAD and from *Fv/Fm*, leaf discs were collected with a punch from the same leaves to determine the relative water content (RWC). The RWC was determined when the plants reached -3.0 MPa (severe stress) and -1.50 MPa after re-irrigation following protocol proposed by Bars and Weatherley (1962). The RWC was measured in ten discs (113 mm$^{-2}$ of each leaf). The leaf discs were obtained in a similar manner to that used for electrolyte leakage. Then, the fresh weight of the discs was determined, and they were immediately placed in Petri dishes approximately 9.0 cm in diameter, containing 10 mL of deionized water. These dishes containing the discs were subjected to a temperature of 25°C and light intensity of 12 $\mu$mol m$^{-2}$ s$^{-1}$ in order to obtain their turgid weight. After 4 hours of exposure, the leaf discs were dried with the aid of filter paper to eliminate excess surface water, and they were then weighed to obtain their turgid weight. To obtain the dry weight, the leaf discs were subjected to a temperature of 60°C for 48h. Based on the fresh weight (FW), dry weight (DW) and turgid weight (TW) of leaf discs, RWC was calculated using the following equation: RWC = FW -DW/ TW - DW x 100.

The carbon isotopic composition of the leaves was determined with a mass spectrometer (DELTA-S Finnigan Mat, Bremen, Germany). The isotopic composition of the atmosphere was considered to be -8‰ in relation to the international standard, PDB (Pee Dee Belemnite), as described by Saranga et al. (1999). Values were expressed as $\delta^{13}C$ (‰) = (R sample /R reference - 1) x 1000, where R is the ratio between $C^{13}/^{12}C$. A secondary standard calibrated against a fossil belemnite from Pee Dee formation was used for comparison.

The analysis of the physical quality of the cotton fiber was performed by the High Volume Instrument, model 900, Spinlab/Zellweger Uster, and the following technological characteristics of the staple were determined: fiber percentage (%Fi), fineness (index micronaire) in µg, strength in tex$^{-1}$ STR (tex is a metric unit of resistance that is equivalent to 9 denier, which is a unit equivalent to the mass of 1 g per 9 km of fiber) and length in mm (UHM) (ASTM D4605-86, 1995). Before the analysis, using the international standard ISO 139/ASTM D 1776/NBR 8428-84 for fiber analysis, the samples were kept for 24 hours in the laboratory, at a temperature of 20 ± 1°C and a relative humidity of 65 ± 2% until arriving at hygrometric equilibrium (acclimatation).

The data were subjected to analysis of variance (ANOVA, $p < 0.05$), and when significant differences were detected, a comparison of means was conducted using Tukey's test ($p < 0.05$). A contrast in the average of the principal effects for genotypes that compared the tolerant group to the water deficit-sensitive group was also made ($p < 0.05$) using SISVAR software (FERREIRA, 2008).

## Results and discussion

In control treatment, leaves were always kept under irrigated leaf water potential (above -0.2 MPa at predawn). The genotypes considered sensitive (CNPA 7H and SU-0450/8909) reached a leaf water potential of -3.0 MPa in the 16th day after suspension of irrigation. However, for the tolerant genotypes, there was variation in the time required to achieve a water potential of -3.0 MPa. Genotype ACALA SJ-4 reached this potential 12 days after suspension of irrigation, while genotype BRS 187 8H reached this foliar water status 18 days after suspension of irrigation (Figure 1A). After re-irrigation, the tolerant genotypes (BRS 187 8H and ACALA SJ-4) recovered 50% of leaf water potential in about 3 hours, while the sensitive genotypes (CNPA 7H and SU-0450/8909) recovered 6 hours after re-irrigation (Figure 1B).

The timing of the progression of drought and the ability of plants to recover after being subjected to short and/or prolonged periods of drought are closely related to crop yield, when considering that processes such as cell expansion, gas exchange, and photochemical efficiency of PSII are affected by increasing water deficit. Marur (1999) verified that the reduction in osmotic potential is a consequence of the net accumulation of solutes in the symplast, resulting in

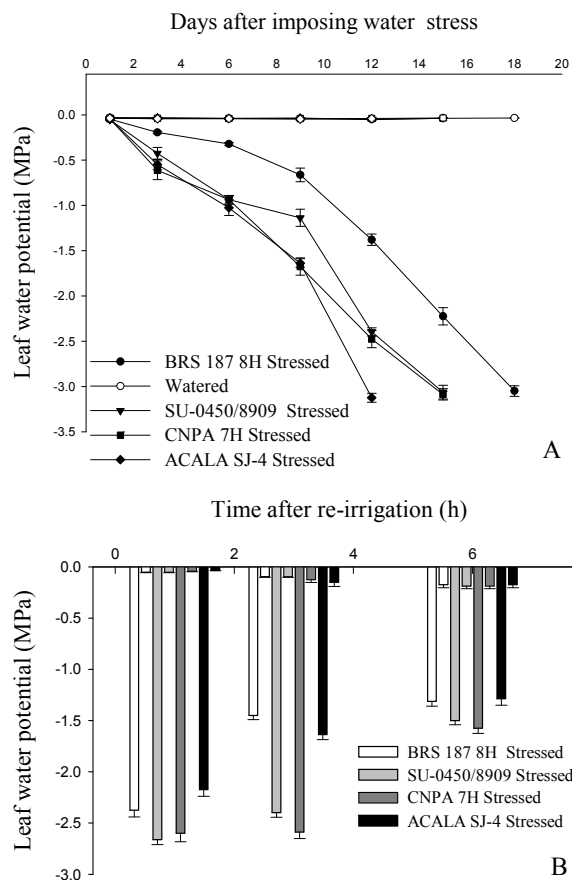reduced sugar content in response to reduced water potential.



**Figure 1.** Leaf water potential in cotton genotypes monitored at predawn, showing a progressive reduction of the leaf water potential of the evaluated genotypes under the two water regimes. The watered treatment was always maintained upper at -0.2 MPa (A). Leaf water potential recovery after re-irrigation action (B).

Subsequently, a summary of the ANOVA of the parameters evaluated in this research is shown in Table 1. For the carbon isotope composition ($\delta^{13}C$), there was significant interaction between genotype and water regime at -3.0 MPa. Whereas, for the membrane leakage, it's occurred both at -3.0 MPa and -1.5 MPa of leaf water potential (Table 1).

Significant statistical differences were not found among the genotypes in the absence of a water deficit. However, when subjected to water stress up to a potential of -3.0 MPa, the tolerant genotypes (BRS 187 and 8H ACALA SJ-4) had average electrolyte leakage rates lower than those of the sensitive genotypes (CNPA 7H and SU-0450/8909) (Table 2). After re-irrigation to -1.50 MPa leaf water potential, the sensitive genotypes continued to show increased electrolyte leakage compared to the tolerant ones, though the magnitude of this difference was reduced (Table 3).

**Table 1.** Summary of the ANOVA from evaluated parameters in cotton genotypes grown under watered and non-irrigated conditions and measured on two leaf water potential.

| Source of variation | | Mean square | | | | |
|---|---|---|---|---|---|---|
| $\Psi_{pd}$ (-3.0 MPa) | DF | *Fv/Fm* | Chlor | RWC | Membrane leakage | $\delta^{13}C$ |
| Genotypes (G) | 3 | 0.000008 [ns] | 97.26★ | 19.00 [ns] | 1336.63★ | 0.5098★ |
| Water regime (W) | 1 | 0.000075 [ns] | 474.32★ | 12481.21★ | 5604.22★ | 1.2246★ |
| G x W | 3 | 0.000204 [ns] | 6.36 [ns] | 84.91 [ns] | 1351.77★ | 2.4948★ |
| Error | 24 | 0.002408 | 10.60 | 6.48 | 4.74 | 0.05 |
| CV (%) | | 1.19 | 6.65 | 3.51 | 8.32 | 0.91 |
| $\Psi_{pd}$ (-1.5 MPa) | | Fv/Fm | Chlor | RWC | Membrane leakage | |
| Genotypes | 3 | 0.000311 [ns] | 136.78★ | 20.36★ | 94.34★ | |
| Water regime | 1 | 0.002278★ | 228.9800★ | 3065.44★ | 1626.21★ | |
| G x W | 3 | 0.000528 [ns] | 10.7808 [ns] | 9.75 [ns] | 84.69★ | |
| Error | 24 | 0.000176 | 9.38 | 5.95 | 6.11 | |
| CV (%) | | 1.60 | 6.26 | 2.95 | 11.89 | |
| Yield and fiber Quality | | Seed cotton yield | %Fibers | UHM | STR | MIC |
| Genotypes | 3 | 91.54★ | 34.80★ | 5.077★ | 39.30★ | 2.35★ |
| Water regime | 1 | 2178.00★ | 9.12 [ns] | 3.38 [ns] | 10.46 [ns] | 0.28 [ns] |
| G x W | 3 | 41.08★ | 11.50 [ns] | 0.81 [ns] | 7.06 [ns] | 0.83 [ns] |
| Error | 24 | 3.75 | 6.86 | 1.19 | 3.06 | 0.34 |
| CV (%) | | 13.44 | 6.82 | 3.58 | 6.34 | 16.26 |

Abbreviated parameters as: Seed cotton yield – g $pl^{-1}$; %Fi – Fibers %; UHM - Length (mm); STR - Strength (g/tex); MIC - Índex micronaire; Fv/Fm - maximum photochemical efficiency; Chlor - estimated chlorophyll content via the SPAD index; RWC - leaf relative water content; $\delta^{13}C$ – Carbon isotope composition. DF- Degree of freedom; $\Psi_{pd}$ – Predawn leaf water potential; MPa - Megapascal; CV (%) - Coefficient of variation. ★significant at 0.05 probability, by the F-test. [ns] not significant at 0.05 probability, by the F-test.

**Table 2.** Maximum photochemical efficiency (Fv/Fm), estimated chlorophyll content (Chlor), membrane leakage, leaf relative water content (RWC) and carbon isotope composition ($\delta^{13}C$) of four cotton genotypes grown under stressed (S) and watered (W) conditions. Measurements were taken at -3.0 MPa at predawn.

| Genotypes | *Fv/Fm* | | Chlor | | Membrane leakage | | RWC | | $\delta^{13}C$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | W | S | W | S | W | S | W | S | W | S |
| 1 CNPA 7H | 0.83a | 0.84a | 51.13a | 57.10a | 13.15a | 55.76a | 92.85a | 51.74b | -26.87b | -26.29a |
| 2 BRS 187 8H | 0.85a | 0.83a | 43.20b | 52.97ab | 11.56a | 24.46b | 90.83a | 57.40a | -27.30ab | -25.62b |
| 3 ACALA SJ-4 | 0.84a | 0.84a | 42.93b | 51.50ab | 14.09a | 11.16c | 94.60a | 46.63c | -27.55a | -26.56a |
| 4 SU-0450/8909 | 0.85a | 0.84a | 43.25b | 49.73b | 13.05a | 66.32a | 91.23a | 55.76ab | -27.02b | -26.52a |
| [1]Contr. 1 and 4 *vs.* 2 and 3 | [ns] | [ns] | ★ | [ns] | [ns] | ★ | [ns] | [ns] | ★ | ★ |

Means for tolerant and sensitive genotypes within each column followed by the same letter are not significantly different at the 5% level by Tukey's statistical test. ★significant at 0.05 probability, by the F-test. [ns] not significant at 0.05 probability, by the F-test. [1]Contr. means - contrast between the average water deficits in tolerant (1 and 4) and sensitive (2 and 3) genotypes. Abbreviated parameters: Fv/Fm - maximum photochemical efficiency; Chlor - estimated chlorophyll content via the SPAD index and RWC- leaf relative water content. W - Watered, S - Stressed.

**Table 3.** Maximum photochemical efficiency (*Fv/Fm*), estimated chlorophyll content (Chlor), membrane leakage and leaf relative water content (RWC) of four cotton genotypes grown under stressed (S) and watered (W) conditions. Measurements were taken at -1.5 MPa, after re-irrigation during recovery of water status.

| Genotypes | *Fv/Fm* | | Chlor | | Membrane leakage | | RWC | |
|---|---|---|---|---|---|---|---|---|
| | W | S | W | S | W | S | W | S |
| 1 CNPA 7H | 0.84a | 0.82ab | 53.33a | 56.88[a] | 14.43a | 28.49b | 92.85a | 71.01a |
| 2 BRS 187 8H | 0.84a | 0.80b | 44.13b | 50.45ab | 12.67a | 21.65c | 90.83a | 74.25a |
| 3 ACALA SJ-4 | 0.83a | 0.83a | 42.75b | 50.93b | 14.09a | 24.60bc | 94.60a | 75.02a |
| 4 SU-0450/8909 | 0.84a | 0.83a | 44.78b | 48.13b | 13.48a | 36.97a | 91.23a | 70.93a |
| [1]Contr. 1 and 4 *vs.* 2 and 3 | [ns] | [ns] | [ns] | [ns] | [ns] | ★ | [ns] | ★ |

Means for tolerant and sensitive genotypes within each column followed by the same letter are not significantly different at the 5% level by Tukey's statistical test. ★significant at 0.05 probability, by the F-test. [ns] not significant at 0.05 probability, by the F-test. [1]Contr. means the contrast between the average water deficit tolerant (1 and 4) and sensitive (2 and 3) genotypes. Abbreviated parameters: *Fv/Fm* - maximum photochemical efficiency; Chlor - estimated chlorophyll content via the SPAD index and RWC - leaf relative water content. W - Watered, S - Stressed.

After re-irrigation to -1.50 MPa leaf water potential, the sensitive genotypes continued to show increased electrolyte leakage compared to the tolerant ones, though the magnitude of this difference was reduced (Table 3).

This study has found that a shorter time period between the re-irrigation and recovery of about 50% of leaf water potential appears to be associated with the stability of cell membranes because the electrolyte leakage was significantly lower in the tolerant genotypes, which recovered leaf water potential much sooner after re-irrigation than did the sensitive genotypes. One of the negative effects of a water deficit involves damage to cell membranes

and the release of ions into the intercellular space (HALLIWEL; GUTTERIDGE, 1984). Electrolyte leakage due to disruption of cell membranes is regarded as one of the consequences of oxidative stress, which leads to lipid peroxidation, membrane permeabilization, and cell death.

For relative water content (RWC), there was no significant interaction between genotype and water regime at both -3.0 and -1.50 MPa of leaf water potential. At a water potential of -1.50 MPa, only the primary factors had an effect (Table 1). For the always watered control treatment, the RWC in the leaf remained higher than 90% during the experimental period, as shown in Table 2. When

subjected to a water deficit at -3.0 MPa, the plants of the sensitive genotypes CNPA 7H and SU-0450/8909 had statistically similar RWCs, with values of 51.7 and 55.7%, respectively. The greatest difference in RWC was recorded between the tolerant genotypes. RWC ranged between 46.62 and 55.75%, respectively, for ACALA SJ-4 and CNPA 187 8H. However, after re-irrigation to a leaf water potential of -1.50 MPa, the tolerant genotypes showed an average RWC of 75%, which was about 8% higher than the sensitive genotypes, although not statistically significant (Table 3). Tolerance to water deficit occurs when a plant recognizes the stress imposed and activates a series of responses that allows it to avoid this condition (BRAY, 1997). As discussed above, an enhanced ability to avoid cell disruption, resulting in greater membrane stability, is associated with the behavior demonstrated by tolerant genotypes. Previous studies have shown that when leaves are subjected to drought, they exhibit large reductions in relative water content and water potential (DECOV et al., 2000; EFEOGLU et al., 2009; NAYYAR; GUPTA, 2006). It is known that dehydration is often reversible. In this study, the RWC was significantly reduced under water stress and then experienced a significant recovery at recovery stage comparing to water deficit conditions in all cultivars (Figure 1A).

The chlorophyll content (Chlor) varied among the genotypes and the water regimes imposed, although it was not significantly different between the sensitive and tolerant genotypes when subjected to the water deficit (Table 1). Regardless of the water regime, the genotype CNPA 7H presented with Chlor values greater than those of the other genotypes, indicating greater leaf chlorophyll content. Under a severe water deficit (-3.0 MPa), there was an increase in the Chlor value in relation to the control treatments (Table 2). After re-irrigation, when the leaf water potential reached -1.5 MPa during recovery from the water deficit, the behavior was similar to that under severe stress (Table 3). In some plant species, chlorophyll degradation has been noted when plants were subjected to water deficits, a behavior attributed to PSII protection under severe photo-inhibitory conditions (BALAGUER et al., 2002). In cotton, this response was not detected because the Chlor index value increased as the water deficit progressed.

Significant differences were not noted among the treatments for maximum photochemical efficiency of PSII at -3.0 MPa of leaf water potential (Table 1). This behavior reveals that the photochemical apparatus was not damaged by the severity of the water deficit imposed, showing that PSII in cotton is highly stable under water deficits. Similar results have been reported by other authors (MASSACCI et al., 2008; SHANGGUAN et al., 2000). After re-irrigation, at -1.50 MPa, different responses were obtained amongst stressed and control plants, but no interaction was found among the genotypes and the water regimes (Table 1), indicating that this parameter may not be useful in differentiating cotton plant genotypes for their tolerance to water deficits.

For the carbon isotopic composition, which was used as an indirect indicator of water use efficiency, a significant interaction was observed between genotype and water regime ($p < 0.05$), which indicated the presence of distinct responses among genotypes (Table 1). Under constant irrigation, the composition of isotopic carbon varied from -26.87 to -27.51‰, with lower values for the tolerant genotypes (BRS 187 8H and ACALA SJ-4) and higher ones for the sensitive genotypes (CNPA 7H and SU-0450/8909). Under -3.0 MPa, the genotype BRS 187 8H showed the highest composition of isotopic carbon (-25.61‰), while ACALA SJ-4 showed the lowest composition of isotopic carbon amongst the genotypes analyzed (-26.56‰) (Table 2). Records showed that improvements in fiber yield in the cotton plant species *G. hirsutum* and *G. barbadense* in the last 50 years were associated with increases in the net rate of photosynthesis and tolerance to elevated temperatures via increased stomatal conductance (RADIN et al., 1994). The lowest composition of isotopic carbon, which was observed in the genotype ACALA SJ-4, may be due to greater stomatal opening under water deficits that allowed a greater rate of $CO_2$ diffusion, which can result in an increased rate of photosynthesis and yield.

For the measure of seed cotton yield, a significant interaction was observed between genotype and water regime (Table 1). Under constant irrigation, the seed cotton yield varied from 24 to 36 g per plant for the genotypes SU-0450/8909 and BRS 187 8H, respectively (Table 4).

When subjected to a water deficit, ACALA SJ-4 showed a greater seed cotton yield, compared to that of the sensitive genotype. More tolerant plants generally show a greater accumulation of dry mass and/or yield under water deficits than when under other environmental conditions (KUMAR et al., 2008). In this sense, the ACALA SJ-4 genotype, which presented with a greater seed cotton yield under water deficit conditions, may be considered more tolerant of a water deficit. This genotype, under water deficits, produced a 39.0% greater yield than did the sensitive genotypes.

**Table 4.** Seed cotton yield and some fiber quality characteristics of four cotton genotypes grown under watered (W) and stressed (S) conditions. Measurements were taken during the harvest period.

| Genotypes | Seed cotton yield | | Fiber % | | UHM | | STR | | MIC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | W | S | W | S | W | S | W | S | W | S |
| 1 CNPA 7H | 30.75b | 13.50b | 38.75a | 34.58b | 30.65a | 32.03a | 28.05a | 29.10ab | 3.80ab | 4.28a |
| 2 BRS 187 8H | 36.25a | 14.25ab | 37.84a | 39.41ab | 30.28a | 30.18a | 28.23a | 31.93a | 2.80b | 3.73ab |
| 3 ACALA SJ-4 | 33.75ab | 18.00a | 37.54a | 36.83ab | 30.63a | 31.03a | 25.33a | 24.53c | 3.33ab | 2.78b |
| 4 SU-0450/8909 | 24.00c | 13.00b | 41.85a | 40.79a | 29.03a | 29.95a | 26.58a | 27.20bc | 4.18a | 4.08a |
| [1]Contr. 1 e 4 *vs.* 2 e 3 | ns | *. | ns | ns | ns | ns | ns | ns | ns | ns |

Means for tolerant and sensitive genotypes within each column followed by the same letter are not significantly different at the 5% level by Tukey's statistical test. *significant at 0.05 probability, by the F-test. ns not significant at 0.05 probability, by the F-test. [1]Contr. means - contrast between average water deficit tolerant (1 and 4) and sensitive (2 and 3) genotypes. Abbreviated parameters: Seed cotton yield – g pl[-1]; %Fi – Fibers %; UHM - Length (mm); STR - Strength (g/tex); MIC - Index micronaire. W - Watered, S - Stressed.

Under conditions of constant irrigation, the tolerant genotypes BRS 187 8H and ACALA SJ-4 maintained higher levels of seed cotton yield when compared to the sensitive genotypes. As proposed by Blum (2009), in this report, if considering the shorter time for achieving a leaf water potential of -3 MPa, the shorter recovery time and higher seed cotton yield, the more efficient water use of ACALA SJ-4 has been an important contribution to drought tolerance in the cotton genotypes evaluated.

Analysis of variance for the technological characteristic of lint indicated that only differences in genotype produced different results (Table 1). Water stress did not alter the quality of the fiber. The observed influence of genotype is understandable because the characteristics of fiber quality are much more influenced by the genetic characteristics of the cotton than by environmental factors.

Classically, drought resistance has been divided into escape, avoidance and tolerance strategies (TURNER, 1986). Nevertheless, these strategies are not mutually exclusive, and plants can combine a range of response types (LUDLOW, 1989). Drought tolerance can occur if plants maintain a favorable water balance through the expression of morphological and/or physiological traits that reduce the loss of water through transpiration by means of mechanisms that regulate stomatal conductance and/or increase water absorption capacity through the growth of a deeper and more extensive root system. In the first case, stomatal closure reduces $CO_2$ diffusion, leading to a reduction in the rate of photosynthesis, consequently reducing growth and economic yield. This strategy is commonly found as an adaptive response in plants surviving under extremely stressful conditions, which allows them to redirect absorptions and energy, which are usually used in metabolic routes of growth and production, to then be used in the synthesis of protective molecules (ZHU, 2002). This strategy used by certain species of crops growing in areas where droughts are severe for the greater part of the plant's life cycle (CHAVES et al., 2003). However, such a trait may be undesirable in areas like Brazil, where only mild and sporadic stress is likely to affect the cotton crop. In the second case, the constitutive characteristics expressed by the roots, such as greater and deeper root mass, may help the plant to maintain its water status at a relatively higher level due to the increased capacity for exploration of the water in the soil. This allows the plant to maintain greater stomatal conductance and allows a higher rate of $CO_2$ diffusion, increasing the rate of photosynthesis, growth, and yield. However, under field conditions, measurements of root characteristics are not practical and use destructive methods, limiting their use in breeding programs (ULLAH et al., 2008). Thus, the adoption of direct screening methods that use variables that are easy to measure and/or non-destructive may lend feasibility to the selection of populations for high yield and may also provide for the selection of germplasms with more robust root systems.

## Conclusion

In this report, we evaluated traits that can reliably distinguish between tolerant and sensitive cotton genotypes, such as membrane leakage and carbon isotopic composition. The identification of these traits is promising because these traits can be quickly assessed and can be used on a large scale in screening for drought tolerance. At -3.0 MPa, the physiological variables of membrane leakage and carbon isotopic composition showed behaviors that were markedly different between the tolerant and sensitive genotypes; thus, they are adequate physiological traits for screening tolerant cotton genotypes. At a recovery of -1.50 MPa, membrane leakage is capable of distinguishing tolerant and sensitive cotton genotypes.

Considering that cotton is a relatively long cycle crop (approximately 180 days) in the Brazilian Savanna-like region, procedures for screening for drought tolerance could be achieved after 50 days from sowing, during the floration phase, when water demand increases significantly.

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