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Field Crops Research 94 (2005) 165–175

Field Crops Research

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Field-grown cotton plants with elevated activity of chloroplastic glutathione reductase exhibit no significant alteration of diurnal or seasonal patterns of excitation energy partitioning and CO_2 fixation

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Received 16 December 2004; accepted 8 January 2005

Abstract

Transgenic cotton plants with elevated activity of chloroplast-targeted glutathione reductase (GR+) were grown in field plots over two seasons in order to compare their photosynthetic performance with that of wildtype plants. We hypothesised that transgenic plants would show enhanced protection of the photosynthetic apparatus against photoinhibition, primarily through an increase in electron transport associated with the role of the chloroplastic antioxidant system as an alternative electron sink. Diurnal measurements of chlorophyll *a* fluorescence from cotyledons, stem leaves, and leaves subtending developing fruits (bolls) were used to estimate the rate of linear electron transport (J_e) and the rates of reversible, regulated (NPD_{REG}) and photoinhibitory (NPD_{PI}) non-photochemical energy dissipation (NPD) at several times (June, July, September, October) during each growing season. GR+ cotyledons exhibited greater J_e than wildtype cotyledons during the middle of a day in early June, while NPD_{PI} was the same for both genotypes. Throughout most days on which measurements were conducted, no genotypic differences in J_e and NPD were observed for stem leaves. Only during the late morning of one day in early October did leaves subtending bolls of GR+ plants exhibit greater J_e compared to that for wildtype plants. As leaves subtending bolls of both genotypes aged, J_e and CO_2 assimilation declined, while NPD increased. Maximum NPD_{PI} and minimum F_v/F_m remained essentially the same for all measurement days. However, the maximum NPD_{REG}, the greatest contributor to NPD, increased with leaf age. We conclude that the rapid rise in leaf temperature during most mornings created conditions in which elevated GR activity conferred no advantage. Also, as light energy absorption became excessive in late morning, cotton leaves exhibited a strong capacity for regulated, non-photochemical energy dissipation that may have served as the major photoprotective mechanism.

Keywords: Antioxidants; Chlorophyll fluorescence; Glutathione reductase; *Gossypium hirsutum*; Photoinactivation; Photosynthesis

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doi:10.1016/j.fcr.2005.01.001

1. Introduction

The capacity to utilize light energy absorbed by the photosynthetic apparatus typically decreases in plants subjected to unfavourable environmental conditions, resulting in greater absorption of light energy in excess of that needed for CO_2 assimilation. Excessive light energy absorption causes damage to the photosynthetic apparatus (photoinactivation) due, in part, to the generation of reactive oxygen species (ROS) that cause oxidative damage to chloroplastic constituents, thereby impairing carbon assimilation during and following stress (Baker, 1994; Allen and Ort, 2001). In addition to dissipating much of this excess energy as heat (Demmig-Adams et al., 1996), plants employ antioxidant defense systems to detoxify the ROS that are formed (Asada, 2000). H_2O_2 is a critical ROS that must be eliminated before it forms the highly reactive hydroxyl radical ($OH^{\bullet}$). Scavenging of H_2O_2 in the chloroplast requires ascorbate. Some processes that regenerate ascorbate require reduced glutathione, whose pools are maintained by glutathione reductase (GR) (Foyer et al., 1994; Asada, 1999).

Cotton, a plant of tropical origin, is especially sensitive to the combination of high photon flux density (PFD) and suboptimal leaf temperatures (Königer and Winter, 1993; Payton et al., 1997). In an attempt to enhance the resistance to photoinactivation, transgenic cotton plants overproducing GR were created (GR+ plants) (Payton et al., 2001). When chilling stress was imposed rapidly in a laboratory, such a modification improved the tolerance of cotton photosynthesis to chilling and high PFD by enhancing photosystem I (PSI) and photosystem II (PSII) protection from photoinactivation (Kornyeyev et al., 2001, 2003). After a 3 h exposure to a PFD of 500 μmol m^{-2} s^{-1} and 10 °C, GR+ leaves exhibited approximately 8–11% higher capacities of PSII (as determined by the ratio of variable to maximum chlorophyll *a* fluorescence, F_v/F_m) and PSI (as determined by the amount of photo-oxidizable P700) than did wildtype leaves. Also, the quantum yield for PSII photochemistry during a chilling stress was more than 30% greater for GR+ leaves than for wildtype leaves. However, the GR+ plants did not outperform wildtype plants when the temperature was gradually decreased from 28 to 14 °C over 9 days in a growth chamber (Logan et al., 2003). Although the laboratory experiments revealed the positive effects of elevated GR activity on the protection of the photosynthetic apparatus over the short term (Kornyeyev et al., 2003), a field trail would be necessary to critically assess the effect of elevated GR activity on photosynthesis under the variable environmental conditions of a growing season. A field study of GR+ plant performance might provide further understanding and reveal potential pitfalls of the strategy of improving crop production through modification of the antioxidant system. Interestingly, despite the development of numerous transgenic plant lines with elevated activities of various antioxidant enzymes since the work of Tepperman and Dunsmuir (1990), only a few of these lines have been subjected to field tests. A field study of the GR+ cotton plants would also be particularly important, since a thorough understanding of how wildtype cotton plants utilise absorbed light energy in the field under variable temperature and PFD conditions is lacking.

We hypothesised that GR+ plants would sustain less photoinactivation during periods of environmental stress, because of enhanced photochemistry attributable to the antioxidant system's role as an alternative electron sink (Asada, 2000). To test this hypothesis, we compared the photosynthetic performance of the transgenic plants with that for wildtype plants in an irrigated field plot for typical days over the entire growing season to determine whether GR overproduction conferred measurable improvement in cotton photosynthesis in the field. By using chlorophyll fluorescence analysis, we were able to investigate the diurnal changes in the contribution of non-photochemical and photochemical mechanisms to the quenching of excitation energy in PSII complexes and assess the extent to which elevated GR activity altered the distribution of the light energy absorbed by PSII antennae under field conditions.

2. Methods

2.1. Plant material

Cotton, *Gossypium hirsutum* L. cv. Coker 312, was transformed to overproduce chloroplast-targeted glutathione reductase (GR+) using the *Arabidopsis* gene for GR as described previously in Payton et al. (2001). Subsequent generations exhibited a 36-fold increase in total leaf GR activity over that of wildtype, with the majority of the increase in GR activity associated with the chloroplasts (Kornyeyev et al., 2001; Payton et al., 2001).

Plants were grown at the Texas Tech University experimental fields in Lubbock, TX (33.6°N, 101.9°W). The irrigated plot was established in mid-May of 2001 and again in 2002. Each row was aligned east to west and consisted of plants from three independently transformed lines of GR+ or wildtype (cv. Coker 312). Each transgenic line was represented by nine plants/line with 20 cm between plants.

The measurements for data presented here were conducted on plants at the cotyledon (June 7, 2002), seedling (July 10, 2001), and boll (fruit) development (August–October 2001 and 2002) stages. No cotyledon measurements were made in 2001, but data for leaves were collected in both years and were similar. The leaves that were chosen for gas-exchange and fluorescence measurements were located on the south-facing side of each plant. Measurements were made on cotyledons prior to the development of stem leaves, on the first fully expanded stem leaf (4th or 5th leaf) of seedlings, and on leaves subtending developing bolls of older plants. Leaves subtending bolls that were measured on October 6, 2001 were ~45 days old. Because no differences in GR activity and photosynthetic performance were found between the transgenic lines in previous laboratory/greenhouse tests (Kornyeyev et al., 2003), as well as in the present field study, the data obtained for the three transgenic lines were combined.

2.2. Assay for GR activity in leaf extracts

To determine the GR activity in cotyledon and leaf extracts, leaf discs were rapidly removed using a cork borer and immediately frozen in liquid N_2. Extracts were prepared and assayed for GR activity spectrophotometrically by monitoring the oxidation of NADPH at 340 nm as previously described (Sen Gupta et al., 1993; Kornyeyev et al., 2001). Aliquots of the extracts were taken before centrifugation for chlorophyll determination in 80% acetone according to Lichtenthaler (1987).

2.3. Gas-exchange measurements

An LCA-4 portable photosynthesis system (ADC Ltd., Hoddesdon, UK) was used to measure CO_2 and H_2O exchange for leaves at ambient CO_2 concentration under natural illumination. For comparisons of CO_2 assimilation and electron transport (photochemistry), the rate of CO_2 assimilation was corrected for the rate of mitochondrial respiration in the dark.

2.4. Chlorophyll fluorescence measurements

Field chlorophyll fluorescence data were collected using a FMS2 portable fluorometer (Hansatech Instruments Ltd., UK). During the measurements, the leaves were kept at their natural angle. The magnitudes of photon flux density (PFD) and temperature were monitored by means of sensors located on the measuring clip of the fluorometer. The leaves (especially cotyledons) of field-grown plants were rather thick, reducing the effect of the size of the thermocouple junction on leaf temperature. In order to allow equilibration of the temperature between the plant tissue and thermocouple, leaf temperature data were taken between 1 and 2 min after the thermocouple was attached to the leaf surface.

The experimental protocol described by Schreiber et al. (1986) and nomenclature of van Kooten and Snel (1990) were employed for the fluorescence analysis. Immediately after measurements of F, F'_m, and F'_o (steady state, maximal, and minimal levels of chlorophyll fluorescence for light-adapted samples, respectively), leaf discs were collected using a cork borer to determine values of F_v/F_m after 1.5 h of dark incubation at room temperature. These values were denoted as $(F_v/F_m)_{PI}$ and used as an estimate of photoinactivation/photodamage of PSII reaction centers.

The rate of linear electron transport (J_e) was estimated by the following equation (Maxwell and Johnson, 2000):

$$J_e = \Phi_{PSII} \times PFD \times 0.5 \times 0.75 \quad (1)$$

Φ_{PSII} is the quantum efficiency of linear electron transport ($\Phi_{PSII} = (F'_m - F)/F'_m$, Genty et al., 1989; Maxwell and Johnson, 2000), PFD is the incident photon flux density, and 0.75 and 0.5 are the coefficients for cotton leaf absorbance (Björkman and Demmig, 1987) and for the sharing of absorbed photons between PSI and PSII, respectively. The value of the latter coefficient (0.5) assumed an equal distribution of light energy between PSI and PSII (Krall and Edwards, 1992).

The ratio of variable to maximal chlorophyll *a* fluorescence measured for light-acclimated leaves (F'_v/F'_m), reflecting the efficiency of excitation energy capture by open PSII reaction centers (Genty et al., 1989), is widely applied as an indicator of the changes in non-photochemical dissipation of the light energy absorbed by PSII antennae (Rohacek, 2002; Oxborough and Baker, 2000). We calculated the parameter $D = 1 - F'_v/F'_m$ in order to estimate the efficiency of non-photochemical dissipation in PSII complexes (Demmig-Adams et al., 1996).

In a manner analogous to the estimation of J_e, the rate of non-photochemical energy dissipation (NPD) was estimated using the following equation (Demmig-Adams et al., 1996):

$$\mathrm{NPD} = D \times \mathrm{PFD} \times 0.5 \times 0.75 \quad (2)$$

Non-photochemical dissipation is known to consist of multiple components (Maxwell and Johnson, 2000; Müller et al., 2001), including the following: (a) a component frequently termed q_E that is dependent on the ΔpH across the thylakoid membrane; (b) a component associated with state transitions (q_T) that is due to the reduction in the absorption cross-section of PSII after phosphorylation of its light harvesting complex (LHCII); (c) a component developing as the result of photoinhibitory damage to PSII (q_I). In addition, even in dark-acclimated, non-stressed leaves, constitutive non-photochemical dissipation in the antennae occurs, decreasing the quantum yield of photochemistry from 1 (the maximal theoretical value) to ~0.8. The following formulae can be used to estimate the quantum efficiency of the processes associated with the different components of non-photochemical dissipation in PSII complexes of light-adapted samples (see Kornyeyev et al., 2002 for additional explanations). For regulated, non-photochemical quenching (D_{REG}) relaxing in darkness:

$$D_{\mathrm{REG}} = 1 - (F'_v/F'_m)/(F_v/F_m)_{\mathrm{PI}} \quad (3)$$

According to this definition, D_{REG} reflects the combined influence of the processes that control the q_T and q_E components of non-photochemical chlorophyll *a* fluorescence quenching described above. Thus, D_{REG} estimates the non-photochemical dissipation that can be relaxed during dark acclimation and can be used to assess the reversible down regulation of PSII activity. The quantum efficiency of non-photochemical dissipation that cannot be reversed (the photoinhibitory component, an analog of q_I) was calculated as:

$$D_{\mathrm{PI}} = (1 - (F_v/F_m)_{\mathrm{PI}}/(F_v/F_m)) \times (F'_v/F'_m)/(F_v/F_m)_{\mathrm{PI}} \quad (4)$$

$(F_v/F_m)_{\mathrm{PI}}$ is the value of F_v/F_m measured after 1.5 h of dark incubation of the samples (leaf discs) taken during the experiment as described above.

We estimated the rate of non-photochemical dissipation controlled by its reversible ($\mathrm{NPD_{REG}}$) and non-reversible ($\mathrm{NPD_{PI}}$) components as:

$$\mathrm{NPD_{REG}} = D_{\mathrm{REG}} \times \mathrm{PFD} \times 0.5 \times 0.75 \quad (5)$$

$$\mathrm{NPD_{PI}} = D_{\mathrm{PI}} \times \mathrm{PFD} \times 0.5 \times 0.75 \quad (6)$$

2.5. Statistical analysis

Analyses were performed on 15 days under different environmental conditions during the 2001 and 2002 field seasons. Data from representative analyses for typical environmental conditions at each stage of plant development (cotyledon, seedling, and fruiting stages) are presented in the figures. Data for transgenic genotypes were compared with data for wildtype using a Student's *t*-test. Means were considered significantly different for $P \leq 0.05$.

3. Results

3.1. Extractable activity of GR

No differences in extractable GR activity were observed between independently transformed lines. For example, GR activities of 1990 ± 557, 2019 ± 302, and 2285 ± 78 μmol (mg Chl)$^{-1}$ h^{-1} were determined for the cotyledons of lines #5 ($N = 4$), #8 ($N = 6$), and #33 ($N = 3$), respectively. Given that the GR activity for wildtype plants was 94 ± 19 μmol (mg Chl)$^{-1}$ h^{-1}, the level of overproduction was high (~22-fold). Leaves subtending bolls exhibited a similar level of overproduction, as well (Table 1). However, with increasing leaf age, extractable GR activity decreased in GR+ but not in wildtype plants, leading to a decrease in the level of overproduction.

Table 1
Changes in the activity of glutathione reductase (GR) during the aging of leaves subtending bolls

Days post-anthesis	Wildtype	GR+	GR + /wildtype
16	73 ± 14	1937 ± 258	26.5
23	60 ± 11	1408 ± 163	23.5
32	61 ± 14	1512 ± 333	24.8
42	66 ± 14	1215 ± 237	18.5

Genotypic differences were significant on all measurement days (Student's *t*-tests; $P < 0.001$). Mean ± 1 standard deviation, $n = 5$–7.

3.2. Photochemistry and non-photochemical energy dissipation during the growing season

We conducted a series of measurements over two growing seasons to analyse the diurnal changes in the activity of the photosynthetic apparatus under typical environmental conditions at three different plant developmental stages. Measurements were made on clear days with a relative humidity at maximum daytime temperatures of 23–27%. The pattern of daily temperature change for cotyledons or stem leaves was similar for plants in early June (Fig. 1A) and early July (Fig. 2A), rising rapidly with increasing PFD and remaining near 35 °C for 6–8 h. Thus, even in the summer, cotton plants experienced a range of 15 °C in leaf temperature at the Texas Tech Experimental Fields. In early October on a sunny day when leaf temperature was 0 °C at dawn, the leaf temperature rose rapidly, reaching 28 °C in late afternoon (Fig. 3A).

For either cotyledons (Fig. 1B) or the first fully expanded stem leaves of seedlings (Fig. 2B) in early June or July, respectively, the rise in the estimated rate of linear electron transport (J_e) paralleled the rise in PFD in the morning for both genotypes. However, the decline of J_e in the afternoon lagged behind the decline in PFD. Cotyledons of GR+ plants exhibited a higher J_e than wildtype cotyledons, but only during the middle of the day (Fig. 1B). J_e for stem leaves of seedlings was similar for both genotypes throughout the day (Fig. 2B).

For leaves subtending bolls that were measured in early October, although J_e increased in parallel with PFD in the first half of the morning, further increases in PFD resulted in only a small increase in J_e, and the maximum J_e for both genotypes was approximately 56% of the maximum J_e attained in July (Fig. 3B). This low J_e for old leaves occurred even when the temperature was near optimum and the PFD was high. In fact, on another October day when the temperature was higher, J_e was also low (data not shown). When

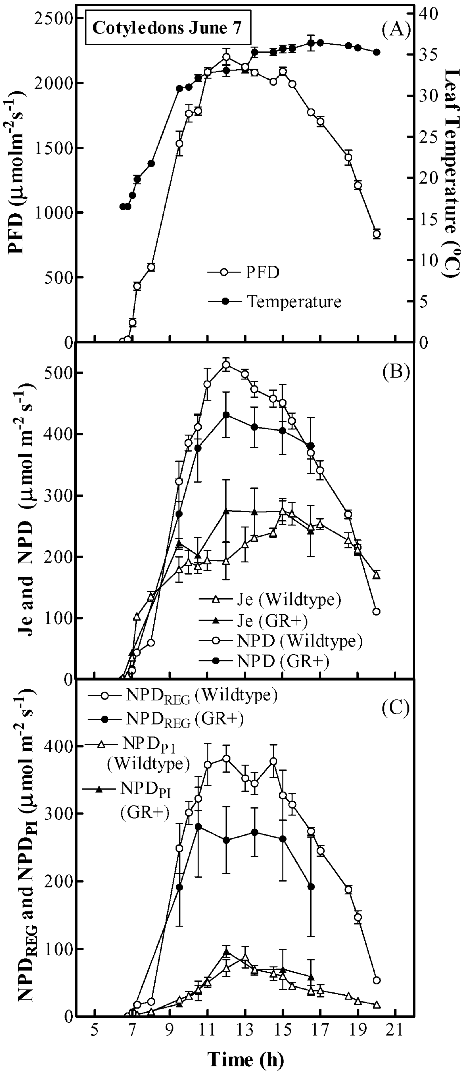

Fig. 1. Changes in leaf temperature and photon flux density (PFD) (A) and the partitioning of light energy, (B and C) for cotyledons of cotton plants overproducing chloroplastic glutathione reductase (GR+) and wildtype plants during June 7, 2002. Rates of linear electron transport (J_e) and non-photochemical energy dissipation (NPD) (B) were estimated using chlorophyll fluorescence analysis. NPD was partitioned into the portion that relaxes over 1.5 h of dark incubation (regulated NPD, NPD$_{REG}$) and the portion that does not relax during dark incubation (photoinhibitory NPD, NDP$_{PI}$) (C). Data are means ± 1 standard deviation, $n = 3$.

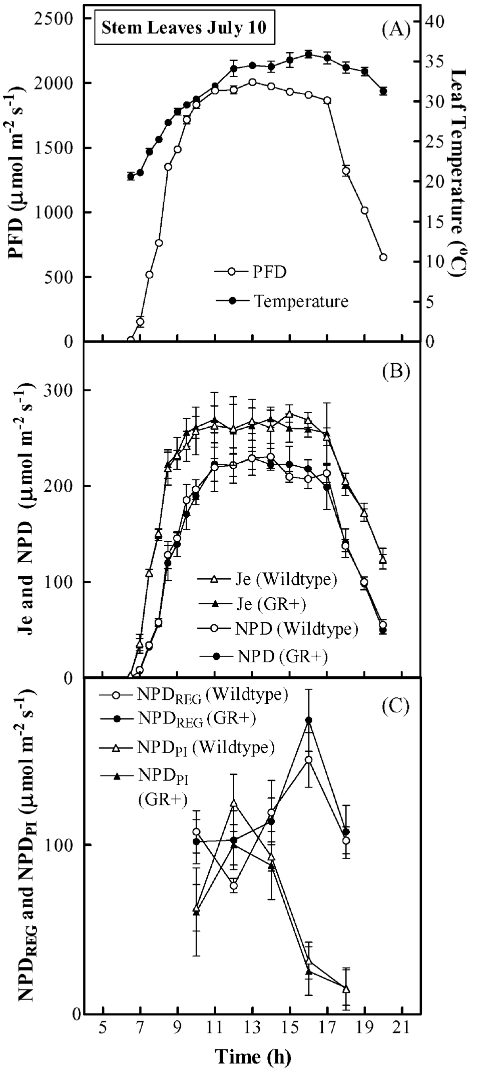

Fig. 2. Changes in leaf temperature and photon flux density (PFD) (A) and the partitioning of light energy, (B and C) for the first fully expanded stem leaves of cotton seedlings overproducing chloroplastic glutathione reductase (GR+) and wildtype plants during July 10, 2001. See the legend of Fig. 1 for details. Data are means ± 1 standard deviation, $n = 4$.

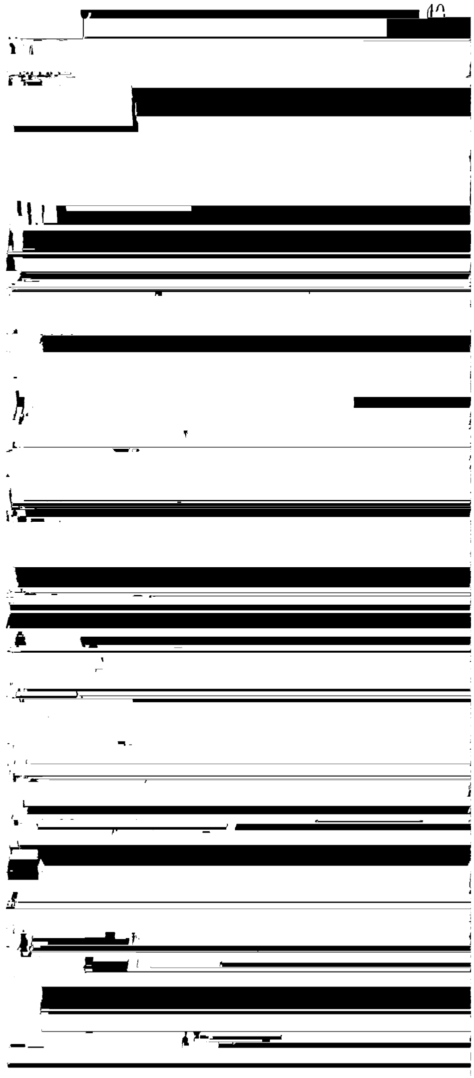

Fig. 3. Changes in leaf temperature and photon flux density (PFD) (A) and the partitioning of light energy, (B and C) for leaves subtending bolls of cotton plants overproducing chloroplastic glutathione reductase (GR+) and wildtype plants during October 6, 2001. See the legend of Fig. 1 for details. Data are means ± 1 standard deviation, $n = 5$.

the morning leaf temperature was low, we observed a slightly, but significantly, higher J_e for GR+ than for wildtype plants (Fig. 3B). However, no significant genotypic differences in J_e were observed between wildtype and GR+ plants on the other October day when the initial leaf temperature was higher (data not shown).

As with J_e, the diurnal pattern of non-photochemical energy dissipation in PSII complexes (NPD) was strongly dependent on PFD, reaching a maximum in the middle of the solar day for every measurement date (Figs. 1–3B). It is interesting to note that the levels of NPD were higher than J_e at the beginning of the growing season (June) for cotyledons and at the end of the season (October) for leaves ~45 days old that were subtending bolls, while electron transport prevailed in the middle of the summer for the first fully expanded stem leaves.

We analysed the diurnal changes in the rate of non-photochemical energy dissipation in PSII controlled by regulatory, readily reversible mechanisms (NPD_{REG}) and photoinhibitory, PSII inactivation (NPD_{PI}). NPD_{REG} played the primary role in the dissipation of excessive light energy absorbed by PSII antennae on each measurement day throughout the growing season (Figs. 1–3C). We observed genotypic differences in NPD_{REG} only for cotyledons during the middle of the day when NPD_{REG} was significantly lower for GR+ in comparison to wildtype plants (Fig. 1C). Note that the small genotypic differences in J_e for cotyledons (in June) and the ~45-day-old leaves that were subtending bolls (in October) had no effect on NPD_{PI} (Figs. 1 and 3C).

The diurnal maximum of NPD_{PI} was usually observed earlier than that of NPD_{REG} (approximately 12:00 h versus early afternoon for maximum NPD_{REG}). The maximum NPD_{PI} was reached during the middle of each measurement day and then decreased in the early part of the afternoon when PFD was still high (Figs. 1–3C). The diurnal change in the maximal efficiency of PSII photochemistry (F_v/F_m) reflected the change in NPD_{PI}, with the minimum F_v/F_m values being reached in the middle of each measurement day (data not shown). As with the maximum levels of NPD_{PI} reached each measurement day, the minimum values of F_v/F_m reached were similar, despite differences in leaf age and environmental conditions, and we observed no significant differences between genotypes (data not shown).

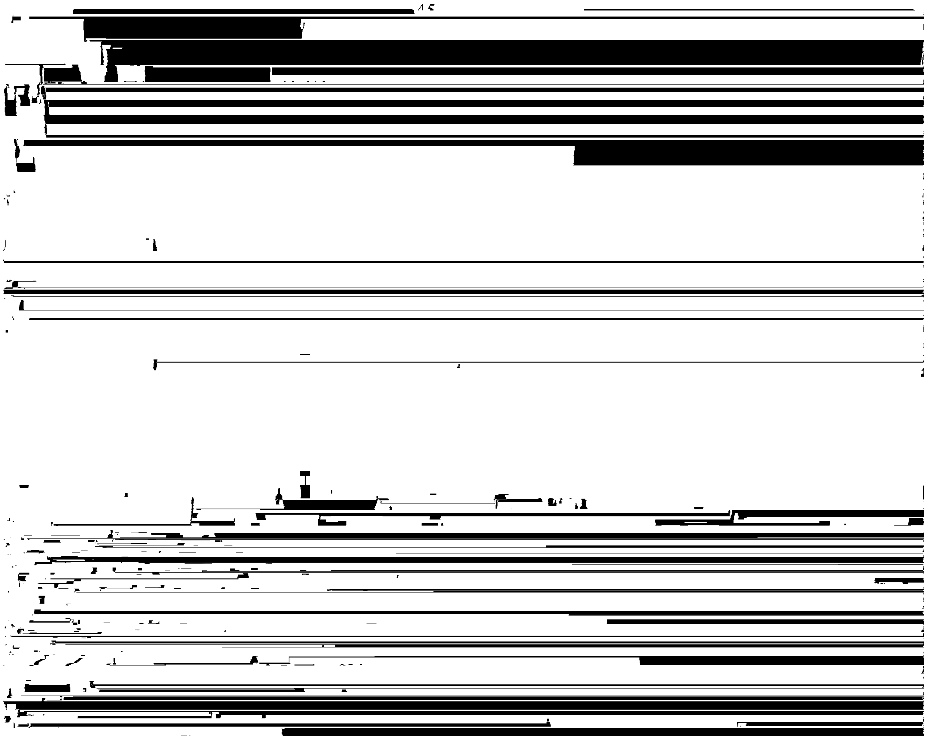

Fig. 4. Changes in photosynthetic parameters with age for leaves subtending bolls on cotton plants overproducing chloroplastic glutathione reductase (GR+) and wildtype plants. Measurements were conducted at maximum photon flux density (PFD) in September 2002. (A) The PFD and leaf temperature when measurements were made. (B) CO_2 assimilation and stomatal conductance. (C) Linear electron transport (J_e) and non-photochemical energy dissipation (NPD). (D) PSII activity estimated as variable to maximal chlorophyll *a* fluorescence (F_v/F_m). Data are means ± 1 standard deviation, $n = 5$.

3.3. Effect of leaf aging on photosynthesis during boll development

Wullschleger and Oosterhuis (1990) reported that net CO_2 assimilation for leaves subtending bolls reached a maximum as the flower opened and declined by about 50% during the boll development period, even though the bolls received much of their photosynthate from these leaves. We investigated the change in gas-exchange parameters, electron transport, and energy dissipation with increasing age of leaves subtending bolls of wildtype and GR+ plants. During the period of greatest boll growth (16–41 days post-anthesis [dpa]), the rate of CO_2 assimilation, stomatal conductance, and J_e declined for these leaves (Fig. 4). The leaf internal CO_2 concentration (C_i) remained constant over this period, averaging 210 μmol CO_2 mol^{-1} (data not shown). As J_e declined, the magnitude of NPD increased proportionately (Fig. 4C). The level of PSII inactivation increased from 16 to 22 dpa and then exhibited no further change with further leaf aging (Fig. 4D). The overproduction of GR in the leaves had no significant effects on the changes in the magnitude of any of these parameters with aging of the leaves.

4. Discussion

4.1. Regulation of excitation energy partitioning in PSII complexes of cotton (How cotton copes with excessive light absorption)

Chlorophyll fluorescence measurements can be used to partition the utilization of absorbed light energy in PSII complexes (Demmig-Adams et al., 1996; Kornyeyev et al., 2002). This analysis applied to our data for cotton leaves in the field under a variety of temperatures and PFDs revealed the important role of non-photochemical energy dissipation in the photoprotection of photosynthesis for cotton plants exposed to natural conditions. The maximum estimated rate of non-photochemical energy dissipation in PSII complexes occurred in the middle of the solar day and was close in magnitude to the rate of linear electron transport for stem leaves of seedlings under non-stressful conditions in July (Fig. 2). Low temperature stress and leaf aging (Figs. 3 and 4) led to a measurable decrease in electron transport, while the level of non-photochemical dissipation of excitation energy in PSII complexes increased. Thus, it appears that these two major routes for energy utilisation are in balance in cotton leaves (Laisk et al., 1997).

CO_2 assimilation by cotton leaves is known to decline with plant and leaf age (Wullschleger and Oosterhuis, 1990; Peng and Krieg, 1991; Pettigrew et al., 2000; Fig. 4). We show here that a decline in electron transport accompanies the decline in CO_2 assimilation as the leaf ages (Figs. 4B and C). However, old cotton leaves compensate well, albeit not perfectly, for low J_e by exhibiting strong non-photochemical quenching of excitation energy (Fig. 4C) that becomes the dominant route for energy dissipation in PSII complexes in October (Fig. 3B). These data are in accordance with those reported for studies on the aging of wheat leaves (Lu et al., 2001).

For cotton in our experiment, the decline in CO_2 assimilation with leaf aging was associated with a large decline in stomatal conductance (Fig. 4B and C), suggesting that stomatal conductance might have constrained CO_2 assimilation with aging. However, the C_i for the leaves as they aged remained virtually constant. Thus, it is likely that CO_2 assimilation of aging leaves is largely limited by biochemical factors. This suggestion is supported by the observation of Pettigrew et al. (2000) that carboxylation capacity declines as cotton plants age.

The deconvolution of non-photochemical energy dissipation in PSII complexes into its regulated (NPD_{REG}) and photoinhibitory (NPD_{PI}) components (Figs. 1–3C) indicates that, for cotton, NPD_{PI} only contributes substantially to excitation energy dissipation during the middle of the photoperiod throughout the growing season on sunny days. The most likely explanation for the decrease in NPD_{PI} in the afternoon at near maximum PFD is that PSII repair rates are high, while NPD_{REG} remains high (in cotyledons) or increases (in stem leaves) to insure substantial protection of PSII complexes from photoinactivation.

Under the conditions experienced by cotton cotyledons or leaves throughout the growing season in our plot, adjustments in NPD were largely realised by altering NPD_{REG} (Figs. 1–3C). This response to rising PFD was most evident for cotyledons and the 45-day-old leaves subtending bolls in October. Even for stem leaves in July between 12:00 and 16:00,

NPD_{PI} declined sharply while NPD_{REG} rose with no change in total NPD (Figs. 2 and 3C). Thus, at least under our experimental conditions, photoinactivation of PSII complexes may not have determined the rate of electron flow from the pool of PSII complexes. The negative effect of PSII photoinactivation on electron flow may only be manifested in situations when there is no demand for thermal dissipation, namely at low PFD and optimal temperature, the very conditions that are favourable for PSII recovery. However, despite the appearance that photoinactivation may not have controlled the actual PSII efficiency in light-acclimated cotton leaves subjected to high light, the recovery processes may have consumed metabolic resources acting in competition with carbon assimilation and, in this way, potentially decreased the productivity of the plants.

4.2. The performance of GR+ plants

From previous studies, it was concluded that resistance to photoinactivation or photoinhibition could be enhanced by a several-fold overproduction of GR in chloroplasts of poplar, tobacco, and cotton (Foyer et al., 1995; Tyystjärvi and Aro, 1996; Payton et al., 2001; Kornyeyev et al., 2003). We showed that GR+ cotton leaves exhibited a greater J_e than wildtype leaves when we exposed the leaves to 10 °C and a moderate PFD in the laboratory or greenhouse (Kornyeyev et al., 2001, 2003). We proposed that it was this enhanced J_e that led to GR+ leaves exhibiting less PSII photoinactivation. Our conclusions were based on results from experiments in which the chilling stress was imposed rapidly on plants that had been grown under favourable conditions. Results from a recent study conducted using a controlled-environment chamber suggested that wildtype cotton acclimated, to some extent, to chilling conditions when the temperature fell slowly over a period of ~2 weeks, abolishing the advantage conferred by GR overproduction (Logan et al., 2003). However, the PFD at which the plants grew in that experiment was low (~300 μmol photons m^{-2} s^{-1}), so the possibility that GR+ cotton could outperform wildtype cotton under the high PFD environment of the field remained.

With few exceptions, our measurements conducted in the field did not reveal a photosynthetic advantage for GR+ over wildtype. With the exception of one short period on an early October day, leaves of both genotypes exhibited similar rates of electron transport, CO_2 fixation, non-photochemical energy dissipation, and photoinactivation of PSII. Thus, there does not appear to be any real advantage to possessing elevated chloroplastic GR activity in cotton leaves in the field. However, it is worth noting that the GR+ cotyledons did exhibit significantly greater J_e than did wildtype cotyledons at high PFD and temperature. Although the significant differences in J_e did not result in any substantial differences in the extent of PSII photoinactivation, as estimated by NPD_{PI} and F_v/F_m, it is possible that genotypic differences in J_e and F_v/F_m would be detected for cotyledons during chilling at high PFD. However, no low temperatures with high PFD occurred in either growing season of this study when plants were in the cotyledon stage.

We offer the following possible explanations for why the GR+ plants generally did not outperform wildtype plants with respect to photoprotection under field conditions:

(1) It is possible that the native GR activity in cotton did not constrain the performance of the antioxidant system under the conditions that the plants experienced in the Texas Tech Experimental Fields. In the laboratory, the genotypic differences in J_e that we observed occurred only at low temperature (Kornyeyev et al., 2003). According to Asada (2000), the rate of the water–water cycle involving GR is regulated by the photoreduction of oxygen, but not its enzymatic reactions, at favourable temperatures. At low temperature, the activity of antioxidant enzymes is decreased while the probability for oxygen photoreduction is increased (Wise and Naylor, 1987). Given the low temperature constraints on enzyme kinetics, it is logical that a greater activity of GR in GR+ leaves would improve the demand for reducing power from electron transport and raise J_e during chilling. During our summer measurements in the field, the leaf temperatures were never at or below the temperature at which we observed genotypic differences in J_e in the laboratory (Kornyeyev et al., 2003). In fact, leaf temperatures rose rapidly as the PFD of the light striking the leaves increased, even during early October (Fig. 3A).

Only for a brief period on one measurement day in October was the combination of leaf temperature (approximately 15 °C) and PFD (nearly 1400 μmol m^{-2} s^{-1}) favourable for observing a genotypic difference in J_e. In addition, it is likely that, with exposure to excessive PFD on a daily basis, acclimatory changes in components of the antioxidant system that were more critical to protection from photoinactivation than GR improved J_e for wildtype plants.

(2) It is possible that the lack of genotypic differences in the intrinsic efficiency of PSII energy transfer (F_v/F_m) under any environmental conditions for cotyledons and leaves was due to the ability of cotton to develop strong non-photochemical energy quenching. In the field, the PFD increased to a maximum over a 2–3 h period. This time allowed for the engagement of photoprotective mechanisms other than ROS scavenging, as indicated by the development of reversible (regulated) non-photochemical energy quenching in PSII complexes. As PFD rose, so did the leaf temperature, increasing the activity of the xanthophyll cycle (Bilger and Bjorkman, 1991), a critical process in non-photochemical quenching of excessive excitation energy (Ruban and Horton, 1999), and increasing PSII repair processes. The leaves in the previous laboratory experiments were exposed to rapid increases in illumination and decreases in temperature (Kornyeyev et al., 2001, 2003). In addition, exposure to the high PFD environment of the field for several weeks may have stimulated acclimation responses associated with non-photochemical quenching.

5. Conclusions

Cotton is a plant that typically grows in a high PFD environment. The data described above show that the major factor contributing to photoprotection in cotton leaves under field conditions is non-photochemical energy dissipation in PSII complexes. Our study shows that the level of non-photochemical dissipation is regulated in response to changes in the rate of photochemistry (electron transport) and PFD. This process is sufficient to prevent extensive damage to PSII complexes over a rather broad temperature range for both young and old leaves. Given the effectiveness of non-photochemical dissipation in protecting the photosynthetic apparatus in cotton leaves, the native ROS scavenging system appears to be sufficient to cope with the level of oxidative stress experienced by the chloroplasts at nearly all times during a normal growing season in western Texas, USA. Thus, for most of the growing season, overexpression of GR in chloroplasts of cotton does not enhance the photosynthetic performance of transgenic plants under field conditions. Only during abruptly imposed chilling do non-photochemical energy dissipation and the native capacity for ROS scavenging fail to substantially protect the photosystems. However, these conditions are rarely, if ever, experienced by cotton in its natural habitat or where it is commercially grown.

Acknowledgements

We thank the Department of Plant and Soil Science, Texas Tech University for providing the field plots and Efrem Bechere for his assistance in maintaining the plots. This study was supported by grant number 99-35100-7630 from the U.S. Department of Agriculture, National Research Initiative, Competitive Grants Program.

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