Effect of Water Stress on the Chloroplast Antioxidant System

I. ALTERATIONS IN GLUTATHIONE REDUCTASE ACTIVITY

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ABSTRACT

The effect of water stress on glutathione reductase and catalase activities was evaluated in leaf blades of field-grown winter wheat (Triticum aestivum L.). Wheat was sown at two seedling rates under both irrigated and dryland conditions. Flag leaves from dryland plants sown at 60 kilograms/hectare showed no change in either glutathione reductase or catalase activities per unit leaf area, while leaves from the basal portion of the canopy exhibited a 273% increase in glutathione reductase activity and a 60% increase in catalase activity. Glutathione reductase activity in dryland plants sown at 120 kilograms/hectare increased 25% in flag leaves and 225% in basal leaves. No change in catalase activity was observed in either flag or basal leaves from these same plants. The increase in glutathione reductase activity in response to water stress was observed when activity was expressed on either a per unit leaf area, protein, or chlorophyll basis. No change in catalase activity was detected when enzyme activity was expressed on a protein basis.

The midday closure of stomates (20) in response to water stress may optimize the water use efficiency of the plant on a daily basis (3), yet result in a reduction in CO₂ assimilation at times when peak irradiances are commonly encountered. Because of stomatal closure, CO₂ fixation is low while photosynthetic electron transport is operating at normal rates. Under these conditions, limited quantities of NADP are available to accept electrons, therefore oxygen can function as an alternative electron acceptor (6). Although this pseudocyclic pathway for electron transport provides additional ATP (15), it can result in the production of superoxide and H₂O₂.

Superoxide and H₂O₂ are toxic oxygen molecules and upon reaction with chloroplast components can form even more reactive oxygen products such as the hydroxyl radical (7) and singlet oxygen (19). These toxic products produced within the chloroplast must be effectively removed to prevent lipid peroxidation, inhibition of CO₂-fixation, and the photooxidation of chloroplast pigments.

Photosynthetic cells can tolerate elevated oxygen levels because of the presence of several endogenous protective mechanisms including glutathione, ascorbate, carotenoids, and enzymes which effectively scavenge and remove the toxic products before cellular damage occurs (13). Plant cells contain millimolar concentrations of reduced glutathione (GSH) which can prevent the inactivation of enzymes by oxidation of essential thiol groups. Because of the availability of GSH, it can be preferentially oxidized thereby protecting the enzymes from inactivation. GSH can also reduce an oxidized sulfhydryl group thereby reactivating certain enzymes. When GSH reacts with oxygen, the glutathione is oxidized to form glutathione disulfide (GSSG). The subsequent re-reduction of GSSG to GSH is catalyzed by the enzyme glutathione reductase in an NADPH-dependent reaction. Glutathione reductase, therefore, plays an essential role in the protection of chloroplasts against oxidative damage by maintaining a high GSH/GSSG ratio.

This study investigates the effect of water stress upon the activity and distribution of the protection enzyme, glutathione reductase, in leaf blades from field-grown winter wheat. The enhanced activity of glutathione reductase during water stress and the importance of canopy position in the elevation of the enzyme will be discussed.

MATERIALS AND METHODS

Winter wheat (Triticum aestivum L., var Kanking) was planted in an Acuff fine sandy loam soil (fine-loamy mixed thermic Aridic Paleustolls) at two seedling rates of 60 and 120 kg/ha on October 18, 1983. The field was located in Lubbock County, Texas. Following initial irrigation of the plots to field capacity, irrigation was withheld from selected plots thus receiving supplemental water by rainfall only. Irrigated plots were furrow irrigated to field capacity on April 11 and May 3, 1984. Daily minimum and maximum temperatures, solar radiation, and rainfall measurements were obtained from the Texas Agricultural Experiment Station at the field site. At the end of the growing season the plants were harvested for grain yield.

Samples from both irrigated and dryland plots were collected for analysis of glutathione reductase and catalase activities seven times throughout the growing season with the first sample being taken after spring regrowth. To determine if blade position within the canopy affected glutathione reductase or catalase activities, blades were excised from the basal and apical portion of the canopy. Three blade samples were analyzed from each canopy position on each sampling date. Prior to blade homogenization for subsequent enzyme analyses, leaf areas were recorded on a Li-Cor® 3000 portable area meter.

 Supernatant fractions utilized for enzyme analyses were prepared by homogenization of the blade with a Polytron (Brinkman Instruments) for 30 s at 4°C. The tissue was homogenized in 20 ml of 0.1 M Tricine-NaOH (pH 7.8) containing 2 g of PVP (insoluble). Homogenates were filtered through four and then 12 layers of cheesecloth and centrifuged at 17,000g for 15 min. The supernatant fraction was assayed for glutathione reductase and catalase activities. Protein content of the supernatant was determined according to the procedure of Lowry et al. (16) using BSA

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as a standard. Chl concentrations were determined according to the procedure of Arnon (1).

Glutathione reductase was assayed according to the method of Schaedle and Bassham (18) with minor modifications. The 1-ml reaction mixture contained 0.1 mM NADPH, 40 mM Tricine-NaOH (pH 7.8), and 0.2 ml of supernatant. The reaction was initiated by the addition of 0.5 mM oxidized glutathione and the rate of NADPH oxidation was monitored at 340 nm. Glutathione reductase activity was expressed as nmol substrate oxidized/min on a leaf area, protein, and Chl basis. Catalase activity was assayed according to the method of Beers and Sizer (2).

The results summarized in this report are for winter wheat grown during the 1983 to 1984 season. Similar results were obtained for spring wheat grown during 1983.

RESULTS AND DISCUSSION

Average monthly meteorological data collected at the field site is presented in Table I. The maximum daily temperature increased from 17°C at spring regrowth to 29°C during the grainfilling period. Concomitant increases in minimum daily temperature from 1°C at spring regrowth to 13°C during grainfilling were observed. The mean solar radiation increased from 23.5 to 26.4 MJ/m²-d during the last 60 d of the study. The total accumulation of rainfall during this growing season was 2.8 cm compared with a 69-year average rainfall of 12.2 cm for this same 3-month period.

The grain yield of the dryland wheat (134 kg/ha) was 2.3-fold less than the yield of the irrigated wheat (308 kg/ha). Previous reports of wheat plants subjected to severe water stress indicated a yield decrease that ranged from 16 to 36% depending upon the ontogenetic stage of the wheat at the time the stress was induced (14). Grain yields of the present study confirm that the lack of rainfall in conjunction with increases in the average daily solar radiation and air temperature induced a severe stress on the dryland plants.

Top leaves from dryland and irrigated plants seeded at the low density (60 kg/ha) had similar levels of glutathione reductase activity (on a leaf area basis) at all sampling dates (Fig. 1). Samples taken from the base (Fig. 1) of the canopy exhibited similar levels of glutathione reductase activity in both dryland and irrigated tissues until day 123 (head emergence). By day 133 (beginning grain-fill to milky ripe stage), glutathione reductase activity in blades of the irrigated plants had declined to 14 nmol NADPH oxidized/min cm², while the activity in the blades of the dryland plants remained at a constant level of 38 nmol NADPH oxidized/min cm² representing a 2.7-fold higher level of activity under dryland conditions. At the high seeding density (120 kg/ha), top leaves from dryland plants showed increased levels of glutathione reductase activity, expressed on a leaf area basis, on days 123, 130, and 133 (Fig. 2). By the end of the study (day 133), top leaves from dryland plants had a 1.25-fold higher level of glutathione reductase activity compared to the irrigated plants. Basal leaves from dryland plants seeded at the high density exhibited a 2.2-fold increase in glutathione reductase activity on the last two sampling dates (Fig. 2). These results indicate that the effect of water stress on the level of glutathione reductase activity is influenced by both canopy position and seeding density. The increases in glutathione reductase activity in plants from the dryland treatment were greater for basal leaves than for leaves harvested from the top of the canopy. In addition, the differences in glutathione reductase activity in top leaves were only observed at the high seeding density while differences in basal leaves were observed under both seeding densities.

The effect of canopy position on the level of glutathione reductase activity may be related to morphological changes that occur in grasses (Gramineae) in response to water stress. Morphological changes, such as leaf rolling, decrease the plant's radiation load and allow more light penetration to the basal portion of the canopy. In addition, leaf rolling may reduce leaf

![Fig. 1. Changes in glutathione reductase activity in leaf blades of winter wheat seeded at the low density (60 kg/ha) under irrigated (O—O) and dryland ( ■ —■) conditions. Leaf samples were homogenized in 0.1 M Tricine-NaOH, pH 7.8. The supernatant was assayed for glutathione reductase activity by following the oxidation of NADPH spectrophotometrically at 340 nm. Enzyme activity is expressed as a function of leaf area. Each data point represents the mean of three samples ± SE.](image-url)
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Fig. 2. Changes in glutathione reductase activity in leaf blades of winter wheat seeded at the high density (120 kg/ha) under irrigated (□—□) and dryland (■—■) conditions. Glutathione reductase activity is expressed as a function of leaf area. Each data point represents the mean of three samples ± SE.

Fig. 3. Changes in glutathione reductase activity, expressed as a function of Chl concentration, in leaf blades of winter wheat seeded at the low density under irrigated (□—□) and dryland (■—■) conditions. Each data point represents the mean of three samples ± SE.

transpiration from 50% to 70% (17). Increased light penetration and reduced transpiration cause increased leaf and air temperatures in the lower portions of the plant canopy. Oats (Avena sativa L.) grown under irrigated and dryland conditions had marked air temperature differences between the two water regimes and within the plant canopy. Dryland plots had 3 to 4°C higher temperatures at the basal portion of the plant canopy as compared with that of the apical portion, while irrigated plots had no difference in air temperature between the apical and basal portion of the canopy (8). Increases in leaf and air temperatures within a dryland plant canopy may affect the level of glutathione reductase activity. Further, as a result of leaf rolling, changes in light quality and quantity within the plant canopy may affect the level of glutathione reductase activity. Light quality and quantity have been shown to influence the level and distribution of glutathione reductase in wheat (11) and mustard (5) seedlings.

The influence of seeding density on the level of glutathione reductase activity in upper leaves from irrigated and dryland plants may be related to the difference in the severity of water stress between the two seeding densities. By day 133 (beginning grain-fill), upper leaves from dryland plants at the high seeding...
density had midday water potentials of $-3.66 \pm 0.12$ MPa compared to $-2.59 \pm 0.04$ MPa for upper leaves from plants at the low seeding density while irrigated plants seeded at both densities had similar midday water potentials ($-1.03 \pm 0.01$ MPa). In addition, dryland plants at the high seeding density had lower grain yields (118 kg/ha) when compared to dryland plants seeded at the low density (150 kg/ha). Midday water potentials and grain yields indicate that dryland plants seeded at the high density were more severely stressed than dryland plants from the low density treatment. The severity of water stress may account for the elevation in glutathione reductase activity observed in upper leaves from dryland plants seeded at the high density (Fig. 2).

To determine if the increases in glutathione reductase activity on a leaf area basis were significant in relation to other changes in leaf metabolism, glutathione reductase activity was expressed on a Chl (Figs. 3 and 4) and protein (Figs. 5 and 6) basis. Glutathione reductase activity, expressed on a Chl basis, is shown in Figures 3 and 4. The overall changes in glutathione reductase activity on a Chl basis parallel the enzyme changes expressed on a leaf area basis. In general, the increases in glutathione reductase activity per mg Chl were greater than the increases in enzyme activity on a leaf area basis because of the maintenance of higher Chl levels (\(\mu g/cm^2\)) in the leaves of irrigated plants compared to dryland plants (Table II). Although changes in Chl content were observed in response to water stress, the changes were not associated with a change in Chl quality as evidenced by an average Chl $a/b$ ratio of 3.23 in top leaves and 2.91 in basal leaves from

**Fig. 4.** Changes in glutathione reductase activity, expressed as a function of Chl concentration, in leaf blades of winter wheat seeded at the high density under irrigated (---) and dryland (---) conditions. Each data point represents the mean of three samples ± SE.

**Fig. 5.** Changes in glutathione reductase activity, expressed as a function of protein content, in leaf blades of winter wheat seeded at the low density under irrigated (---) and dryland (---) conditions. Each data point represents the mean of three samples ± SE.
both irrigated and dryland treatments. Despite the lower Chl levels, dryland plants maintained elevated glutathione reductase activities.

Glutathione reductase activity, expressed on a protein basis, is shown in Figures 5 and 6. The increase in glutathione reductase activity per mg protein in leaves from dryland plants is consistent with the trends observed when glutathione reductase activity is expressed on a leaf area basis. The increase in glutathione reductase activity per mg protein in the basal leaves was not as great as the increase in enzyme activity on a leaf area basis because of the elevation of protein content (mg/cm²) in the leaves of dryland plants (Table III). While the soluble protein levels (mg/cm²) increased in basal leaves of dryland plants, the increases observed did not equal the increases seen in glutathione reductase activity. Thus, the elevation of glutathione reductase activity in dryland plants is not completely ascribable to a general increase in leaf protein content.
Table IV. Enzyme Activities of Leaf Blades for the Last Sampling Date (Day 133) from Irrigated and Dryland Plants

<table>
<thead>
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<th>Low Density</th>
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<th>High Density</th>
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<tr>
<td></td>
<td>Top Leaf</td>
<td>Base Leaf</td>
<td>Top Leaf</td>
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<tr>
<td></td>
<td>Irrigated</td>
<td>Dryland</td>
<td>Irrigated</td>
<td>Dryland</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>71 ± 15</td>
<td>54 ± 3</td>
<td>27 ± 2</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Catalase</td>
<td>138 ± 8</td>
<td>145 ± 4</td>
<td>120 ± 14</td>
<td>124 ± 5</td>
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* A unit of catalase activity is defined as that amount of enzyme required to decompose 1 µmol H₂O₂/min at 25°C. A unit of glutathione reductase activity is defined as that amount of enzyme required to oxidize 1 nmol NADPH/min at 25°C. Data for each enzyme are expressed as the average for three samples ± se.

Glutathione reductase and catalase activities in leaves from dryland plants (expressed as a percent of the irrigated control) are presented in Figure 7. At the first sampling date, levels of both enzymes in top and basal leaves from dryland plants were similar to levels observed in leaves harvested from the irrigated controls. By the end of the study (day 133), basal leaves harvested from dryland plants seeded at the low density exhibited a 60% increase in catalase activity compared to a 273% increase in glutathione reductase activity when expressed as a function of leaf area. When expressed on a per mg protein basis, however, no difference in catalase activity was detected between basal leaves from dryland and irrigated plants on the last sampling date (Table IV). This is in contrast to an 82% increase in glutathione reductase activity (expressed on a protein basis) in basal leaves from dryland plants seeded at the low density (Fig. 5; Table IV). Therefore, unlike the elevation in glutathione reductase activity, increases in catalase activity can be accounted for by a general increase in leaf protein content.

Environmental factors have been reported to cause an increase in the level of glutathione reductase activity in plants. Increased levels of glutathione reductase activity were observed in leaf extracts from spinach (Spinacia oleracea L.) in response to frost-hardening (4, 12). In addition, leaves from both corn (Zea mays L.) and cotton (Gossypium herbaceum L.) exposed to a 75% oxygen concentration showed a 2- to 3-fold increase in glutathione reductase activity within 48 h (9, 10). Foster and Hess (10) have proposed that increases in glutathione reductase activity in response to elevated oxygen concentrations suggest a prominent role for this enzyme in the protection of leaf tissue against oxidative damage. The observed increases in glutathione reductase activity in water-stressed wheat may also serve to protect chloroplasts against damaging oxygen products. Upon midday stomatal closure in response to water stress, elevated levels of glutathione reductase may serve to ensure the availability of NADP to accept electrons derived from photosynthetic electron transport, thereby directing electrons away from oxygen and minimizing the production of superoxide (10). Glutathione reductase may also function in the removal of H₂O₂ generated within chloroplasts via an ascorbate-glutathione cycle in which ascorbate peroxidase and glutathione reductase are key enzymes (13). In the present study, we have shown that elevated levels of glutathione reductase activity were produced in response to water stress. This increase in enzyme activity may constitute an adaptive response of wheat plants to low water potentials.

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