PHYSIOLOGICAL CHARACTERIZATION OF COTTON (*G.HIRSUTUM*) IN RESPONSE TO WATER DEFICIT

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Abstract

To tolerate water deficits, some plants osmotically adjust their cells to maintain both turgor and water potential gradient. In this study four cotton genotypes were characterized in relation to their osmotic adjustment, photosynthetic rate, relative water content, carbon discrimination and other physiological parameters. The results of this study have been used to guide our work in the molecular characterization and gene isolation in these same genotypes by Differential Display (RT-PCR). Cultivars Siokra L-23 and the wild type (T-1521) presented higher water deficit tolerance when compared with CS 50 and Stoneville 506. Osmotic adjustment, higher photosynthetic rate, and higher carbon discrimination played a key role in these results.

Introduction

The development of water deficit in leaves and roots of higher plants generates a series of plant responses that enable them to tolerate/resist the decrease in Ψ_w. Associated with the decline in Ψ_w is a drop in cell volume and cell turgor. When turgor is maintained, processes influenced directly by turgor, such as stomatal conductance, assimilation rate and expansive growth are fully or partially maintained (Ludlow, 1987). Osmotic adjustment is believed to be a primary adaptation response, because by increasing the concentration of cellular solutes, the Ψ_w gradients needed to ensure continued uptake of water during the stress period are maintained (Ingram and Bartels, 1996) and plant turgor (Ψ_P) are also maintained for continued growth (Kramer and Boyer, 1995).

Bohnert and Jensen (1996) suggested that, although the increase in the osmolyte concentration may intuitively be recognized as helpful for water retention, radical scavenging may also be a requirement. Water stress disrupts cellular redox homeostasis and, therefore, chloroplast functions, which inevitably leads to the generation of oxygen-radical species. Thus, osmotic adjustment probably performs additional functions in plant tolerance to stress, beyond helping to retain water and cell turgor.

Carbon isotope discrimination ($\Delta\ ^{13}C$) analysis can be used as a long term factor for studying water use efficiency (WUE). Differences in diffusion rates of $^{13}CO_2$ and $^{12}CO_2$ and fractionation by ribulose bisphosphate carboxylase/oxygenase (Rubisco) cause preferential incorporation of ^{12}C over ^{13}C in C_3 plants. Thus, comparison among genotypes can give an estimation of WUE over all growth periods (Knight et al., 1994).

The objectives of this work were to characterize physiologically the differences empirically observed among four cotton genotypes in relation to their tolerance to water deficit, and to identify and isolate gene transcripts responsible for these differences.

Material and Methods

Seeds of four cotton (*G.hirsutum* L.) genotypes with diverse water deficit tolerance (Siokra L-23 and T-1521, tolerant; CS 50 and Stoneville 506, sensitive) were germinated in pots containing sand. Irrigation was conducted using balanced nutrient solution (pH 6.6) at half strength (Henvitt, 1963). The volume of nutrient solution to be added was calculated to maintain the pots of the stressed treatments at 5% gravimetric humidity (GH) and non-stressed treatments at 15% GH. All treatments were kept at 15% GH until the 20^{th} day after germination, after which irrigation was withheld in the stressed treatments until sand humidity reached 5% GH. Measurements started only after stressed treatments reached 5% GH. Water and Osmotic potential were measured by collecting leaf discs (1 cm diameter) at 1 h pre-dawn and sealing them in thermocouple psychrometer chambers. The µV readings were recorded after 4 h equilibration at 25^0C (Oosterhuis and Wullschleger, 1989). After water potential readings, the osmotic potential was measured by placing the thermocouple psychrometers in liquid nitrogen for three minutes to disrupt membranes and remove turgor. An equilibration time of 4 h at 25^0C was again used before taking µV readings. Net photosynthesis, stomatal conductance, transpiration and short term WUE were measured using a LI-6200 portable photosynthesis system (Licor Inc., Lincoln, NE). After the last photosynthesis measurement, plants were harvested and oven dried at 60^0C to a constant weight. Dry weights were measured and a 0.5 g sample was taken from each treatment. Carbon discrimination analysis was conducted with an Isotope Ratio Mass Spectrometer at the University of Utah.

Differential display was used to analyze gene expression during the stress. Total RNA was extracted from leaves of the four genotypes after the last measurement. A reverse transcription followed by a polymerase chain reaction (PCR) was done using anchor and 10-mer primers according to Liang and Pardee (1992, 1995). PCR products were separated in a 6% denaturing polyacrylamide gel. Bands that appeared to be differentially displayed were recovered from the gel, reamplified and cloned in a pGEM-T vector.

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Results and Discussion

Total water potential can be maintained during mild drought by osmotic adjustment, which involves utilizing sugars or other compatible solutes (Bohnert et al., 1995). Siokra L-23 and T-1521 showed a significant decrease in their osmotic potential during the applied stress. At the last measurement, these reductions reached 36% and 42%, respectively for Siokra L-23 and T-1521. Although water potential was also significantly reduced in these two genotypes (Siokra L-23, 35%; T-1521, 42%) the magnitude of this reduction was not as big as in Stoneville 506 and CS 50, 66% and 59%, respectively (Figure 1). When Ψ_s is reduced and Ψ_w is maintained**,** cell turgor is also maintained.

Compared with Stoneville 506 and CS 50, Siokra L-23 andT-1521 had small reductions in water potential. This condition enabled both Siokra L-23 and T-1521 to maintain photosynthesis at higher rates during stress than Stoneville 506 and CS 50. Figure 2 shows net photosynthesis rates for the four genotypes during the course of the stress. Stoneville 506 and CS 50 had significant reductions in their net photosynthesis throughout the stress period. The differences at 1 day between stressed and non-stressed indicate that the susceptible genotypes were already responding to the onset of stress more than the resistant genotypes. Whereas significant reductions for Siokra L-23 only intensified by the third day at 5% sand gravimetric humidity, and for T-1521 the reduction in photosynthesis became significant only after the 3th day compared with the control.

One of the effects of keeping photosynthesis active during longer periods can be visualized by the carbon discrimination results. In Figure 3, T-1521 displays higher ^{13}C discrimination than the other three genotypes. This indicates that stomates were able to stay open for longer periods of time, allowing discrimination to continue, since less ^{13}C was incorporated (Knight et al., 1994)**.** Although Siokra L-23 did not show this same behavior, by looking at the slope of the curves in Figure 3 it is possible to see that short term WUE of Siokra L-23 was less affected as in Stoneville 506 and CS 50. Figure 3 shows short term WUE plotted against carbon discrimination. Carbon discrimination gives information about long term WUE (Knight et al., 1994). Differences between T-1521 and Siokra L-23 might be due to differences in the timing when photosynthesis reduction starts, as discussed above and showed in Figure 2. All genotypes had significant reductions in short term WUE, however these differences were much more severe in Stoneville 506 and CS 50. In the last measurement (Figure 3), Stoneville 506 and CS 50 had WUE(short term) reductions of 64% and 30%, respectively. Siokra L-23 and T-1521, otherwise, showed only 5% and 14% reduction, respectively, when compared with their non-stressed controls.

One hundred and nine cDNA fragments were identified as differentially displayed bands and from these 65 were cloned in the vector for sequencing and confirmation by ribonuclease protection assay (RPA).

Conclusion

These results show that Siokra L-23 and T-1521 partially tolerate water deficit stress, whereas CS 50 and Stoneville 506 show sensitivity, which supports previous field observations. This information is essential for further studies where these genotypes have been compared in terms of their differential gene expression during water stress. Empirical observations by growers and producers on differential responses of genotypes to drought are an important source of information to researchers. However, these observations must be confirmed by physiological characterization of the genotypes. With confirmed genotypic differences, molecular studies can be conducted to identify and isolate genes responsible for the diverse responses to drought. The development of new water-deficit tolerant cultivars can be significatively improved with this information. In this regard, differentially displayed mRNA transcripts from this work were sequenced and show interesting homology with know genes (see “Physiological and molecular responses during water deficit in cotton” in this issue). These sequences can know be used to identify genotypes with similar genetic characteristics.

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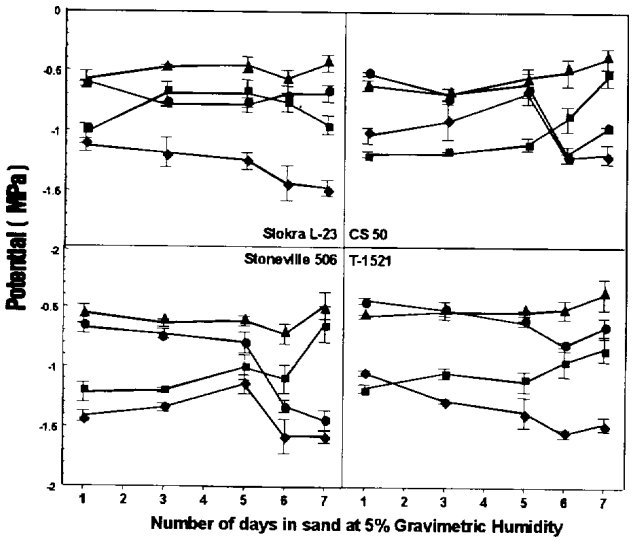

Figure 1. Water and osmotic potential in leaves of cotton genotypes. Stressed treatments were kept at 5% gravimetric humidity (GH) and non-stressed treatments at 15% GH. Readings were taken at 1 h pre-dawn. Lines represent water potential in non-stressed plants (-▲-), water potential in stressed plants (-●-), osmotic potential in non-stressed plants (-■-) and osmotic potential in stressed plants (-◆-). Each point represents the mean of five observations ± standard error (SE).

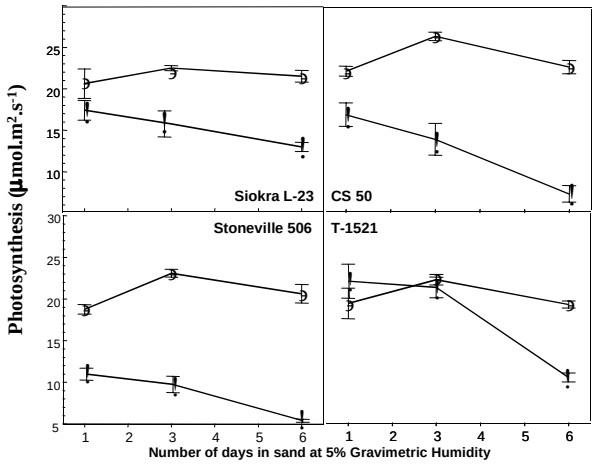

Figure 2. Net photosynthesis in leaves of four cotton genotypes during water deficit. Stressed treatments (-●-) were kept at 5% gravimetric humidity (GH) and non-stressed treatments (-▲-) at 15% GH. Each point represents the mean of five observations ± standard error (SE).

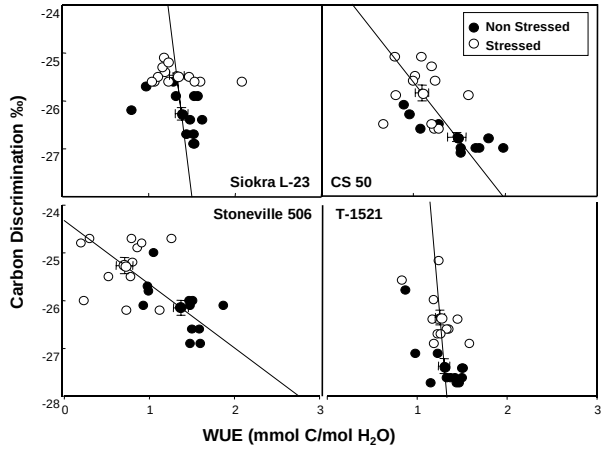

Figure 3. Carbon discrimination in leaves of four cotton genotypes during water deficit. Stressed treatments were kept at 5% gravimetric humidity (GH) and non-stressed treatments at 15% GH.