Georgia Journal of Science

Volume 79 *No. 2 Scholarly Contributions from the Membership and Others* | Article 7

2021

PHYSIOLOGICAL RESPONSES TO WATER DEFICIT BY SORGHUM BICOLOR GENOTYPES VARYING IN EXPRESSION OF THE LIMITED TRANSPIRATION TRAIT

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Acknowledgements

We would like to acknowledge the following funding source: Georgia Southern University Undergraduate Research Funding. We thank Dr Sarah Gremillion for reviewing the manuscript.

PHYSIOLOGICAL RESPONSES TO WATER DEFICIT BY S*ORGHUM BICOLOR* GENOTYPES VARYING IN EXPRESSION OF THE LIMITED TRANSPIRATION TRAIT.

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Abstract

The ability by land plants to partially close their stomata in response to high vapor pressure deficit, called the limited transpiration trait, is a rare phenomenon in crop plants. The characteristic has been demonstrated in several crop species including *Sorghum bicolor*. The molecular and physiological basis for the limited transpiration trait is however, not clear. This study was conducted to determine the physiological attributes associated with the limited transpiration trait in three sorghum genotypes SC1345, SC35 and Macia. Plants of these three sorghum genotypes were established in a greenhouse and subjected to water deficit stress. Chlorophyll fluorescence and relative water content were assessed after exposing plants to water deficit. The two genotypes with an ability to express the limited transpiration trait (SC35 and Macia) were able to maintain a higher water status while genotype SC1345, which does not have a transpiration breakpoint, had a significantly lower water status compared to controls. In addition, an interesting pattern for chlorophyll fluorescence was observed in the genotypes expressing the limited transpiration trait. The results confirm that the limited transpiration trait helps to maintain plant water status, and also suggest that chlorophyll fluorescence could be used to screen for the trait.

Key words: sorghum bicolor, limited transpiration trait, vapor pressure deficit

INTRODUCTION

Grain sorghum, *Sorghum bicolor* (L) Moench is an important food crop for more than 500 million people in Africa and Asia. In the U.S., sorghum is mostly grown for livestock feed. There are also emerging interests to grow sorghum for biofuel (Mathur et al. 2017). Sorghum is most valuable for its tolerance to drought and marginal growing conditions. The crop has also been shown to exhibit a unique ability whereby plants partially or completely close their stomata when temperatures rise and humidity drops during the course of the day (Sinclair et al. 2017). This characteristic, referred to as the limited transpiration trait, is a stomatal response to the difference between the vapor pressure of the atmosphere and that of the leaf, called the vapor pressure deficit (VPD) (Fletcher et al. 2007; Shekoofa et al. 2017). The limited transpiration trait so far has been demonstrated in a number of crop species including sorghum, corn, chickpea, pearl millet, soybean and peanut (Sinclair et al. 2017; Sinclair et al. 2018). These crops exhibit a breakpoint in transpiration flow in response to increasing VPD (Choudhary and Sinclair 2014; Gholipoor et al. 2010; Riar et al. 2015) resulting in yield benefits even under water-limited conditions.

There are variations across species, and even among sorghum genotypes, in the way the limited transpiration trait is expressed (Choudhary et al. 2013). Three different transpiration patterns have been reported in genotypes exposed to increasing VPD (Gholipoor et al. 2010; Shekoofa et al. 2014). In one sorghum genotype SC1345, there is a continuous and steady transpiration rate with no breakpoint in transpiration as VPD increases. Contrary to this, the SC35 genotype has a low transpiration break point, while the Macia genotype has a considerably high breakpoint. Expression of the limited transpiration trait is temperature-dependent and some sorghum genotypes have been shown to lose the trait when temperatures rise above a certain level (Riar et al. 2015). The molecular and physiological basis for these differences in the pattern of stomatal response is unknown (Grossiord et al. 2020).

The objective of this study was to establish other physiological differences exhibited by these sorghum genotypes. This information could possibly lead to identification of molecular markers for the trait. The study looked at two physiological parameters, chlorophyll fluorescence and the water status of the plants, how they vary in three sorghum genotypes, SC1345, SC35 and Macia in response to water deficit. Chlorophyll fluorescence (Fv/Fm) is a reliable measure of the efficiency of PSII when plants are subjected to environmental stress (Tsai et al. 2019). When plants are under stressful conditions, the D1 subunit of PSII is the most vulnerable protein to degradation, resulting in compromised photosynthetic performance. The relative water content of the leaves is, on the other hand, a good indicator of the water status of the plants, showing the balance between water supply to the shoots and water loss through transpiration (Lugojan and Ciulca 2011). The origins of the sorghum genotypes (U.S. and Africa) seem to provide a basis for natural variation.

MATERIALS AND METHODS

Plant culture

For all experiments, the three genotypes of *Sorghum bicolor,* SC1345, SC35 and Macia, were selected from the parents of sorghum NAM population (Bouchet et al. 2017). Seeds were obtained from the USDA germplasm bank (Griffin, GA). Plants were grown in a greenhouse with partially controlled conditions in 1.5-gallon pots (both 22 cm in diameter and depth) in Sta-green potting soil mix. Plants were kept well-watered up to three leaf stage and Miracle-Gro plant nutrient solution was administered once a week, 50 mL per pot. After full establishment of the third leaf, watering was withheld for 12 days. Soil moisture was monitored using a soil moisture meter (Vegetronix. Inc, Riverton UT) during the period of water withdrawal. The measured moisture content at field capacity for the potting mix was between 35 - 40%. The greenhouse temperature and humidity over the course of the experiment are shown in Figure 1. It is important to note that the greenhouse climate was only partially controlled with a fan blowing cool air and an evaporative misting system maintaining humidity, hence the daily fluctuations in Figure 1.

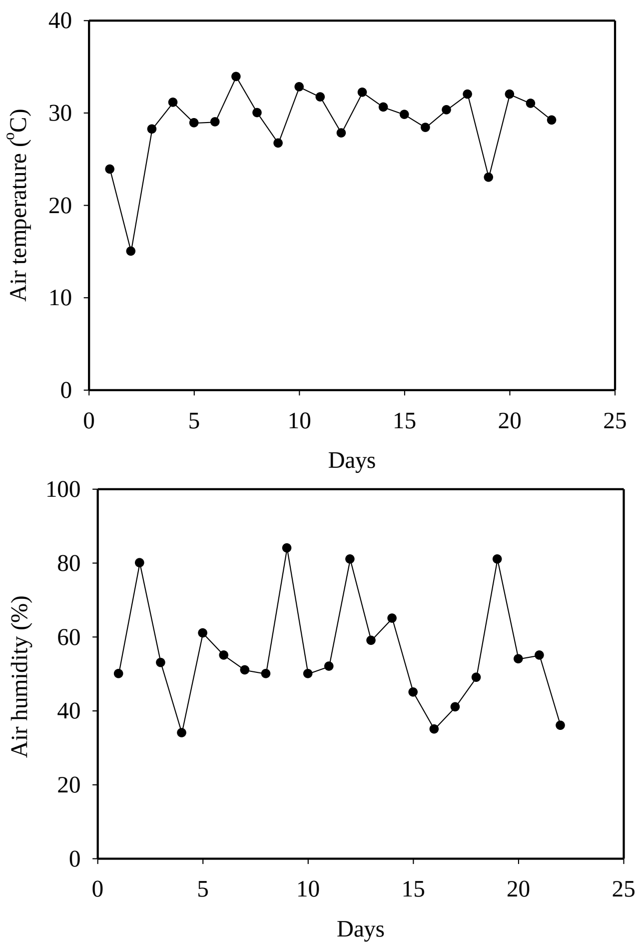

Figure 1 Greenhouse temperature and relative humidity measured during the growth period of the experiment April- May 2019 and 2020.

Physiological measurements

After the stress period, relative water content (RWC) of leaves was determined according to Barrs and Weatherley, (1962). Leaf samples from the third fully expanded leaf were detached from the plant by cutting with a scissors and folded to fit into a 50mL vial. Fresh weight (FW) of approximately 1 g was measured immediately. After fresh weight determination, the leaf samples were submerged in DI water in 50 mL vials, overnight at 4°C in the dark. After 12 hr, leaf samples were pampered dry and weighed to determine turgid weight (TW). The samples were oven-dried at 55°C for 24 hr. The constant mass and dry weight (DW) was then obtained. The formula for relative water content was applied:

RWC (%) = $\frac{FW-DW}{TW-DW} * 100$ (Barrs and Weatherley 1962).

Chlorophyll fluorescence measurements were taken using a handheld Plant Efficiency Analyzer (Hansatech Instruments, Norfolk England) following the manufacturer's instructions. All readings were taken between 11 am and 12 noon on the 3rd fully expanded leaf. Leaves were dark-adapted for 20 min using the manufacturer's clips prior to determination of chlorophyll fluorescence. Statistical analyses (t-tests) were done using the software SigmaPlot v12.

RESULTS

The limited transpiration trait is arguably, one of the physiological attributes related to the drought tolerance of sorghum. Three genotypes (SC1345, SC35 and Macia) from the sorghum global germplasm were selected for this study because they vary in the way they display the limited transpiration trait in response to increased VPD. The SC1345 genotype does not have a breakpoint in transpiration, the SC35 genotype is a sibling of BTX623 with a low (2.01 kPa) breakpoint, and the Macia genotype has a high (2.58 kPa) breakpoint at 37°C (Gholipoor et al. 2010; Riar et al. 2015). This study sought to establish whether there are other physiological characteristics differentially expressed in these sorghum genotypes, and possibly identify ways to identify the limited transpiration trait in a larger population.

Physiological responses vary in response to soil drying

To establish differences in the behavior of the three sorghum genotypes SC1345, SC35 and Macia, two physiological attributes were assessed in response to water stress. First, the relative water content of the leaves was measured after withholding water for 12 days. The soil moisture content of the pots initially at 35-40%, fell to <20% (Figure 2). In addition, during the day when the temperature was high, some leaves showed a curling phenotype (Fig. 3). The SC1345 genotype showed a significant difference in the RWC between the water-stressed plants and the well-watered plants ($P = 0.029$), with lower RWC in the stressed plants (Fig. 4). There was however, no difference in the SC35 and Macia genotypes control compared to the stressed plants as they displayed an ability to maintain their leaf water status even after 12 days of water withdrawal.

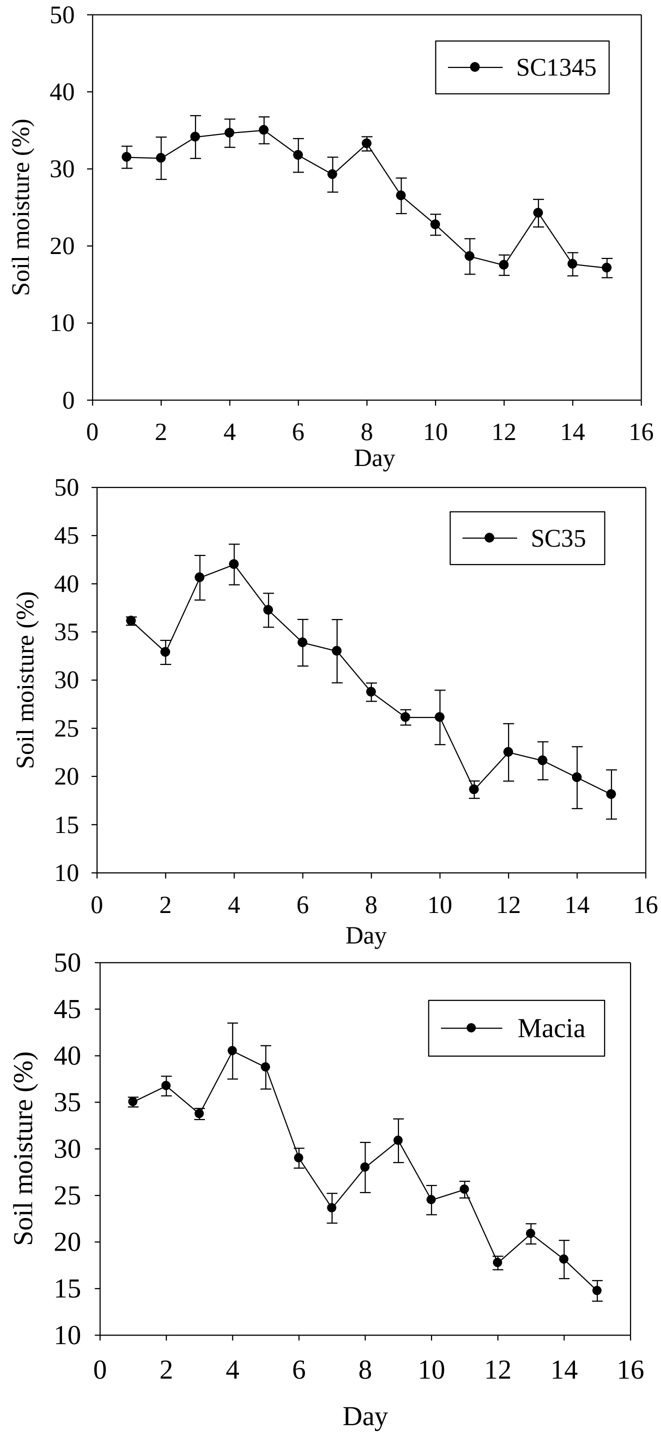

Figure 2. Percent soil moisture content in the drying pots of each sorghum genotype was determined using a soil moisture probe over the course of the experiment. Moisture content at field capacity of the media is 35-40%.

Figure 3. Leaf curling phenotype observed in stressed sorghum plants. Water was withheld from for 12 days.

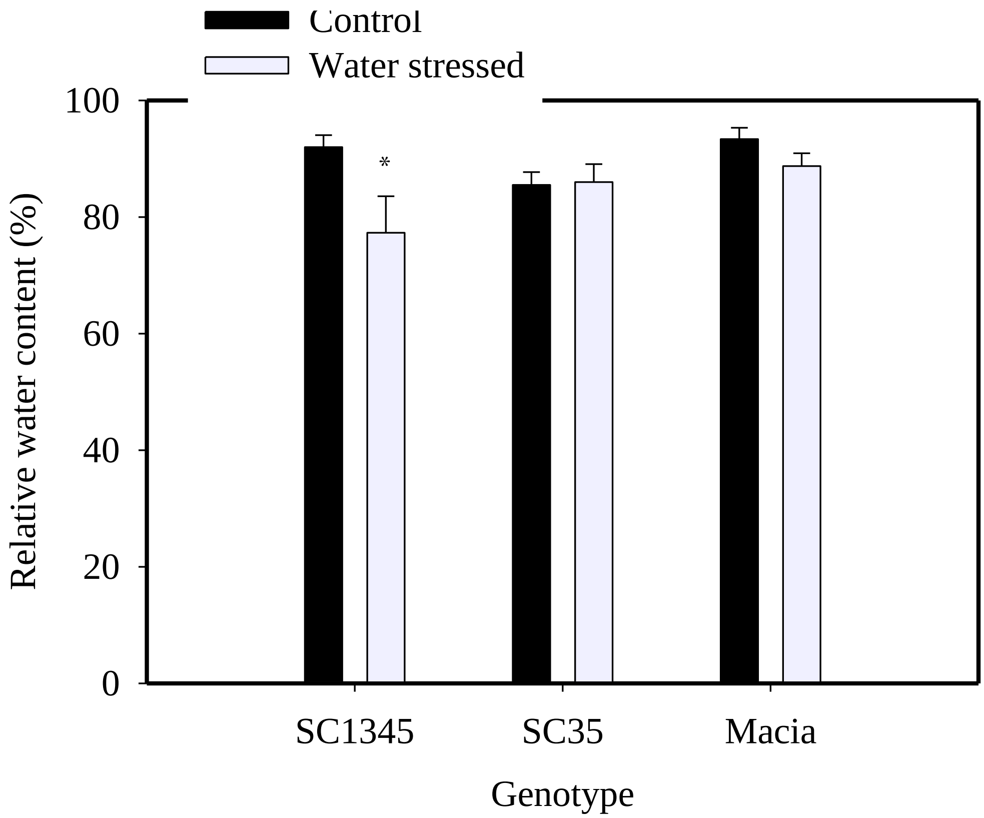

Figure 4. Relative water content of leaf tissues of three sorghum genotypes SC1345, SC35, and Macia. Three week old plants grown in 2-gallon pots in Sta-Green potting soil mix were subjected to water deficit stress by withdrawing water for 12 days. * denotes a significant T-test (P = 0.029). Each bar represents the mean and standard error (n=4).

Chlorophyll fluorescence ratio (*Fv/Fm*) was measured every day after imposing the water stress treatment. As expected, the control plants for all the three genotypes showed a steady *Fv/Fm* ratio ranging between 0.76 and 0.79 (Fig. 5). After exposure to water deficit, the three genotypes showed differential patterns of response on the *Fv/Fm* ratio. There was no significant difference in the *Fv/Fm* ratio between the control and stressed plants for the SC1345 genotype (Fig. 5A). The *Fv/Fm* ratios of the stressed plants in SC35 and Macia genotypes fell in the first 4 days and thereafter the plants started to acclimate. The stressed plants had surprisingly higher fluorescence ratios compared to the controls at the end of the stress period (Fig. 5B & C). Looking across the genotypes, the SC1345 line had consistently lower ratios compared to SC35 and Macia, in stressed plants (Fig. 5A-C).

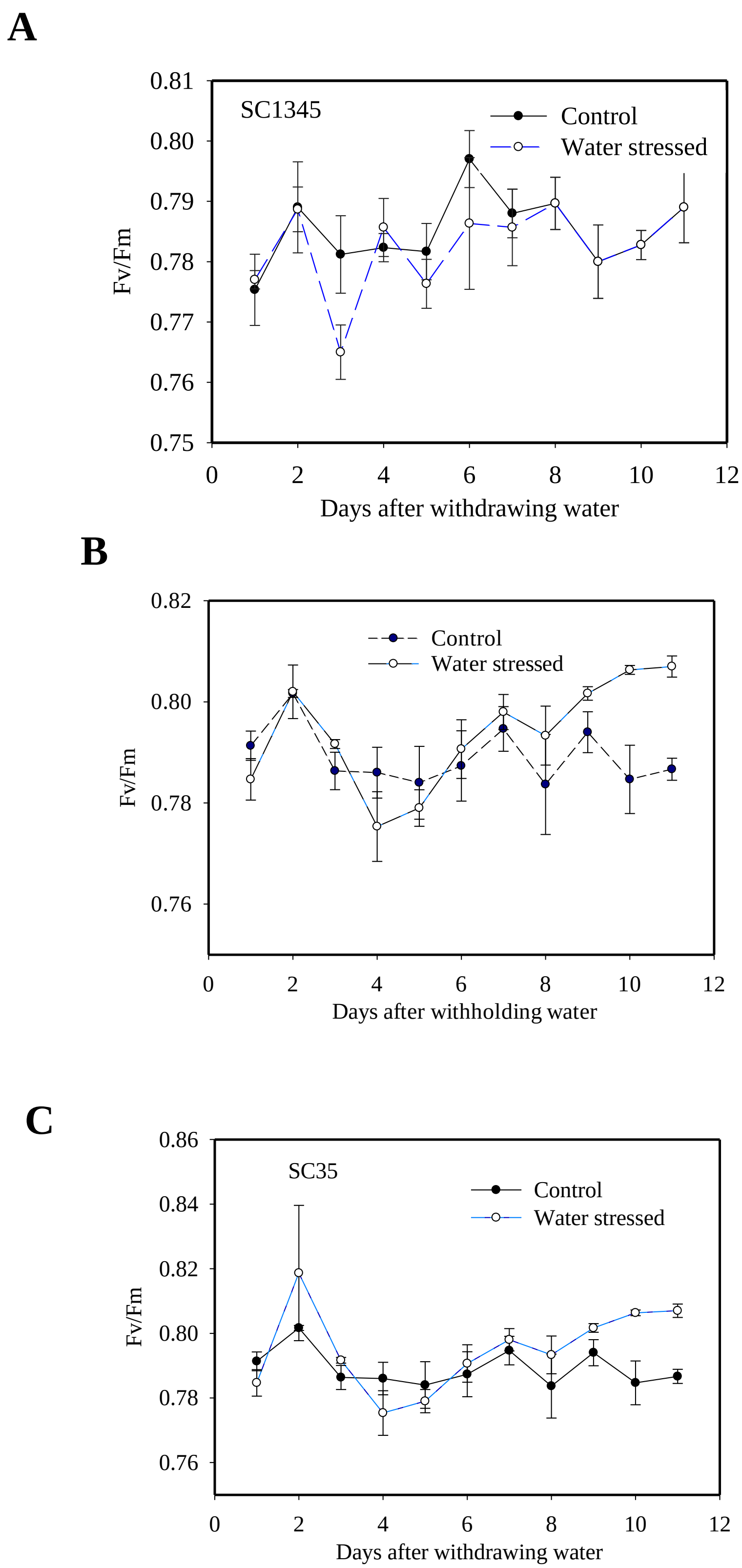

Figure 5. Chlorophyll fluorescence as measured by *Fv/Fm* of sorghum genotypes (A) SC1345, (B) SC35 and (C) Macia. Measurements were taken on control and treatment plants after watering was withdrawn from treatment plants over a period of 12 days. Each point represents the mean and standard error (n=4).

DISCUSSION

The VPD at which plants limit the rate of transpiration to retain water has been shown to vary across species. Genotypes SC1345, SC35 and Macia with three different patterns of transpirational responses to high VPD have been observed in sorghum genotypes (Gholipoor et al. 2010; Shekoofa et al. 2014), in response to water deficit. This study was conducted to establish other physiological variations possibly present in the sorghum genotypes. The relative water content is a physiological attribute, reliably used to show the water status of the plant. It is reasonable to presume that the limited transpiration trait plays a central role in the ability of plants to conserve water. In this study, the water status in the SC1345 sorghum genotype was significantly lower than the well-watered plants after 12 days of withholding water (Fig. 4). This result is consistent with other studies, considering that the SC1345 genotype does not break transpiration at high VPD (Gholipoor et al. 2010). In contrast, the water status of the other two genotypes, SC35 and Macia, did not differ from the control plants, indicating the effectiveness of the breakpoint in maintaining plant water status as VPD is increased. Reduced transpiration at high VPD implies that the plant conserves its water, making it available in later developmental stages. That the RWC was still high (±80%) could be explained by the high humidity conditions in Savannah and the closed environment in a partially controlled greenhouse, such the water loss was much less than 60-70% which would be expected in a drier environment or in an open field. A misting pad helps to keep humidity higher in the greenhouse than what would be expected in an open field.

The chlorophyll fluorescence ratio, *Fv/Fm* is generally expected to be above 0.8 under non-stressful conditions. The *Fv/Fm* ratio for the SC1345 genotype quickly fell below 0.8 and was consistently lower than the SC35 and Macia genotypes. The results show a pattern of chlorophyll fluorescence unique for each genotype, an indication of some inherent differences in the genetic ability to synthesize and replace polypeptides of the D1 unit of PS II which is the most vulnerable protein to degradation. Another interesting aspect was that the stressed plants of SC35 and Macia recovered after day 4 and outperformed the control plants. This result suggests an inherent ability for SC35 and Macia to acclimate to stressful conditions, probably an advantage imparted by or related to the transpiration trait. It would be interesting to follow up on a larger scale and establish whether chlorophyll fluorescence response patterns could be used to identify or detect genotypes with the limited transpiration trait.

A number of studies have investigated the genetic diversity of sorghum using quantitative traits, and expression divergence of genes has been reported (Jiang et al. 2013). Similar variation has been reported in *Arabidopsis thaliana* where over 300 accessions showed 10-fold range of variation in the abscisic acid levels in response to water stress (Kalladan et al. 2017). The basis for divergence in gene expression is probably related to the geographic origins of these genotypes. It would be interesting to determine whether this variation alone is sufficient to account for the different patterns of transpirational responses to high VPD. In conclusion, this study has confirmed that the limited transpiration trait helps to maintain plant water status and that chlorophyll fluorescence patterns vary among genotypes expressing different breakpoints in transpiration. It would be interesting to establish these physiological differences at on a larger scale, such as field level.

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