OSMOTIC ADJUSTMENT AND ACCUMULATION OF PROLINE IN COTTON GENOTYPES UNDER WATER-DEFICIT STRESS

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Abstract

Cotton has several mechanisms to alleviate the effects of drought stress; however, the physiological and metabolic functions vary among all available genotypes. Osmotic adjustment through accumulation of compatible solutes is one of the major mechanisms plants use to acclimate to water-deficit conditions but information on osmotic adjustment in reproductive units of cotton plants has not been clarified. The objective of this study was to characterize the osmotic adjustment in leaves and reproductive units of a range of cotton genotypes grown under water-deficit stress. A growth room experiment was conducted at the University of Arkansas in Fayetteville, AR. Treatments consisted of four cotton (*Gossypium hirsutum* L.) genotypes, DP0912, PHY499, Siokra L23, and ST5288, and two water regimes, an untreated control with no water-deficit stress, and water deficit. Water-stress was imposed at flowering by withholding water from the water-stressed plants group until the plant's stomatal conductance (gs) reach approximately 20 mmol $m^{-2}s^{-1}$. Osmotic potential was measured and samples were collected for proline content from the upper first position white flowers and their subtending leaves. Variability in osmotic adjustment exists among the cotton genotypes and between the plant organs under water-deficit stress. PHY499 had higher osmotic adjustment in the leaves of water-stressed plants through more negative osmotic potential and higher proline accumulation. ST5288 appeared to have higher osmotic adjustment in both leaves and ovaries of water-stressed plants due to the higher proline accumulation in the leaves and ovaries and more negative osmotic potential in the ovaries. DP0912 and Siokra L23 accumulated higher amount of proline in the leaves and ovaries, but it did not increase the osmotic potential of the plant organs in both genotypes.

Introduction

Cotton is considered as a relatively drought tolerant crop as it is originated from perennial species grown in areas with scarcity of water (Lee, 1984). However, the domestication and cultivation of cotton as an annual crop has resulted in reduced tolerance to drought conditions and consequently lower yields (Quisenberry et al., 1981). Even though cotton has several mechanisms to alleviate the effects of drought stress (e.g. increase in antioxidant enzymes activity, heat shock proteins, accumulation of osmolytes and osmotic adjustment), physiological and metabolic functions vary among all available genotypes. Under drought conditions, osmotic adjustment is an important mechanism of acclimation in plants. It occurs through accumulation of compatible solutes in the cytosol in order to maintain turgor pressure and ensure cell enlargement (Hsiao et al., 1976). Proline, an amino acid, is the most frequent compatible solute accumulate in cells of water-stressed plans (Fumis et al., 2002). Research has indicated that proline content is significantly increased in plants grown under water-deficit stress (Pilon et al., 2014; Crusciol et al., 2009; Nikolaeva et al., 2010). However, information on osmotic adjustment and proline in reproductive units of cotton plants has not been elucidated. Therefore, the objective of this study was to characterize the osmotic adjustment in leaves and reproductive units of a range of cotton genotypes grown under water-deficit stress.

Materials and methods

A growth room experiment was conducted at the Altheimer Laboratory, University of Arkansas in Fayetteville, AR. Treatments consisted of four cotton (*Gossypium hirsutum* L.) genotypes, DP 0912 B2RF, PHY 499 WRF, Siokra L23, and ST 5288 B2F, and two water regimes, an untreated control with no water-deficit stress, and water deficit imposed at the flowering stage. The experimental design was a complete randomized design with five replications.

Cotton genotypes were planted using one plant per 2-L pot containing a horticultural mix (Sun-Gro horticulture mix). Half-strength Hoagland's nutrient solution was applied daily in order to maintain adequate nutrients and water during conduction of the experiment, except for the water-deficit treatment when imposed. Mepiquat chloride was applied as needed to control vegetative growth. The growth chamber was set for normal conditions of 32/24°C (day/night), ±60% relative humidity, and 14h photoperiod. Water-stress was imposed at flowering by withholding

water from the water-stressed plants group at approximately eight weeks after planting (depending on each genotype development) until the plant's stomatal conductance (g_s) reach approximately 20 mmol $m^{-2}s^{-1}$. Well-watered control plants received optimum quantity of water throughout the duration of the experiment.

Stomatal conductance was measured daily from the fourth main-stem leaf from each plant using a leaf porometer Decagon SC-1during induction of the stress. Once the plants reached the desired stress level they were re-watered and osmotic adjustment was estimated. Osmotic potential (MPa) was measured 12 hours after the plants were re-watered from the upper first position white flowers and their subtending leaves using a end-window leaf cutter psychrometer (model 74 series, J.R.D. Merrill Specialty Equipment, Logan, UT) equipped with stainless steel sample chambers using the technique described by Oosterhuis (1987). Osmotic potentials were determined after the psychrometer-chambers were frozen in liquid nitrogen for 5 minutes, thawed at room temperature for 30 minutes, and then allowed to equilibrate in a waterbath at 25°C for 4 hours. Readings were made using a micro-voltmeter and chart recorder. Samples from the upper first-position white flowers and their subtending leaves were collected for determination of proline concentration (Bates et al., 1973). For the colorimetric test, 1 mL of extract, 1 mL of acid ninhydrin, and 1 mL of glacial acetic acid were pipetted. After samples were maintained in a waterbath at 95°C for 60 minutes, tubes were cooled and readings were made in a spectrophotometer at 520 nm. L-proline p.a. was used as standard curve.

Results

Stomatal conductance

On the last day of the stress, plants of all genotypes grown under water-deficit stress were expected to show stomatal conductance of approximately 20 mmol $m^{-2}s^{-1}$ (Fig. 1). Stomatal conductance of water-stressed plants was significantly lower than the well-watered plants.

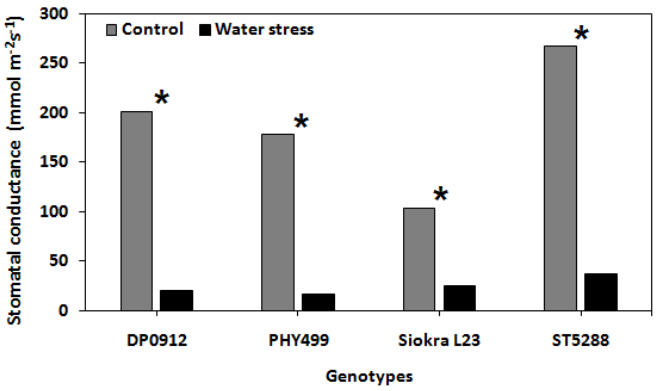

Figure 1. Stomatal conductance of four genotypes on the last day of the stress. Asterisks indicate significant difference (P≤0.05) between the water regimes within the same genotype.

Osmotic potential

The genotype PHY499 showed osmotic potentials more negative in the leaves of water-stressed plants compared with the control with an adjustment of 90.5% (Fig. 2).

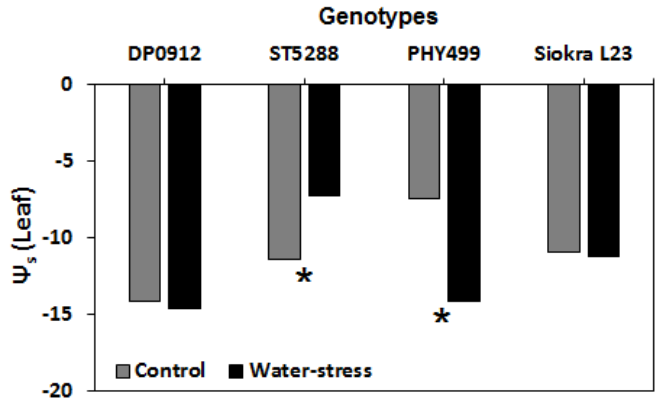

Figure 2. Osmotic potential (Ψs) in the leaves of four genotypes under two water regimes. Readings were taken 12 hours after re-watering. Asterisks indicate significant difference (P≤0.05) between the water regimes within the same genotype.

The genotypes DP0912, ST5288, and PHY499 showed osmotic potentials more negative in the ovaries of water-stressed plants compared with the control (Fig. 3). Siokra L23 had similar osmotic potential in ovaries of both water-stressed and well-watered plants, which indicates that this genotype does not use osmotic adjustment as a mechanism to acclimate to water-deficit stress. The highest osmotic adjustment in the ovaries was found in ST5288 with an adjustment of 580%.

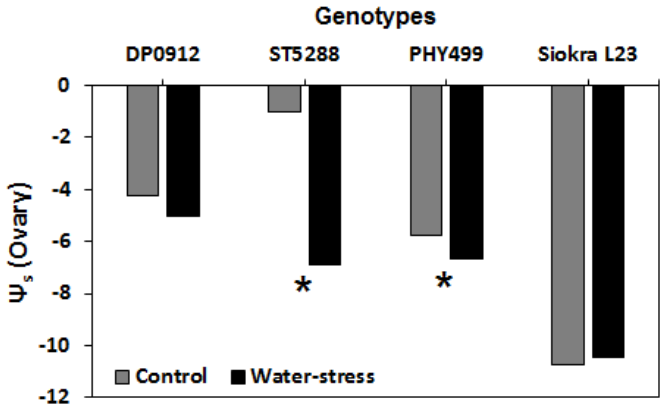

Figure 3. Osmotic potential (Ψs) in the ovaries of four genotypes under two water regimes. Readings were taken 12 hours after re-watering. Asterisks indicate significant difference (P≤0.05) between the water regimes within the same genotype.

Proline concentration

Proline content in the leaves was increased by the water-deficit stress in all genotypes (Fig. 4). Proline content was significantly higher in the leaves of water-stressed plants of DP0912, PHY499, and ST4288.

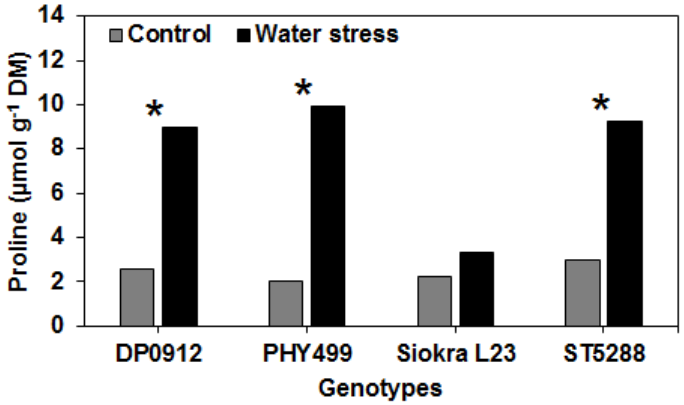

Figure 4. Proline content in the leaves of four genotypes under two water regimes. Asterisks indicate significant difference (P≤0.05) between the water regimes within the same genotype.

Proline content in the ovaries was significantly increased in the water-stressed plants of the genotypes DP0912, Siokra L23, and ST5288 (Fig. 5). The genotype PHY499 showed higher proline content in the ovaries of water-stressed plants; however, it was not significantly different from the control.

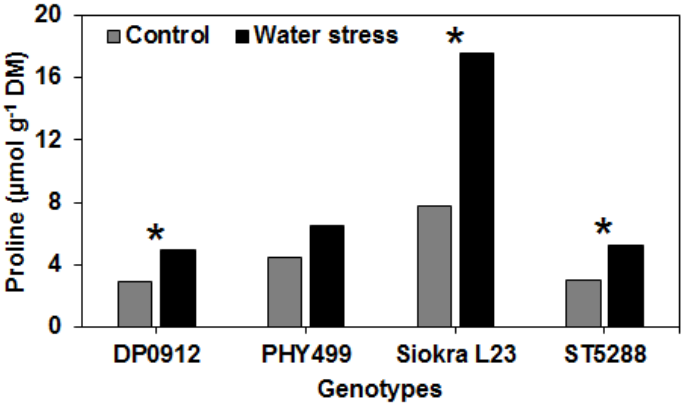

Figure 5. Proline content in the ovaries of four genotypes under two water regimes. Asterisks indicate significant difference (P≤0.05) between the water regimes within the same genotype.

Conclusions

Results suggest variability in acclimation through osmotic adjustment among the cotton genotypes and between the plant organs under water-deficit stress. PHY499 seem to have higher osmotic adjustment in the leaves of water-stressed plants through more negative osmotic potential and higher proline accumulation. ST5288 seems to have higher osmotic adjustment in both leaves and ovaries of water-stressed plants due to the higher proline accumulation in the leaves and ovaries and more negative osmotic potential in the ovaries. DP0912 and Siokra L23 accumulated higher amount of proline in the leaves and ovaries, but it did not increase the osmotic potential of the plant organs in both genotypes.

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