# PHYSIOLOGICAL CHARACTERIZATION OF FOUR GENOTYPES REPRESENTING DIVERSE WATER STRESS TOLERANCE A. L. Nepomuceno, D.M. Oosterhuis and J. M. Stewart. University of Arkansas, Agronomy Department Favetteville, AR

#### Abstract

To tolerate water deficits, some plants osmotically adjust their cells to maintain both turgor and cell volume. 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 and a higher photosynthetic rate played a key role in these results.

#### **Introduction**

The most common environmental factor that limits plant productivity is water deficit. To tolerate this stress plants have evolved biochemical pathways that enable them to retain or/and obtain more water, protect their cytoplasmic functions and maintain ion homeostasis. In spite of plant responses to environmental stress involving adaptation at several levels of organization, all biochemical pathways that confer more tolerance, must have a genetic basis. Based on this, our objectives were to physiologically characterize four cotton genotypes in relation to their tolerance to periods of water deficit, and by using differential display, identify and isolate genes differentially expressed during those periods of stress.

# Material and Methods

Seeds of four cotton (*Gossypium hirsutum* L.) genotypes (Siokra L-23, CS-50, Stoneville 506 and T-1521) were germinated and the seedlings fixed in pots containing aerated nutrient solution. At 23 days after germination plants were placed in pots with dilute nutrient solution containing non penetrating PEG 6000 at -0.3 MPa water potential. The plants stayed in the stress treatment for 4 hours, during the dark period, for four consecutive days. Leaf discs were collected (4 hours after each stress period) and sealed in the thermocouple psychrometer chamber. The  $\mu$ V readings were recorded after an equilibration time of 4 hours (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. Photosynthetic rate was measured using a LI-6200 portable photosynthesis system (Licor system, Lincon, NE). Total RNA was extracted from leaves of the four genotypes after the last period of stress. 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% non-denaturing polyacrylamide gel. Bands that appear differentially displayed were recovered from the gel, reamplified and cloned in a pGEM vector.

# **Results and Discussion**

Siokra L-23 and T-1521 exhibited little or no change in their water potential. However, a significant decrease in the osmotic potential occurred after each period of stress. This osmotic adjustment maintained leaf turgidity, and consequently increased their water stress tolerance. On the other hand, CS-50 and Stoneville 506 did not show the same behavior. Both genotypes exhibited a significant reduction in their leaf water potential. The higher water stress tolerance in Siokra L-23 and T-1521 can be analyzed in terms of net photosynthesis and the relative water content taken 12 and 24 hours after the last period of stress. Both Siokra L-23 and T-1521 showed small, non-significant reductions in photosynthesis of 1.4% to 5.1%. Whereas, CS-50 and Stoneville 506 had reductions that ranged from 9.3% to 24.5%. The relative water contents were also less in these two genotypes, mainly 24 hours after the last period of stress.Water and osmotic potential in the four genotypes 24 hours after the last period of stress. These two genotypes had a reduction of 25.8% and 20.7%, respectively. CS-50 and Stoneville 506, on the other hand, show reductions of 6.5% and 14%, respectively.

# **References**

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