Seasonal Patterns of Glutathione and Ascorbate Metabolism in Field-Grown Cotton under Water Stress

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ABSTRACT

The goal of this study was to identify water-stress-related limitations in the antioxidant protection in cotton (Gossypium hirsutum L.) in the field. Cotton was grown under full irrigation and severe-to-moderate water deficits in both years of the study. The amount and form of glutathione (5-L-glutamyl-L-cysteinylglycine) were monitored in both years, while the antioxidant enzymes ascorbate peroxidase and glutathione reductase, the amount of ascorbate, and the amount of malondialdehyde (MDA) were monitored in the second year of the study. The amount of glutathione varied (> 2×) during the sampling interval, though there was no effect of water treatment on the variation. The ratio of reduced/oxidized glutathione varied seasonally, but not in response to water deficits. Glutathione reductase activity varied (< 5×) seasonally, but again not in response to water deficits. The amount of ascorbate and the activity of ascorbate peroxidase were increased under water stress (< 5× and 1.5×, respectively). The amount of MDA (an indicator of oxidative damage) varied seasonally (< 2×), though not in response to water stress. While glutathione metabolism appears sufficient for oxidative stresses resulting from field-water deficits, altered ascorbate metabolism may be a response to water deficits in the field. Malondialdehyde amount did not increase with water stress, suggesting that antioxidant metabolism was sufficient for the oxidative stresses. It is apparent that antioxidant metabolism in these plants exposed to water deficits under production conditions was sufficient to protect against oxidative damage.

Environmental stress adversely affects plant performance and results in significant reductions in crop yield and quality worldwide (Boyer, 1982). The exposure of plants to such environmental stresses as water deficits, high and low air temperatures, and certain air pollutants can result in the production of reactive oxygen species (ROS) that are thought to contribute to diminished plant performance (Grill et al., 2001; Noctor and Foyer, 1998). Acclimation to growth at high light intensities has been associated with higher levels of ROS scavenging enzymes (Gillham and Dodge, 1986; Schöner and Krause, 1990; Grace and Logan, 1996). Gossett et al. (1994) have shown that salt-tolerant cotton cultivars have higher constitutive levels of catalase, α-tocopherol, ascorbate peroxidase, and glutathione reductase than salt-sensitive cultivars when grown under salt stress.

Oxidative stress is a term commonly used to describe the adverse effects of ROS on plants. A variety of enzymatic and nonenzymatic mechanisms exist to metabo-

lize ROS into less harmful chemical species. The term antioxidant metabolism describes the detoxification of ROS, and the chemicals involved are generally referred to as antioxidants. Recent reviews by Foyer (2001) and Blokhina et al. (2003) summarize a great deal of the current knowledge of antioxidant metabolism in plants.

Efforts to limit water-stress related oxidative damage in plants generally fall into two broad areas: management of water to moderate the water stresses, and the development of germplasm with enhanced antioxidant metabolism. Success in both approaches will be enhanced by improved knowledge of when and to what extent antioxidant metabolism is a limiting factor in the plant’s response to environmental stress.

Glutathione is one of several chemical compounds in plants that are involved in the detoxification of ROS. Glutathione can be oxidized directly by oxidants and also as a component of the Halliwell-Asada cycle that maintains the cellular ascorbate pool in a reduced state (Noctor et al., 1994). Previous studies on the role of glutathione in oxidative stress protection have generally been based on oxidative stresses generated by acute exposures to chemical oxidants (Iturbe-Ormaetxe et al., 1998), gaseous pollutants (Grill et al., 1982; Foster and Hess, 1980), high air temperatures (Nieto-Sotelo and Ho, 1986), and water stresses (Loggini et al., 1999). However, studies involving antioxidants in plants grown under field conditions and subjected to stresses in the field are rare.

The goal of this study was to determine the timing and extent of limitations in glutathione metabolism in cotton grown under water stresses typical for a production environment. The study involved a seasonal analysis of antioxidant metabolism in cotton grown under water stress in Lubbock, TX, during the summers of 1996 and 1997. Glutathione was the only antioxidant monitored in Year 1. The absence of water-stress related variation in glutathione in that year prompted the expansion of the study in 1997 to include the imposition of another level of water stress, and additional measurements of antioxidant metabolism. In both years, cotton plants were grown in the field under conditions representative of cotton production in semiarid regions like the Texas High Plains, and the imposed water treatments resulted in the development of relatively severe water stresses.

It is hypothesized that oxidative stress resulting from water stress will occur during the growing season. Knowledge of the extent and pattern of oxidative stress in field-grown cotton will provide valuable information on the

Abbreviations: DOY, day of year; GSH, reduced glutathione; GSSG, oxidized glutathione; PET, potential evapotranspiration; ROS, reactive oxygen species.
best means of improving antioxidant activity in cotton. It will provide an understanding of when, how much, and for how long antioxidant metabolism should be altered to improve plant performance under stress in the field.

**MATERIALS AND METHODS**

**Plant Material and Cultural Practices**

Cotton (‘Paymaster HS 26’) was planted in Lubbock county, TX, on 26 Apr. 1996 [Day of Year (DOY) 117] and on 15 May 1997 (DOY 136) in 1-m rows with a John Deere 7300 Max-Emerge 2 Vacuum Planter. Fifty percent of the final stand was established in Year 1 by 7 May 1996 (DOY 128) and in Year 2 by 23 May 1997 (DOY 143), with a final plant population of approximately 135,000 plants ha\(^{-1}\). Liquid fertilizer, formulated as 28–0–0 N–P–K, was chiseled in every furrow approximately 30 d before planting at the rate of 22 kg N ha\(^{-1}\). The study was irrigated by a surface drip system with laterals placed in alternate furrows. The study area received a preplant furrow irrigation of 127 mm in alternate furrows before planting. With each irrigation, 0.09 kg N mm\(^{-1}\) of water was applied through the drip system. Lint yield was determined from four replicates of two single-row sections that were hand-harvested in each treatment.

**Irrigation 1996**

Two irrigation treatments were established in Year 1: dryland and full irrigation. Both treatments received preplant irrigations of 127 mm and rain of 300 mm. The full-irrigation treatment also received subsequent irrigations on a regular basis throughout the season. Irrigation scheduling was automated on a 3-d interval and was initiated on DOY 173. The full-irrigation treatment received 16 irrigations of 21 mm. Estimates of potential evapotranspiration (PET) were provided by the PET Network of the Texas Agricultural Experiment Station, Lubbock, TX.

**Irrigation 1997**

Three irrigation treatments were established in Year 2: dryland, deficit irrigation, and full irrigation, receiving rain, a fraction of PET, and full PET, respectively. All treatments received preplant irrigations of 127 mm and rainfall of 256 mm. The deficit and full-irrigation treatments also received irrigations on a regular basis throughout the season. Irrigation scheduling was automated on a minimum interval of 3 d, and irrigation was initiated on DOY 181. The full-irrigation treatment received 21 irrigations of 21 mm, and the deficit irrigation treatment received 21 irrigations of 7 mm. Estimates of PET were provided by the PET Network of the Texas Agricultural Experiment Station, Lubbock, TX.

**Leaf Water Potential**

Leaf water potential was determined in Year 2 on a weekly basis in all treatments between 1400 and 1500 h (when differences among treatments would be largest). Measurements were made on leaves collected from the third node below the apex using a pressure chamber (Model 3005 Plant Water Status Console from Soilmoisture Equipment Corp., Santa Barbara, CA). A minimum of three samples were analyzed for each measurement.

**Collection of Leaf Samples**

All leaf samples for metabolic analyses were collected between 0800 and 0830 h. Samples were collected early in the day to minimize diurnal differences in antioxidant metabolism that were not related to water status. Leaves were collected from the third node below the apex from plants in all treatments. Ten samples per treatment were placed in plastic bags with a wet paper towel for transport from the field to the laboratory (generally < 15 min), frozen in liquid nitrogen, and stored at −90°C until analysis.

**Glutathione Reductase Assay**

Glutathione reductase was extracted from cotton leaf material by grinding the tissue in a mortar and pestle to a powder in liquid nitrogen. The powder was then ground to a paste with ice-cold buffer [0.1 \(M\) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.5, 20% (v/v) glycerol, 1% (w/v) polyvinylpyrrolidone, 1% (v/v) Triton X, 5 \(M\) ethylene-diamine tetraacetic acid (EDTA), 1 \(mM\) dithiothreitol (DTT), and 1 \(mM\) phenylmethylsulfonyl fluoride] at a ratio of 4 \(mL\) extract to 1 \(g\) fresh wt. The extract was centrifuged (15,000 \(×\) g) for 30 min at 4°C. Polyethylene glycol (8000 MW) was added to 20% saturation, and the mixture stirred slowly for 3 h at 4°C. The mixture was centrifuged (15,000 \(×\) g) for 30 min at 4°C. The pellet was resuspended in 2 \(mL\) of buffer (0.05 \(M\) Tricine pH 7.5 containing 0.012 \(mM\) flavin adenine dinucleotide, 0.065 \(mM\) DTT, and 20% glycerol (prepared at time of use). The resuspended pellet was used in the assays.

The glutathione reductase assay was modified from Mahan et al. (1990). The 1-mL assay contained 0.5 \(mM\) oxidized glutathione (GSSG) and 0.1 \(mM\) NADPH in a buffer (0.125 \(M\) HEPES pH 8). The assay was initiated with the addition of 0.5 \(mL\) of the leaf extract that contained approximately 0.03 units of glutathione reductase. Enzyme activity was monitored by the decrease in absorbance at 340 nm for 30 s at 25°C and the initial rate determined. A unit of activity is the amount of enzyme that will catalyze the reduction of 1 \(\mu\)mol of GSSG min\(^{-1}\) at 25°C.

**Glutathione Measurements**

The amount and form of glutathione were determined by a modification of the method of Anderson (1985), which measured total glutathione [reduced glutathione (GSH), and GSSG] by a glutathione reductase catalyzed reaction. Removal of GSH from the sample by chemical derivatization provided for the quantification of GSSG in the sample.

Leaf tissue was ground to a powder in liquid nitrogen in a precooled mortar and pestle. The powder was then ground to a paste with 10% 5-sulfosalicylic acid (5 \(mL\) \(g\)\(^{-1}\) fresh wt. of tissue) to reduce the oxidation of GSH. The extract was centrifuged (15,000 \(×\) g) for 30 min at 4°C and used in subsequent assays.

The 1-mL volume assay for total glutathione contained the following: 0.28 \(mM\) NADPH, 0.6 \(mM\) 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB), the plant extract or glutathione standard, and 0.2 units per assay glutathione reductase from yeast in 0.143 \(M\) potassium phosphate pH 7.5, with 6.3 \(mM\) EDTA). The assay mixture (minus glutathione reductase and leaf extract) was allowed to equilibrate at 30°C for 5 min. After the addition of the glutathione-containing extract and glutathione reductase, the decrease in absorbance at 412 nm was monitored for 30 s. A reaction blank was prepared by replacing the plant extract with 10% 5-sulfosalicylic acid. The concentration of glutathione was determined through comparison with a standard curve of reaction rate as a function of the concentra-
tion of GSSG (0.07–1.68 μg mL\(^{-1}\) in 10% 5-sulfosalicylic acid). Glutathione values are expressed as GSH equivalents (1 mol GSSG is equivalent to 2 mol GSH). Each reported value represents the mean of a minimum of three determinations.

The amount of oxidized glutathione in the extract was determined by removing GSH by derivatization with 2-vinylpyridine. The derivatizations were performed immediately after leaf extractions to minimize the oxidation of GSH in the sample. Plant extract (300 μL) and 6 μL of 2-vinylpyridine were mixed in a microcentrifuge tube, and 34 μL of 1 M triethanolamine was added to the side of the tube and the solution was mixed vigorously. The final pH of the mixture was measured, and if >7, the derivatization was repeated using less triethanolamine. The derivatizations were performed at room temperature for 1 h, and the resultant solution was then used as plant extract for the glutathione assay previously described.

**Ascorbate Assay**

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Ascorbate Peroxidase Assay

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dase are described in Allen (1995). Cotton leaf material (0.5 g) was ground to a powder in a mortar and pestle using liquid nitrogen. The powder was homogenized in 2 mL extraction buffer with a tissue homogenizer on ice. The extraction buffer consisted of 50 mM HEPES, pH 7.0, 1 mM ascorbate, 1 mM EDTA, and 1% (v/v) Triton X, and was prepared immediately before the extraction. The extract was centrifuged (15 000 × g) for 15 min at 4°C. The supernatant fraction was used for the assays. All reagents and the extract were prepared fresh before the assay. The assay reaction mixture contained 50 mM HEPES, pH 7.0, 1 mM EDTA, 1 mM hydrogen peroxide, 0.5 mM ascorbate, and 30 μL of the extract in a total volume of 1 mL. The assay was allowed to equilibrate at 25°C for 1 min before the addition of hydrogen peroxide, which initiated the reaction. The reaction was monitored as a decrease in absorbance at 290 nm for 60 s at 25°C, and the initial rate determined. A control reaction was prepared by replacing the ascorbate with the HEPES buffer. A unit of ascorbate peroxidase is defined as the amount necessary to oxidize 1 μmol of ascorbate min\(^{-1}\) at 25°C (290 nm extinction coefficient of 2.8 L mmol\(^{-1}\) cm\(^{-1}\)).

**Malondialdehyde Quantification**

The lipid peroxidation assays were performed according to a modification of the method of Draper and Hadley (1990). Previously frozen cotton leaf tissue (0.1 g) was ground in liquid nitrogen and homogenized in 1.2 mL 5% tetraethyloctanooic acid plus 0.12 mL of 0.05% methanolic butylated hydroxytoluene with a tissue homogenizer. The homogenate was heated for 30 min at 100°C to release protein-bound MDA. After cooling, the homogenate was centrifuged (15 000 × g) for 15 min and the extract was used for the assays. The extract (0.5 mL) was reacted with 0.5 mL of 0.67% thiobarbituric acid at 100°C for 30 min. A sample blank consisted of 0.5 mL of the extract plus 0.5 mL of water incubated at 100°C for 30 min. The assay mixture was cooled to room temperature and the absorbance of each sample was determined at 532 nm and 600 nm. The absorbance at 600 nm was subtracted from the 532-nm reading, and the concentration of MDA was calculated using an extinction coefficient of 155 L mmol\(^{-1}\) cm\(^{-1}\).

**RESULTS AND DISCUSSION**

**Irrigation and Rainfall Patterns**

The quantity and distribution of rain and irrigation during Year 1 (1996) are shown in Fig. 1. The dryland and irrigated treatment received 336 mm of rain during the season, with slightly more than one-third of the total occurring in late-season (DOY 235–240) rainfall events, which totaled 120 mm. The full-irrigation treatment received 356 mm of irrigation (16 irrigation events and deficit irrigation treatment generally showed the lowest leaf water potential in Year 2 treatments. As a result of rain, the deficit irrigation treatment received 163 mm was ground to a powder in a mortar and pestle using liquid nitrogen. The powder was homogenized in 2 mL extraction buffer with a tissue homogenizer on ice. The extraction buffer consisted of 50 mM HEPES, pH 7, 1 mM ascorbate, 1 mM EDTA, and 1% (v/v) Triton X, and was prepared immediately before the extraction. The extract was centrifuged (15 000 × g) for 15 min at 4°C. The supernatant fraction was used for the assays. All reagents and the extract were prepared fresh before the assay. The assay reaction mixture contained 50 mM HEPES, pH 7.0, 1 mM EDTA, 1 mM hydrogen peroxide, 0.5 mM ascorbate, and 30 μL of the extract in a total volume of 1 mL. The assay was allowed to equilibrate at 25°C for 1 min before the addition of hydrogen peroxide, which initiated the reaction. The reaction was monitored as a decrease in absorbance at 290 nm for 60 s at 25°C, and the initial rate determined. A control reaction was prepared by replacing the ascorbate with the HEPES buffer. A unit of ascorbate peroxidase is defined as the amount necessary to oxidize 1 μmol of ascorbate min\(^{-1}\) at 25°C (290 nm extinction coefficient of 2.8 L mmol\(^{-1}\) cm\(^{-1}\)).

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The quantity and distribution of rain and irrigation during Year 2 (1997) are shown in Fig. 1. All treatments received 258 mm of rain during the season in five rain events. The deficit irrigation treatment received 163 mm of irrigation (20 events) and rain for a total of 421 mm. The full-irrigation treatment received 404 mm of irrigation (20 irrigation events) and rain for a total of 662 mm during the experimental period. Comparison of these amounts with total PET of 558 mm for the full-irrigation treatment indicated that the full-irrigation treatment, which received 118% of PET, should not have experienced significant water stress, while the deficit and dryland treatments, which received 75 and 46% of their potential consumption, were subjected to water stress. The lint yields of the full irrigation, deficit irrigation, and dryland treatments were 1514, 845, and 365 kg ha\(^{-1}\), respectively, again underscoring the differences in water stresses among the irrigation treatments.

Figure 2 shows the seasonal pattern of leaf water potential in Year 2 treatments. As a result of rain, the water potentials in all three treatments were similar before DOY 205. From DOY 205 to 269, the dryland treatment generally showed the lowest leaf water potential values, with the deficit irrigation treatment intermediate between dryland and full irrigation. At the end of the season, by DOY 269, the differentials were eliminated by a combination of rain events and reduced evaporative demand due to cooler air temperatures at the end of the season.
Fig. 2. Leaf water potential measured in three water treatments during 1997. Measurements were made between 1400 and 1500 h. Values are means of a minimum of three leaves. DOY = day of year.

Glutathione and Glutathione Reductase

Seasonal variation in the amount and form of glutathione during Year 1 is shown in Fig. 3. The amount of glutathione varied across the season in both the dryland and full-irrigation treatments with no significant (P > 0.05) differences associated with the amount of water received. In both treatments at all dates, the reduced form was predominant, with no pronounced differences in the reduced and oxidized forms that could be associated with the water treatments.

The experiment was repeated in an expanded form in the summer of Year 2 with three water treatments: full and deficit irrigation, and dryland. Seasonal variation in the amount and form of glutathione during Year 2 is shown in Fig. 4. While the amount of glutathione varied during the season in all treatments, there were no significant (P > 0.05) differences associated with the amount of water received. Additionally, there were no important differences in the proportion of reduced and oxidized forms that could be associated with the water treatments.

Two changes in glutathione metabolism were anticipated in response to water deficits in this study: increased glutathione content and/or an increase in the fraction of the glutathione pool in the oxidized form. Neither response was observed in relation to water stress treatments in either year of the study. The absence of differences in total glutathione or the fraction in the oxidized form resulting from the water treatments is somewhat surprising in light of the results of previous studies on the response of glutathione metabolism to water deficits.

Poole and Rennenberg (1992) studied variation in the glutathione content of needles on a seasonal basis from spruce trees grown at three altitudes. Higher levels were found at the highest altitude, and lower levels at the lower altitudes, and it was concluded that increased glutathione content was an adaptive response to increased stresses experienced by the plants at the higher altitudes.
It might be expected that the antioxidant metabolism of plants would respond differently to high altitude oxidative stress that is constant and long term, as compared with water stress that is variable and transient.

In general, the majority of glutathione in an unstressed plant cell is found in the reduced form (GSH) typically comprising 70 to 90% of the total (Bielawski and Joy, 1986; Smith, 1985). Under oxidative stress, if the rate of glutathione oxidation exceeds the rate of glutathione reduction, an increase in the fraction of glutathione in the oxidized form (GSSG) can result (Sgherri and Navari-Izzo, 1995; Smith, 1985). Sgherri and Navari-Izzo (1995) reported changes in the glutathione metabolism of sunflowers under water stress in the field. Changes in glutathione content and form resulting from oxidative stresses were identified in experiments involving glutathione metabolism in catalase-deficient barley by Smith et al. (1985), who reported increases in the total glutathione content and oxidized glutathione in H$_2$O$_2$-stressed barley plants. Increased glutathione content has been reported to be related to a feedback inhibition of glutathione synthesis by GSH (Richman and Meister, 1975; Hell and Bergmann, 1990; Schneider and Bergmann, 1995) and the depletion of GSH has been postulated to initiate increased glutathione synthesis under oxidative stress. Since there was no water-stress-related increase in oxidized glutathione in this study, an increase in total glutathione pool would not be anticipated.

Seasonal variation in glutathione content was reported
in spruce \textit{[Picea abies (L.) H. Karst.]} needles by Schmieden et al. (1993). They reported variation in total glutathione content with the highest levels (up to 300 $\mu$g g$^{-1}$ dry wt.) during the winter and the lowest values (as low as 63 $\mu$g g$^{-1}$ dry wt.) during the summer. The ratio of GSH/GSSG varied as well with a value of 12 in the winter and a lower value of 2 in the summer. They concluded that lower values of the ratio indicated increased oxidative stress in the tissues. The changes in glutathione metabolism that occur during the transition from summer to winter are in response to gradual and long-term changes in environmental conditions. Seasonal changes in oxidative stress are most probably different from those of transient and continuously variable water and temperature stresses seen in this study, thus the failure to see similar changes in response to water stress is understandable.

**Glutathione Reductase Activity**

Figure 5 demonstrates glutathione reductase activity during the growing season. The activity generally increased during the season. With the exception of the elevated activity in the dryland treatment on the second sampling date, there were no clear differences in glutathione reductase activity that appeared to be associated with the water treatments.

In some studies, glutathione reductase has been reported to vary in response to environmental stresses. Foster and Hess (1980) found that glutathione reductase was increased in cotton leaves exposed to an oxygen-enriched atmosphere. Increased glutathione reductase activity was reported in spinach plants exposed to low air temperatures by Schöner and Krause (1990). Loggini et al. (1999) reported increased glutathione reductase activity in the leaves of wheat cultivars that were subjected to short-term water deficits.

Stress-related changes in both the amount and form of glutathione, as well as the activity of glutathione reductase, were reported by Sgherri and Navari-Izzo (1995). They found increases in glutathione amount and glutathione reductase activity in the leaves of sunflower following exposure to water stresses in the field. When exposed to severe water deficits, they found that the glutathione pool became more oxidized, and both the total glutathione pool and activity of glutathione reductase declined. In another study that involved stress exposures under field conditions, Gamble and Burke (1984) monitored the activity of glutathione reductase in the leaves of wheat grown under different levels of water and temperature stress. They reported that glutathione reductase activity was increased under water deficits in what they suggested was an adaptive response. With the exception of the studies by Gamble and Burke (1984) and Sgherri and Navari-Izzo (1995), the above-cited studies all involved stresses imposed on relatively young plants grown in pots with limited water, as compared with this study, in which the water deficits developed gradually as the growing plants consumed the available water in much larger soil volumes in the field.

**Ascorbate and Ascorbate Peroxidase**

Figure 6 shows the activity of ascorbate peroxidase during the growing season. The ascorbate peroxidase activity generally increased as the season progressed with evidence of higher activity in the two water-deficit treatments, as compared with the full-irrigation treatment.
The amount of ascorbate across the season is shown in Fig. 7, which shows relatively high amounts at the initial sampling dates before the onset of water stress. The amount declines in all three treatments by DOY 212, when water stress had developed. Ascorbate was elevated in the water deficit treatments relative to the full irrigation at all subsequent sampling dates, even on DOY 269, when differences in leaf water potential were no longer detected (Fig. 2).

The changes in ascorbate metabolism associated with water deficits are in agreement with the findings of Sgherri et al. (2000), who reported increased ascorbate peroxidase activity in the leaves of wheat exposed to a cycle of water deficit and rewatering. At the same time, their finding that the activity of superoxide dismutase was diminished indicates that different antioxidant components can respond differently to water deficits. The elevations in ascorbate and ascorbate peroxidase activity do not agree with the results of Iturbe-Ormaetxe et al. (1998), who found that both ascorbate peroxidase activity and ascorbate concentration declined in peas exposed to water deficits or paraquat. Both studies involved water deficits that developed quickly in relatively small soil volumes as opposed to gradually developing field water deficits in this study.

**Malondialdehyde**

Malondialdehyde is an indicator of oxidative-stress related peroxidation of membrane lipids, and its concentration has been shown to correlate with peroxides resulting from temperature- and water-stress-related membrane damage (Halliwell and Gutteridge, 1989). Figure 8 shows the seasonal pattern of MDA in leaves from the three water treatments in Year 2. There was no indication of elevated MDA levels related to the water stress and, in fact, the highest levels of MDA that were observed early in the season were found in the full-irrigation treatment. Iturbe-Ormaetxe et al. (1998) reported elevated MDA levels in peas that had been exposed to acute short-term water stress in containers. Huang et al. (2001) reported increased MDA content in response to high soil temperatures in two cultivars of creeping bentgrass that were grown in sand-filled tubes for 42 d. While MDA was elevated by stress in both heat-sensitive and tolerant cultivars, it was higher in the heat-sensitive cultivar. The similar levels of MDA in well-watered plants in their study and both well-watered and water-stressed plants in our study suggests that oxidation of membranes was not enhanced by the water stresses imposed in this study.

**Enzyme Activity and Canopy Temperature**

While glutathione reductase activity, as measured by assays at 25°C, did not increase in response to water stress, the elevated leaf temperatures that are common in water-stressed plants have the potential to alter enzyme activity in the plant. Burke and Hatfield (1987) reported that because of differences in foliage temperatures in plants exposed to water deficits of varying severity, measurements of enzyme activity at a single temperature are not necessarily reflective of the activity during a day. In an effort to account for a temperature effect in this study, a Q10 of 2 was used to describe the thermal dependencies of glutathione reductase and ascorbate peroxidase between 15 and 45°C. By using enzyme activity measured at 25°C as a base, and a Q10 of 2, a temperature-adjustment equation was derived for the 15 to 45°C
range of canopy temperatures. For each 15-min period between 0800 and 2000 h on each sampling date, canopy temperature was multiplied by an appropriate adjustment factor to predict enzyme activity at that canopy temperature. Daily adjustment factors (averages of 48 15-min adjustments) varied from a high of 1.93 for the rainfed on DOY 227 to a low of 0.86 for the fully irrigated on DOY 253. The average temperature adjustments across all six days of measurement were 1.4, 1.3, and 1.1 for rainfed, deficit irrigation, and full irrigation, respectively, and the temperature-adjusted activities of glutathione reductase and ascorbate peroxidase were higher than the 25°C assay values in all cases. Though glutathione reductase assays did not detect increased activity with stress, it is possible that water-stress-related increases in leaf temperature could result in enhancement of antioxidant protection. The potential increases in this study are postulated to be on the order of 30 to 40%, and it is possible that such an increase would ameliorate the negative effects of stress-induced oxidative stress.

CONCLUSIONS

The initial goal of this study was to document the seasonal pattern of glutathione content in leaves of water-stressed cotton. Glutathione amount and form changed during the season in the first year, but not in response to water treatments imposed. Our observed lack of clear differences in response to different water levels in the first year prompted a second season with a larger range of water stress treatments and antioxidant indicators. In the second year of the study, the amount and form of glutathione, the activity of glutathione reductase and the amount of the oxidative stress indicator MDA did not vary in response to the water treatments. Ascorbate peroxidase activity and the amount of ascorbate were elevated in water deficit treatments relative to the full irrigation during Year 2.

The failure to observe water-stress-related glutathione differences in either the first or second year might be explained as a failure to develop water stress of sufficient intensity and duration to result in the increased production of ROS in the plant. In both years of the study, the fraction of PET replaced by irrigation and rain and the reductions in yield were similar. The PET values and lint yields indicate the development of moderately severe water stresses in both years, and the yield differences between the treatments are sufficiently large to indicate impaired plant performance resulting from environmental stresses. The development of water stresses, coupled with the observed changes in ascorbate metabolic indicators, suggest that the failure to observe changes in glutathione are not related to the failure to develop water deficits in the plants.

Glutathione amount and form, and glutathione reductase activity in cotton did not vary in response to differential water status of the magnitude and timing imposed in this study. If altered glutathione metabolism is a de facto indicator of increased oxidative stress, then the absence of alterations suggests that oxidative stress was the same in the dryland and irrigated treatments. Since very few studies of the response of oxidant metabolism to stress have been performed using field stressed plants, it is possible that some of the glutathione inductions previously reported are the result of oxidative stresses that are different from those induced in this study. An alternative explanation of the results is that the plants in the dryland treatment did indeed experience increased oxidative stress, but that cotton has only limited ability to alter glutathione metabolism in an adaptive manner in response to oxidative stress. Under this scenario, the lack of an increase in the foliar pool of glutathione in cotton under water stress might indicate an avenue for the improvement of cotton performance under field water stresses through the enhancement of glutathione metabolism. However, the failure to detect water-stress-related increases in MDA levels suggests that the oxidative stress damage that occurred was mitigated before membrane damage could occur.

Several studies have already demonstrated that glutathione metabolism can be altered, and that such alterations can improve plant performance under certain types of stress (Foyer et al., 1994; May et al., 1998; Noctor et al., 1998; Foyer and Noctor, 2001). The more than two-fold seasonal changes in the glutathione pool found in this study indicate that there is a mechanism for altering the pool size. The absence of variation in glutathione in response to water stress suggests that it may not be a vital component of the cotton plant’s response to water stress. In light of the relative constancy of at least some degree of oxidative stress in plants even under optimal water conditions, there would be a strong advantage for the plant to have antioxidant activity in excess of the level needed for protection under normal conditions. It is possible that the amount of glutathione present in...
the leaves was adequate for the oxidative compounds present in both the irrigated and dryland treatments. If indeed there was sufficient reduced glutathione present in the leaves, it is possible that transgenic approaches to increasing glutathione levels may not be very productive. This possibility is supported by the failure to improve plant protection by transgenic modification of glutathione metabolism reported in some studies.

REFERENCES


