

Minireview

The role of antioxidant enzymes in photoprotection

Barry A. Logan^{1,*}, Dmytro Korniyev^{2,3}, Justin Hardison¹ & A. Scott Holaday²

¹Department of Biology, Bowdoin College, 6500 College Station, Brunswick, ME 04011, USA; ²Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA; ³Institute of Plant Physiology and Genetics, Vasylykivska St. 31/17, 03022 Kyiv, Ukraine; *Author for correspondence (e-mail: blogan@bowdoin.edu; fax: +1-207-725-3405)

Received 6 October 2005; accepted in revised form 11 January 2006

Key words: antioxidants, photoinhibition, stress tolerance, transgenic plants, Water–Water cycle

Abstract

The enzymatic component of the antioxidant system is discussed as one of the defensive mechanisms providing protection against excessive light absorption in plants. We present an analysis of attempts to improve stress tolerance by means of the creation of transgenic plants with elevated antioxidant enzyme activities and conclude that the effect of such transgenic manipulation strongly depends on the manner in which the stress is imposed. The following factors may diminish the differences in photosynthetic performance between transgenic plants and wild type under field conditions: effective functioning of the thermal dissipation mechanisms providing a primary line of defense against excessive light, long-term adjustments of the antioxidant system and other photoprotective mechanisms, the relatively low level of control over electron transport exerted by the Water–Water cycle, especially under warm conditions, and a decrease in the content of the transgenic product during leaf aging.

Abbreviations: APX – ascorbate peroxidase; DCMU – 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; GR – glutathione reductase; GSH – reduced glutathione; MV – methyl viologen; ROS – reactive oxygen species; SOD – superoxide dismutase

Introduction

Photosynthetic electron transport can reduce molecular oxygen (O₂) in the so-called 'Mehler reaction' (Mehler 1951; Mehler and Brown 1952), yielding superoxide, a reactive oxygen species (ROS) capable of damaging or inactivating essential macromolecules (Halliwell and Gutteridge 1999). As atmospheric O₂ concentrations increased over geologic timescales, O₂ photoreduction may have arisen as an unavoidable consequence of the mechanism of electron transport. Although superoxide itself may be damaging under some circumstances, it may cause greater harm by leading to the formation of the highly reactive hydroxyl radical from H₂O₂, the product

of superoxide dismutation in the chloroplast (Bowler et al. 1992). In addition, singlet O₂, another unstable ROS, can be formed via resonance energy transfer from triplet excited-state chlorophyll to ground-state O₂ (Asada 1996; Niyogi 1999). ROS-mediated inactivation is thought to be one of the primary causes of the slowly reversible loss of photosynthetic activity commonly termed 'photoinhibition,' which is sometimes observed in leaves exposed to light in combination with environmental stresses (Allen 1995; Niyogi 1999). In fact, the physiological effects of many environmental stresses, such as chilling temperatures, can be appreciated in terms of their ability to disrupt the balance between light energy absorption and light energy utilization via

the Calvin–Benson cycle in a manner that favors O_2 reduction and singlet O_2 formation.

Plants possess multiple means of minimizing the deleterious effects of ROS. These include an integrated array of antioxidant enzymes and metabolites that detoxify those ROS that form, as well as a mechanism that safely dissipates excess absorbed light before it can lead to singlet O_2 formation. This latter mechanism is referred to as *thermal energy dissipation* or *feedback de-excitation* and involves energy transfer to a chlorophyll–zeaxanthin dimer, which loses excitation energy as heat after charge separation followed by recombination (Holt et al. 2005). Collectively, thermal energy dissipation and antioxidants are thought to be ‘photoprotective’ because they protect photosynthetic cells from the dangers of excess light absorption. Excellent reviews of the molecular biology and physiological ecology of thermal energy dissipation have been published (Demmig-Adams and Adams 1992, 1996; Gilmore 1997; Niyogi 1999) and we shall concentrate this review on the photoprotective role of antioxidant enzymes. However, it should be noted that thermal energy dissipation and antioxidation are closely connected. Xanthophylls can directly scavenge certain ROS (reviewed by Havaux and Niyogi 1999). Violaxanthin deepoxidase, the enzyme that transforms violaxanthin into zeaxanthin (the xanthophyll that can facilitate thermal energy dissipation), requires ascorbic acid, a linchpin in the chloroplast antioxidant system (Bratt et al. 1995). In addition, electron flow to O_2 photoreduction contributes to the low thylakoid lumen pH required for thermal energy dissipation (Li et al. 2002; Makino et al. 2002).

Unlike thermal energy dissipation, which proactively prevents ROS formation, antioxidants react with those ROS that have formed. In other words, they represent the second line of defense against photoinhibition. One may suggest that if thermal dissipation is capable of keeping excessive energy flow under control, the antioxidant system may not be especially important for photoprotection. However, a considerable body of literature suggests that this is not so. Plants adjust levels of antioxidants when acclimating to prevailing environmental conditions in concert with changes in the xanthophyll cycle. Adjustments in both processes are best understood as responsive to levels of excess light absorption. In addition, O_2 photo-

reduction and subsequent ROS detoxification have been shown to influence chloroplast metabolism, as well as nuclear and chloroplastic gene expression, in a manner that suggests that they are tightly interwoven into the regulatory regimes that preserve the balance between light use to power carbon assimilation and protection against the potentially harmful effects of absorbing too much light energy. In this review, we describe the current state of understanding of the antioxidant enzyme pathways for chloroplastic ROS detoxification, their role in photoprotection and their influence on the rate of photosynthetic electron transport, and assess attempts to enhance the stress tolerance of plants via transgenic overproduction of antioxidant enzymes.

The Water–Water cycle

Chloroplastic superoxide is detoxified via a pathway referred to as the ‘Water–Water cycle’ (Asada 1999). The name derives from the fact that water acts as both the source of electrons (at the oxygen-evolving complex associated with PS II) and the final product of the pathway. Thus, the Water–Water cycle produces nothing, but consumes considerable photogenerated reductant while ridding the chloroplast of potentially damaging ROS. Two enzymes functioning in series convert superoxide to water. Superoxide dismutase (SOD) first catalyzes the dismutation of superoxide to molecular oxygen and H_2O_2 (McCord and Fridovich 1969; Bowler et al. 1992; Alscher et al. 2002). Ascorbate peroxidase, in turn, uses ascorbate to reduce H_2O_2 to water (Jablonski and Anderson 1982). It is critical that H_2O_2 be removed, because it can potentially inactivate Calvin–Benson cycle bisphosphatases via thiol oxidation (Charles and Halliwell 1981) and because it can readily interact with reduced transition metal cations via the Fenton reaction to form the highly reactive hydroxyl radical (Halliwell and Gutteridge 1999). PS I and its vicinity would seem acutely vulnerable to hydroxyl radical generation and subsequent attack, since PS I binds several Fe–S clusters and is also the principal site for generation of superoxide, which can reduce iron and other transition metal cations (Terashima et al. 1998). Immunogold labeling experiments suggest that, at least in some preparations, certain isoforms of SOD and APX

are found in close association with PS I. This led Asada (1996) to propose the existence of a thylakoid super-enzyme complex, which could greatly minimize the potentially harmful effects of ROS generation by catalyzing superoxide detoxification in an assembly line fashion, limiting ROS escape and the possibility that they could damage cellular constituents.

The one-electron oxidation product of ascorbate, monodehydroascorbate, formed by APX activity, can be recycled back to the reduced form via at least three mechanisms, two of which ultimately derive their reducing power from photosynthetic electron transport. Monodehydroascorbate can accept electrons directly from the electron transport chain, a reaction that is thought to occur at either the cytochrome b_6/f complex or at PS I (Miyake and Asada 1992; Grace et al. 1995). It can also be reduced via the activity of monodehydroascorbate reductase, an enzyme that utilizes NADH, or to a lesser extent NADPH, as a reductant (Hossain et al. 1984). Lastly, monodehydroascorbate radicals can participate in a non-enzymatic dismutation yielding reduced ascorbate and the two-electron oxidation product of ascorbate, dehydroascorbate. Dehydroascorbate can, in turn, be reduced to ascorbate via the activity of dehydroascorbate reductase, which utilizes glutathione (GSH) as a reductant (Hossain and Asada 1984). Glutathione reductase completes this cadre of redox reactions by recycling GSH using NADPH as a reductant (Smith et al. 1989).

In addition to the largely enzyme-driven Water–Water cycle described above, ascorbate and GSH may also participate in superoxide scavenging via a reaction sequence that is non-enzymatic, with the exception of GSH recycling. Ascorbate can detoxify superoxide non-enzymatically (Halliwell and Gutteridge 1999) and is found at high concentrations in the chloroplast (>10 mM) (Foyer 1993). Furthermore, GSH can reduce dehydroascorbate to ascorbate under the alkaline conditions that are likely to prevail in the stroma during illumination (Foyer and Halliwell 1976; Winkler et al. 1994; Foyer and Noctor 2000, Noctor et al. 2000). The relative contributions of enzymatic versus non-enzymatic superoxide detoxification pathways are not known (see Asada 1999; Polle 2001) and may depend upon environmental conditions and the taxon.

The response of the Water–Water cycle to environmental stress

Plants adjust constituents of the Water–Water cycle in response to prevailing environmental conditions. Change in antioxidant activities/contents can be observed hours or days after a change in growth conditions (Logan et al. 1998b, 2003). The adjustment of levels of leaf antioxidants to a range of abiotic stresses, such as growth light intensity or chilling temperatures, can be best understood in terms of the effect that these stresses have on the absorption of light that exceeds the needs of photosynthetic electron transport, so-called excess light.

In a broad range of plant species, a strong correlation has been reported between the intensity of the light environment and foliar activities of a number of antioxidant enzymes and contents of ascorbate and GSH (Gillham and Dodge 1987; Mishra et al. 1993, 1995; Grace and Logan 1996; Logan et al. 1996, 1998a). However, it does not appear that the light intensity, *per se*, is as important as is the extent of excess light absorption. In a direct comparison of light acclimation in *Vinca major* and pumpkin, leaves of full-sun acclimated *V. major* possessed 31% greater SOD activities, 49% greater APX activities, and 61% greater reduced ascorbate contents than full-sun acclimated pumpkin leaves (when expressed per unit leaf fresh weight) (Logan et al. 1998a). Since *V. major* maintained 50% lower rates of photochemistry under ambient, full-sunlight exposed conditions in comparison to pumpkin (estimated from chlorophyll fluorescence emission) and absorbed greater levels of excess light as a consequence, these findings further support the hypothesis that antioxidant systems acclimate in response to the level of excess light, not simply to the intensity of the light environment. Conversely, leaves of hydroponic spinach grown in a growth chamber at $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ possessed levels of SOD, APX, GR, and ascorbate that were statistically indistinguishable from levels found in leaves of spinach grown under similar conditions but only $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (B. Logan, T. Rosenstiel, B. Demmig-Adams, W. Adams, unpublished data). However, spinach grown at the higher light intensity maintained 2-fold greater photosynthetic capacities, which presumably reduced the portion of the absorbed energy in

excess of that needed for CO₂ assimilation, thus explaining the absence of an acclimatory response of the Water–Water cycle components to a doubling in the growth light intensity.

Exposure to chilling temperatures has the potential to greatly increase the absorption of excess light. Chilling has a greater inhibitory effect on the enzyme-catalyzed reactions of the Calvin–Benson cycle than on the biophysical and redox reactions that make up light harvesting and electron transport. Thus, chilling can perturb the balance between the production and consumption of photogenerated reductant in a manner that favors O₂ photoreduction. Many long-lived evergreens respond to the onset of wintertime cold temperatures by decreasing chlorophyll content (Adams and Demmig-Adams 1994; Verhoeven et al. 1996; Logan et al. 1998c; Burkle and Logan 2003) and reorganizing their light harvesting antennae into an energy dissipating state (Gilmore and Ball 2000; Matsubara et al. 2002; Adams et al. 2002, 2004). However, profound up-regulation of levels of antioxidants is another hallmark of acclimation to cold temperatures (Schöner and Krause 1990; Anderson et al. 1992; Mishra et al. 1993; Logan et al. 1998c, 2003). Cold-induced increases in the levels of antioxidant enzymes compensate for the effect of lower temperatures on their activities and also presumably help cope with an enhanced rate of ROS formation (Lohrmann et al. 2004). In addition to up-regulation in overall activity, some species have been shown to respond to chilling with preferential expression of antioxidant enzyme isoforms with lower temperature optima and other biochemical features that would favor activity at colder temperatures (Guy and Carter 1984).

The size of the O₂ photoreduction electron ‘sink’

The extent to which the Water–Water Cycle can serve a photoprotective role depends upon the magnitude of the electron flux to O₂ photoreduction. However, measuring this flux has proven methodologically difficult. Some of the first attempts to do so used radiolabeled ¹⁸O₂ to distinguish O₂ derived from water splitting at the oxygen-evolving complex from O₂ consumed via photoreduction. Early measurements employing this method reported that electron flow to oxygen

accounted for 10–30% of the overall electron flow (Canvin et al. 1980; Furbank et al. 1982).

Electron transport that ultimately leads to O₂ photoreduction is undetectable when measuring net O₂ fluxes, but measurable via chlorophyll fluorescence-based estimates of electron transport. Therefore, one can compare electron transport rates derived from these two measures to estimate the rate of O₂ photoreduction. In tropical trees measured at light saturation using this approach, 30% of the overall electron transport was dedicated to O₂ photoreduction (Lovelock and Winter 1996).

The two approaches above used to estimate O₂ photoreduction demand that measurements be made under non-physiological conditions that suppress photorespiration. More recently, Ruuska et al. (2000a) have applied transgenic technology to this problem by suppressing Rubisco activity in tobacco via the expression of an antisense construct to the Rubisco small subunit (reviewed in Badger et al. 2000). Antisense tobacco exhibit differing capacities for CO₂ assimilation without parallel alterations in the capacities for photosynthetic electron transport (Ruuska et al. 2000a). Across a range of O₂ and CO₂ concentrations, calculated rates of electron transport closely matched the requirements for reductant, suggesting that O₂ photoreduction is, at most, a minor flux, even under circumstances where the capacity to generate reductant greatly exceeds the capacity to consume it (Ruuska et al. 2000a).

An increase in the ratio of the quantum yields for electron transport (estimated from fluorescence) and CO₂ fixation has been interpreted as a manifestation of greater fluxes to alternative electron sinks, in particular to the Water–Water cycle (Fryer et al. 1998). Adopting this approach, Miyake and Yokota (2000) consistently measured electron fluxes to alternative sinks in leaves of watermelon, ranging up to 20% of the overall electron fluxes. Hirotsu et al. (2004) determined that the acclimation of rice to low temperature led to an increase in the portion of photon energy utilized by alternative sinks from 10 to 15%.

Yet another method used to estimate O₂ photoreduction via the Mehler reaction involves analyses of fluxes in the presence of glyceraldehyde, which prevents CO₂ and O₂ assimilation in the Calvin–Benson cycle by inhibiting the formation of ribulose biphosphate via phosphoribulo-

kinase (Wu et al. 1991). The data obtained by Wu et al. (1991) suggest that the Mehler reaction is not a substantial sink for electron flow.

None of the methods described above is free of complicating factors, including pleiotropic effects, the use of non-physiological gas concentrations, and the possibility that chlorophyll fluorescence analysis may over-emphasize the contribution of the uppermost layer of chlorophyllous cells. This, and the general lack of agreement among methods, leaves the size of the electron sink represented by O₂ photoreduction unresolved. Multiple methods do agree, however, that significant electron flow to O₂ occurs during photosynthetic induction (Neubauer and Yamamoto 1992; Ruuska et al. 2000b). Electron flow to O₂ during induction may contribute to the low luminal pH required to induce thermal energy dissipation (Gilmore 1997; Makino et al. 2002). Even if O₂ photoreduction ultimately proves to be a relatively minor sink at steady state, the balance between photosynthetic ROS production and scavenging could still have profound effects on gene expression, as ROS and the redox status of certain chloroplastic constituents have recently been shown to regulate the transcription and post-transcriptional processing of nuclear and chloroplastic genes (reviewed in Barnes and Mayfield 2003; Link 2003; Baier and Deitz 2005; Fey et al. 2005; Pfannschmidt and Liere 2005). The dramatic responses of antioxidants to the imposition of environmental stresses indirectly, but strongly, suggests that O₂ photoreduction is sensitive to environmental conditions and that adequate ROS scavenging capacity is essential for the proper functioning of the chloroplast.

Transgenic manipulations of photoprotection

Numerous studies imply that generation of ROS under illumination is one of the primary factors contributing to photoinactivation of the photosynthetic apparatus (Prasil et al. 1992; Andersson and Barber 1996; Melis 1999). Pigment-protein complexes of PS I and PS II, which are considered to be the main targets for photoinhibition, can be a source of ROS (Bradley et al. 1991; Foyer and Harbinson 1994; Osmond and Grace 1995). Moreover, ROS produced by PS I can augment photo-induced inactivation to PS II (Krieger et al. 2000; Tjus et al. 2001). Consequently, photopro-

tection of chloroplast constituents should depend on the efficiency of ROS removal by antioxidants. Indeed, the addition of antioxidants *in vitro* decreases the extent of photoinactivation (Barényi and Krause 1985; Richter et al. 1990; Tschiersch and Ohmann 1993; Tjus et al. 2001), and the lack of antioxidant enzymes is associated with a higher sensitivity to light treatment (Danna et al. 2003). These findings taken together with the observation that the response to many environmental stresses, particularly chilling, includes up-regulation of antioxidant systems have led plant geneticists to attempt to improve the stress tolerance of some crop species by transforming plants with genes for chloroplast-targeted antioxidant enzymes (for reviews see Foyer et al. 1994; Allen 1995). Such attempts have met with mixed results, as indicated by the findings described in Table 1. Where enhanced environmental stress tolerance has been observed, its mechanism has rarely been probed in depth. In addition, artificially induced oxidative stress, for instance, the treatment with MV, is frequently imposed to study the effect of a transgene (see Table 1). Those experiments, however, do not provide information about the performance of transgenic plants under field conditions.

Increased tolerance to cold-induced photoinhibition was shown for transgenic poplar possessing between 100- and 1000-fold overexpression of chloroplast-targeted GR (Foyer et al. 1995). Enhanced cold tolerance was explained by more rapid recycling of ascorbate and GSH pools, the influence of higher levels of GSH on the stabilization of the enzymes requiring thiol groups for their activity, and by the possible influence of altered GSH concentration on protein synthesis and gene expression. Tobacco plants (cv. Samsun) possessing increased GR activities (5- to 8-fold overexpression) have a lower rate constant of PS II photoinhibition at 20 °C (Tyystjärvi et al. 1999). However, this effect was not observed in another cultivar (cv. Bel W3), nor in transgenic poplar plants with a similar level of FeSOD overexpression. Transgenic tomato plants with a 60-fold increase in chloroplastic GR activity also did not show an improved resistance to photoinhibition (Bruggemann et al. 1999).

Research conducted on cotton plants indicates that the protective effect of transgenic overexpression of antioxidant enzymes may, in part, result from their ability to maintain the photo-

Table 1. A review of transgenic plants with elevated activities of antioxidant enzymes

Enzyme	Cellular compartment	Source species	Target species	Stress	Performance	Reference
Cu/Zn SOD	Chloroplast	Petunia	Tomato	High light Low temperature Low CO ₂	No effect No effect No effect	Tepperman and Dunsmuir (1990)
Cu/Zn SOD	Chloroplast	Petunia	<i>Nicotiana tabacum</i>	Ozone	No effect	Pitcher et al. (1991)
Fe-SOD	Chloroplast	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	MV ^a H ₂ O ₂	Enhanced No effect	Van Camp et al. (1996)
Fe-SOD	Chloroplast	<i>Arabidopsis thaliana</i>	<i>Populus tremula</i> × <i>P. Alba</i>	Singlet O ₂ Chilling High light	No effect No effect No effect	Tyystjärvi et al. (1999) Arisi et al. (1998)
Mn-SOD	Mitochondrion	<i>Nicotiana plumbaginifolia</i>	<i>Nicotiana tabacum</i>	MV Chilling	No effect No effect	Slooten et al. (1995)
Cu/Zn-SOD	Chloroplast	<i>Pisum sativum</i>	<i>Nicotiana tabacum</i>	Chilling	Enhanced	Gupta et al. (1993)
Fe-SOD	Chloroplast	<i>Arabidopsis thaliana</i>	<i>Medicago sativa</i>	Winter survival Freezing	Enhanced No effect	McKersie et al. (2000)
Mn-SOD	Chloroplast		<i>Gossypium hirsutum</i>	MV Chilling + high light Chilling	No effect No effect Enhanced	Payton et al. (1997) Payton et al. (2001), Kornyeyev et al. (2001) Kwon et al. (2003)
DHAR	Chloroplast	Human	<i>Nicotiana tabacum</i>	MV + H ₂ O ₂ Chilling NaCl	Enhanced Enhanced Enhanced	Wang et al. (1999)
APX3	Not determined	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	Aminotriazole ^b treatment	Enhanced	Yan et al. (2003)
APX	Cytosol	<i>Pisum sativum</i>	<i>Nicotiana tabacum</i>	Drought	Enhanced	Torsethaugen et al. (1997)
APX	Chloroplast	<i>Pisum sativum</i>	<i>Gossypium hirsutum</i>	Ozone	No effect	Kornyeyev et al. (2001, 2003a)
tAPX	Chloroplast	Spinach	<i>Nicotiana tabacum</i>	Chilling Chilling	Enhanced Enhanced	Yabuta et al. (2002)
tAPX	Chloroplast	<i>Arabidopsis thaliana</i>	<i>Arabidopsis thaliana</i>	MV MV Photoinhibition	Enhanced Enhanced No effect	Murgia et al. (2004)
APX	Cytosol	<i>Pisum sativum</i>	<i>Lycopersicon esculentum</i>	Low temperature Fe or Cu overload Chilling NaCl	No effect No effect Enhanced Enhanced	Wang et al. (2005)

GR	Chloroplast	Bacteria	<i>Populus tremula</i> × <i>P. Alba</i>	Chilling + high light Ozone	Enhanced	Foyer et al. (1995)
GR	Chloroplast	Bacteria	<i>Nicotiana tabacum</i> cv. Samsun	High light	Enhanced	Strohm et al. (1999)
GR	Cytosol	<i>Pisum sativum</i>	cv. Bel W3	High light	No effect	Tyystjärvi et al. (1999)
GR	Chloroplast	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i> <i>Gossypium hirsutum</i>	No data Chilling	No data Enhanced	Stevens et al. (2000) Payton et al. (2001), Kornyejev et al. (2003b)
GR	Chloroplasts	<i>Escherichia coli</i>	<i>Lycopersicon esculentum</i>	Gradual chilling	No effect	Logan et al. (2003)
GR	Cytosol	<i>Brassica campestris</i>	Rice	High light	No effect	Bruggemann et al. (1999)
GR and SOD	Cytosol		<i>Nicotiana tabacum</i>	MV	Enhanced	Kouril et al. (2003)
SOD and APX	Chloroplast		<i>Nicotiana tabacum</i>	MV	Enhanced	Aono et al. (1995)
				MV	Enhanced	Kwon et al. (2002)

^a MV = methyl viologen (Paraquat).

^b Aminotriazole inhibits catalase and induces oxidative stress.

synthetic apparatus in a more oxidized state (Kornyejev et al. 2001, 2003a, b). PS II is more likely to experience photoinactivation when its final electron acceptor, Q_A , is reduced (Melis 1999). Similarly, light energy has a greater probability of leading to photoinhibition if it is absorbed by the antennae of 'closed' PS II units (i.e. those already processing an electron) (Kornyejev et al. 2004). Transgenic cotton with elevated activities of SOD, APX or GR (approximately 3-, 5-, and 35-fold overexpression, respectively) all maintained lower levels of Q_A reduction than the wild type when exposed to illumination under chilling conditions (Kornyejev et al. 2001). No evidence for a direct effect of the transgenic modifications of ROS metabolism on PS II protection was found (Kornyejev et al. 2001), although the enhanced PS I protection observed in GR and APX overproducing plants could be the direct result of ROS scavenging.

Consistent with the report that an effect of transformation is evident at only certain levels of a stress (Rubio et al. 1997), differences between transgenic and wild-type cotton were observed only at low temperatures (10–15 °C) (Kornyejev et al. 2003b). At warmer temperatures (i.e. 20–30 °C) antioxidant overproduction brings about no enhancement in resistance to photoinhibition. It may be that as the leaf temperatures rise, the contribution of CO₂ fixation to the maintenance of electron flow overwhelms the effect of the transgenic manipulation. It may also be that native enzyme activity is adequate at warm, but not at chilling temperatures. These observations suggest the existence of a 'thermal window' in which the effect of increased antioxidant activity may have a meaningful effect on PS II reduction state and thereby influence the level of PS II photoinhibition.

The effect of a single-enzyme transgenic modification may depend on the overall state of the antioxidant system. For example, a decrease in the activity of the key antioxidant enzymes caused by shading resulted in the disappearance of the beneficial effect of MnSOD overproduction on the methyl viologen resistance of transgenic tobacco plants (Slooten et al. 1995). The overproduction of SOD itself can cause an increase in the activity of other antioxidant enzymes (Sen Gupta et al. 1993a, b; Kingston-Smith and Foyer 2000); however, this effect is not always observed (Payton et al. 2001).

Most studies seeking to examine the stress tolerance of transgenic plants employ abruptly imposed, severe stresses. Typically, leaf tissues are detached from plants grown under near-optimal conditions and placed under stresses that exceed those that the species under study might encounter in the field. Experiments of this sort have yielded important insights into the mechanisms of chilling tolerance and the regulation of oxidative metabolism. However the enhanced stress tolerance that is occasionally observed under such conditions may not be predictive of enhanced stress tolerance under conditions typical of the field. For example, when leaf discs of warm-grown transgenic cotton that possessed ~4-fold higher chloroplastic APX activities were abruptly exposed to 10 °C at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, they sustained lower levels of PS II and PS I photoinhibition in comparison to wild type (Figure 1a) (Kornyejev et al. 2001, 2003a). However, chilling tolerance was not enhanced when this same cotton genotype was grown in a growth chamber in which temperatures were lowered from 28 to 14 °C over 9 days and held for a subsequent 9-day period at 14 °C (Figure 1b) (J Hardison, B Logan, AS Holaday, unpublished data). The absence of an effect of APX overproduction under longer-term, gradually imposed chilling may be explained, in part, by the fact that wild-type cotton acclimated to the latter chilling regime by up-regulating native APX activity and abolishing significant genotypic differences in APX activity. Under similar experimental conditions, transgenic GR overproduction

also failed to improve cotton chilling tolerance (Logan et al. 2003).

In field trials conducted in west Texas, where cotton is a primary crop, transgenic overproduction of GR (Kornyejev et al. 2005) or APX (D Kornyejev, B Logan, R Allen, AS Holaday, unpublished data) failed to meaningfully improve photosynthetic performance. Over the course of the growing season from early June until mid-October, in Lubbock, Texas, the environmental conditions that enhance photoinactivation, namely, high light intensities in combination with chilling temperatures, were rarely observed because high light intensities quickly led to leaf warming. In addition, the wild-type and transgenic cotton plants had high capacities for non-photochemical, thermal dissipation of excitation energy that were rapidly engaged as light intensities climbed in the morning, most likely reducing the need for elevated levels of antioxidant enzymes (Kornyejev et al. 2005). Thermal energy dissipation, which safely eliminates a large fraction of the photon energy absorbed by PS II antennae, may be the dominant photoprotective mechanism in cotton. The elevated activity of single antioxidant enzymes does not affect the levels of non-photochemical quenching of chlorophyll fluorescence nor the extent of de-epoxidation of xanthophyll cycle pigments (Kornyejev et al. 2003a, b). In the case of APX overproduction, these data indicate that there is no noticeable competition between APX and violaxanthin de-epoxidase for reduced ascorbate, at least under chilling, moderate light

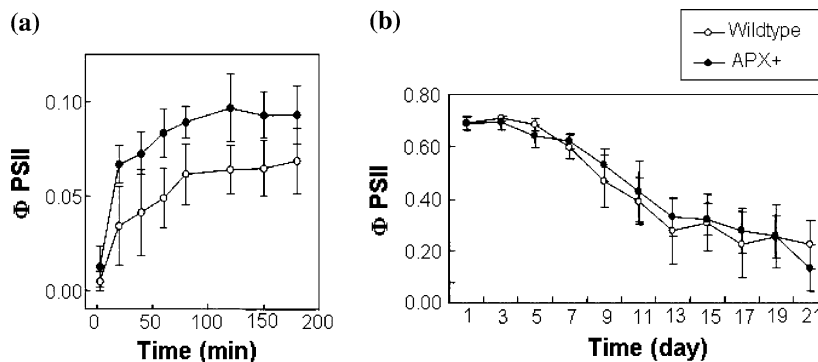


Figure 1. Photosystem II quantum yields of transgenic cotton that overexpress chloroplastic ascorbate peroxidase (closed circles) and wild type (open circles) during exposure of leaf discs to short-termed, abruptly imposed exposure to 10 °C at 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (a) or a gradual reduction of temperature on whole plants from 28 to 14 °C over 9 days followed by continuous exposure to 14 °C (all at $\sim 300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (b). Panel (a) redrawn from Kornyejev et al. (2003a).

conditions. The extent of transgenic enzyme overproduction also fell with leaf age, diminishing the difference in enzyme activity between transgenic and wild-type plants at the time of boll development (Kornyejev et al. 2005).

Clearly, the nature and timing of the stress profoundly influences the response of transgenic plants with increased ROS scavenging capacity. This should be taken into account when assessing the utility of manipulating these systems as a strategy for developing more stress-tolerant crop varieties for agricultural use and underscores the need to design experiments that examine the performance of transgenic genotypes under realistic conditions of stress. We do not recommend basing the value of a transformation on its ability to enhance photosynthetic protection in the presence of MV or during a rapidly imposed environmental stress. In addition, the effect of transgenic up-regulation of antioxidant enzymes on plant defense deserves in-depth examination, as it may influence plant performance in the field.

The influence of reactive oxygen metabolism on electron transport and chloroplast redox state

As stated above, transgenic cotton plants overproducing chloroplast-targeted antioxidant enzymes maintained the leaf pool of Q_A in a more oxidized state (Kornyejev et al. 2001). The reduction state of Q_A is determined by the balance between light energy inputs into PS II and downstream electron flow. Any process that consumes reducing equivalents and thereby increases downstream electron flow will serve to lower the reduction state of PS II and lower its vulnerability to photoinhibition. Under conditions of stress, such as chilling, where Calvin–Benson cycle consumption of reducing equivalents is compromised, electron flow through the Water–Water cycle may serve in this capacity. This effect has been demonstrated in a series of experiments examining the performance of transgenic cotton with elevated activities of GR and APX in their chloroplasts (Kornyejev et al. 2003a, b). In comparison to the wild-type plants, the transgenic plants maintain lower Q_A reduction states (Kornyejev et al. 2001), higher rates of electron transport through PS II, and sustain less PS II photoinhibition (Kornyejev et al. 2003a, b) during exposure to 10 °C and 500 μ mol

photons $m^{-2} s^{-1}$. The protective effect of APX or GR overproduction on PS II function can be abolished by inhibiting electron transfer from PS II with 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) (Kornyejev et al. 2001), indicating that the effect of elevated antioxidant enzyme activity is exerted downstream of PS II. Thus, the PS II photoprotection conferred by antioxidant overexpression is not due to the direct effects of enhanced ROS scavenging, instead it is due to the effect this enhancement has upon the redox state of Q_A . Kornyejev et al. (2003a, b) propose that the enhanced photochemistry maintaining Q_A in a more oxidized state than in wild-type leaves is largely due to an enhanced demand for reducing power in the chloroplasts of the transgenic plants during chilling. This hypothesis is supported by the following information: (a) Although transgenic plants maintain greater PS I capacity (Kornyejev et al. 2003a, b), as estimated by measuring the relative amount of oxidizable P700 present, the genotypic differences in PS I capacity are only observed after 2 h of chilling, while the greatest genotypic differences in electron transport are observed during photosynthetic induction, the very period when O_2 photoreduction is likely to be substantial (Neubauer and Yamamoto 1992; Ruuska et al. 2000b); (b) Enhanced photochemistry is not due to an increase in Calvin–Benson cycle activity, since rates of CO_2 assimilation at 10 °C do not differ between transgenic and wild-type plants despite differences in electron transport; (c) Electron transport through PS II is greater for transgenic plants than for wild-type plants in the presence of glyceraldehyde that inhibits the Calvin–Benson cycle (Kornyejev et al. 2003b); (d) The content of GSH in leaves overproducing GR is higher than that of wild-type leaves during chilling (Kornyejev et al. 2003b); and, (e) in leaves overproducing APX, the illumination-dependent rise in H_2O_2 during chilling is dramatically reduced (Kornyejev et al. 2003a). Thus, the *in vivo* activity of these enzymes is higher in transgenic than in wild-type leaves during chilling. Taken together, these data strongly suggest that the Water–Water cycle can serve as an alternative electron sink, at least under chilling conditions. Antioxidant enzyme overproduction is not likely to affect the rate of O_2 photoreduction (i.e. the Mehler reaction). Rather, it increases the rate of ROS scavenging and, in doing so, increases

the demand for reducing equivalents to recycle ascorbate and glutathione.

Concluding remarks

Although the rate of O₂ photoreduction and subsequent ROS formation may not be substantial under optimum, steady-state conditions, data from a variety of experimental analyses indicate that the detoxification of ROS by antioxidants during exposure to stressful environments is essential for the protection of the photosynthetic apparatus. The capacity of the native antioxidant systems is highly responsive to the growth environment; nonetheless, native antioxidant systems can be overwhelmed by stresses such as chilling temperatures when they are abruptly imposed. However, transgenic overproduction of chloroplastic antioxidants has generally proven to be a poor strategy for protecting plants against stress in the field or experimental settings that simulate field conditions. Other strategies should be investigated if agriculturally meaningful gains in photosynthetic stress tolerance are to be realized. Identification of those sites which constrain electron transport and CO₂ assimilation during and, perhaps more importantly, following the stress will be needed to develop these strategies.

References

- Adams WW and Demmig-Adams B (1994) Carotenoid composition and down regulation of Photosystem II in three conifer species during the winter. *Physiol Plant* 92: 451–458
- Adams WW, Demmig-Adams B, Rosenstiel TN, Brightwell AK and Ebbert V (2002) Photosynthesis and photoprotection in overwintering plants. *Plant Biol* 4: 545–557
- Adams WW, Zarter CR, Ebbert V and Demmig-Adams B (2004) Photoprotective strategies of overwintering evergreens. *Bioscience* 54: 41–49
- Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol* 107: 1049–1054
- Alscher RG and Hess JL (eds) (1993) *Antioxidants in Higher Plants*, CRC Press, Boca Raton, 174 pp
- Alscher RG, Erturk N and Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53: 1331–1341
- Anderson JV, Chevone BI and Hess JL (1992) Seasonal variation in the antioxidant system of eastern white pine needles. *Plant Physiol* 98: 501–508
- Andersson B and Barber J (1996) Mechanisms of photodamage and protein degradation during photoinhibition of Photosystem II. In: Baker NR (ed) *Photosynthesis and the Environment*, pp 101–121. Kluwer Academic Publishers, Dordrecht, The Netherlands
- Aono M, Saji H, Sakamoto A, Tanaka K, Kondo N and Tanaka K (1995) Paraquat tolerance of transgenic *Nicotiana tabacum* with enhanced activities of glutathione reductase and superoxide dismutase. *Plant Cell Physiol* 36: 1687–1691
- Arisi AC, Cornic G, Jouanin L and Foyer CH (1998) Overexpression of iron superoxide dismutase in transformed poplar modifies the regulation of photosynthesis at low CO₂ partial pressures or following exposure to the prooxidant herbicide methyl viologen. *Plant Physiol* 117: 565–574
- Asada K (1996) Radical production and scavenging in chloroplasts. In: Baker NR (ed) *Photosynthesis and the Environment*, pp 123–150. Kluwer Academic Publishers, Dordrecht, The Netherlands
- Asada K (1999) The Water–Water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu Rev Plant Physiol Plant Mol Biol* 50: 601–639
- Badger MR (1985) Photosynthetic oxygen exchange. *Annu Rev Plant Physiol* 36: 27–53
- Badger MR, von Caemmerer S, Ruuska S and Nakano H (2000) Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. *Phil Trans Royal Soc Lond, Ser B* 355: 1433–1446
- Barényi B and Krause GH (1985) Inhibition of photosynthetic reactions by light. A study with isolated spinach chloroplasts. *Planta* 163: 218–226
- Baier M and Deitz K-J (2005) Chloroplasts as a source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. *J Exp Bot* 56: 1449–1462
- Barnes D and Mayfield SP (2003) Redox control of posttranscriptional processes in the chloroplast. *Antioxidants Redox Signal* 5: 89–94
- Bowler C, Van Montagu M and Inze D (1992) Superoxide dismutase and stress tolerance. *Ann Rev Plant Physiol Plant Mol Biol* 43: 83–116
- Bradley RL, Long KM and Frasch WD (1991) The involvement of Photosystem II generated H₂O₂ in photoinhibition. *FEBS Lett* 286: 209–213
- Bratt CE, Arvidsson P-O, Carlsson M and Åkerlund HE (1995) Regulation of violaxanthin de-epoxidase activity by pH and ascorbate concentration. *Photosynth Res* 45: 169–175
- Bruggemann W, Beyel V, Brodka M, Poth H, Weil M and Stockhaus J (1999) Antioxidants and antioxidative enzymes in wild-type and transgenic *Lycopersicon* genotypes of different chilling tolerance. *Plant Sci* 140: 145–154
- Burkle LA and Logan BA (2003) Seasonal acclimation of photosynthesis in eastern hemlock and partridgeberry growing in different light environments. *Northeastern Naturalist* 10: 1–16
- Canvin DT, Berry JA, Badger MR, Fock H and Osmond CB (1980) Oxygen exchange in leaves in the light. *Plant Physiol* 66: 302–307
- Charles SA and Halliwell B (1981) Light activation of fructose bisphosphatase in isolated spinach chloroplasts and deactivation by hydrogen peroxide. *Planta* 151: 242–246
- Danna CH, Bartoli CG, Sacco F, Ingala LR, Santa-Maria GE, Guiamet JJ and Ugalde RA (2003) Thylakoid-bound ascorbate peroxidase mutant exhibits impaired electron transport and photosynthetic activity. *Plant Physiol* 132: 2116–2125

- Demmig-Adams B and Adams WW (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43: 599–626
- Demmig-Adams B and Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci* 1: 21–26
- Fey V, Wagner R, Bräutigam K and Pfannschmidt (2005) Photosynthetic redox control of nuclear gene expression. *J Exp Bot* 56: 1491–1498
- Foote CS (1976) Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: Pryor WA (ed) *Free Radicals in Biology*, pp 8–124. Vol. 2 Academic Press, New York, United States
- Foyer CH (1993) Ascorbic acid. In: Alscher RG and Hess JL (eds) *Antioxidants in Higher Plants*, pp 31–58. CRC Press, Boca Raton, United States
- Foyer CH and Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133: 21–25
- Foyer CH and Harbinson J (1994) Oxygen metabolism and the regulation of photosynthetic electron transport. In: Foyer CH, Mullineaux PM (eds) *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, pp 42. CRC Press, Boca Raton, United States
- Foyer CH, Descourvieres P and Kunert KJ (1994) Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant Cell Environ* 17: 507–523
- Foyer CH, Souriau N, Perret S, Lelandais M, Kunert KJ, Pruvost C and Jouanin L (1995) Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. *Plant Physiol* 109: 1047–1057
- Foyer CH and Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. *New Phytol* 146: 359–388
- Fryer MJ, Andrews JR, Oxborough K, Blowers DA and Baker NR (1998) Relationship between CO₂ assimilation, photosynthetic electron transport, and active O₂ metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiol* 116: 571–580
- Furbank RT, Badger MR and Osmond CB (1982) Photosynthetic oxygen exchange in isolated cells and chloroplasts of C₃ plants. *Plant Physiol* 70: 927–931
- Gamble PE and Burke JJ (1984) Effect of water stress on the chloroplast antioxidant system. I. Alterations in glutathione reductase activity. *Plant Physiol* 76: 615–621
- Gillham DJ and Dodge AD (1987) Chloroplast superoxide and hydrogen peroxide scavenging systems from pea chloroplasts: seasonal variations. *Plant Sci* 50: 105–109
- Gilmore AM (1997) Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum* 99: 197–209
- Gilmore AM and Ball MC (2000) Protection and storage of chlorophyll in overwintering evergreens. *Proc Natl Acad Sci, USA* 97: 11098–11101
- Grace S, Pace R and Wydrzynski T (1995) Formation and decay of monodehydroascorbate radicals in illuminated thylakoids as determined by EPR spectroscopy. *Biochim Biophys Acta* 1229: 155–165
- Grace SC and Logan BA (1996) Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant Physiol* 112: 1631–1640
- Grace SC and Logan BA (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Phil Trans Royal Soc Lond, Ser B* 355: 1499–1510
- Grace SC, Logan BA and Adams WW (1998) Seasonal differences in foliar content of chlorogenic acid, a phenylpropanoid antioxidant, in *Mahonia repens*. *Plant Cell Environ* 21: 513–521
- Gupta AS, Heinen JL, Holaday AS, Burke JJ and Allen RD (1993) Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. *Proc Natl Acad Sci USA* 90: 1629–1633
- Guy CL and Carter JV (1984) Characterization of partially purified glutathione reductase from cold-hardened and non-hardened spinach leaf tissue. *Cryobiology* 21: 454–464
- Halliwell B and Gutteridge JMC (1999) *Free Radicals in Biology and Medicine* (3rd ed.). Oxford University Press, Oxford
- Havaux M and Niyogi K (1999) The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc Natl Acad Sci USA* 96: 8762–8767
- Hirotsu N, Makino A, Ushio A and Mae T (2004) Changes in the thermal dissipation and the electron flow in the water/water cycle in rice grown under conditions of physiologically low temperature. *Plant Cell Physiol* 45: 635–644
- Holt NE, Zigmantas D, Valkunas L, Li XP, Niyogi KK and Fleming GR (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. *Science* 307: 433–436
- Hossain HA and Asada K (1984) Purification of dehydroascorbate reductase from spinach and its characterisation as a thiol enzyme. *Plant Cell Physiol* 25: 85–95
- Hossain HA, Nakano Y and Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol* 25: 385–395
- Jablonski PP and Anderson JW (1982) Light-dependent reduction of hydrogen peroxide by ruptured pea chloroplast. *Plant Physiol* 69: 1407–1413
- Karpinski S, Escobar C, Karpinska B, Creissen G and Mullineaux PM (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during light stress. *Plant Cell* 9: 627–640
- Kornyevev D, Holaday A and Logan BA (2004) Minimization of the light energy absorbed by 'closed' reaction centers of photosystem II as a photoprotective strategy in higher plants. *Photosynthetica* 42: 377–386
- Kornyevev D, Logan BA, Payton P, Allen RD and Holaday AS (2001) Enhanced photochemical light utilization and decreased chilling-induced photoinhibition of Photosystem II in cotton overexpressing genes encoding chloroplast-targeted antioxidant enzymes. *Physiol Plant* 113: 323–331
- Kornyevev D, Logan BA, Allen RD and Holaday AS (2003a) Effect of chloroplastic overproduction of ascorbate peroxidase on photosynthesis and photoprotection in cotton leaves subjected to low temperature photoinhibition. *Plant Sci* 165: 1033–1041
- Kornyevev D, Logan BA, Payton PR, Allen RD and Holaday AS (2003b) Elevated chloroplastic glutathione reductase activities decrease chilling-induced photoinhibition by increasing rates of photochemistry, but not thermal energy dissipation, in transgenic cotton. *Funct Plant Biol* 30: 101–110

- Kornyeyev D, Logan BA, Allen RD and Holaday AS (2005) Field-grown cotton plants with elevated activity of chloroplastic glutathione reductase exhibit no significant alteration of diurnal and seasonal patterns of excitation energy partitioning and CO₂ fixation. *Field Crops Res* 94: 165–175
- Kouril R, Lazar D, Lee H, Jo J and Naus J (2003) Moderately elevated temperature eliminates resistance of rice plants with enhanced expression of glutathione reductase to intensive photooxidative stress. *Photosynthetica* 41: 571–578
- Kurepa J, Hérouart D, Van Montagu M and Inzé D (1997) Differential expression of CuZn- and Fe-superoxide dismutase genes of tobacco during development, oxidative stress and hormonal treatments. *Plant Cell Physiol* 38: 463–470
- Kwon SY, Choi SM, Ahn YO, Lee HS, Lee HB, Park YM and Kwak SS (2003) Enhanced stress-tolerance of transgenic tobacco plants expressing a human dehydroascorbate reductase gene. *J Plant Physiol* 160: 347–353
- Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY, Allen RD and Kwak SS (2002) Enhanced tolerance of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. *Plant Cell Environ* 25: 873–882
- Li X-P, Phippard A, Pasari J and Niyogi KK (2002) Structure-function analysis of Photosystem II subunit S (PsbS) in vivo. *Funct Plant Biol* 29: 1131–1139
- Link G (2003) Redox regulation of chloroplast transcription. *Antioxidants Redox Signal* 5: 79–87
- Lohrmann N, Logan B and Johnson A (2004) Seasonal acclimatization of antioxidants and photosynthesis in *Chondrus crispus* and *Mastocarpus stellatus*, two co-occurring red algae with differing stress tolerances. *Biol Bull* 207: 225–232
- Logan BA, Barker DH, Demmig-Adams B and Adams WW III (1996) Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rainforest. *Plant Cell Environ* 19: 1083–1090
- Logan BA, Barker DH, Demmig-Adams B and Adams WW (1997) The response of xanthophyll cycle-dependent energy dissipation in *Alocasia brisbanensis* to sunflecks in a subtropical rainforest. *Aust J Plant Physiol* 24: 27–33
- Logan BA, Demmig-Adams B, Adams WW III and Grace SC (1998a) Antioxidation and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* and *Vinca major* acclimated to four growth irradiances in the field. *J Exp Bot* 49: 1869–1879
- Logan BA, Demmig-Adams B and Adams WW III (1998b) Antioxidation and xanthophyll cycle dependent energy dissipation in *Cucurbita pepo* and *Vinca major* during a transfer from low to high irradiance in the field. *J Exp Bot* 49: 1881–1888
- Logan BA, Grace SC, Adams WW III and Demmig-Adams B (1998c) Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* 116: 9–17
- Logan BA, Demmig-Adams B and Adams WW III (1999a) Acclimation of photosynthesis to the environment. In: Singhal GS, Renger G, Sopory SK, Irrgang KD and Govindjee (eds) *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*, pp 477–512. Narosa Publishing House, New Dehli, India
- Logan BA, Demmig-Adams B, Adams WW III and Rosenstiel TN (1999b) Effect of nitrogen limitation on foliar antioxidants in relationship to other metabolic characteristics. *Planta* 209: 213–220
- Logan BA, Monteiro G, Kornyeyev D, Payton P, Allen R and Holaday A (2003) Transgenic overproduction of glutathione reductase does not protect cotton, *Gossypium hirsutum* (Malvaceae), from photoinhibition during growth under chilling conditions. *Am J Bot* 90: 1400–1403
- Lovelock CE and Winter K (1996) Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical tree species. *Planta* 198: 580–587
- Makino A, Miyake C and Yokota A (2002) Physiological functions of the Water–Water cycle (Mehler reaction) and the cyclic electron flow around PS I in rice leaves. *Plant Cell Physiol* 43: 1017–1026
- Matsubara S, Gilmore AM, Ball MC, Anderson JM and Osmond CB (2002) Sustained downregulation of Photosystem II in mistletoes during winter depression of photosynthesis. *Funct Plant Biol* 29: 1157–1169
- McCord JM and Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *J Biol Chem* 244: 6049–6055
- McKersie BD, Murnaghan J, Jones KS and Bowley SR (2000) Iron-superoxide dismutase expression in transgenic alfalfa increases winter survival without a detectable increase in photosynthetic oxidative stress tolerance. *Plant Physiol* 122: 1427–1438
- Mehler AH (1951) Studies on the reaction of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. *Arch Biochem Biophys* 33: 65–77
- Mehler AH and Brown AH (1952) Studies on the reactions of illuminated chloroplasts. III. Simultaneous photoproduction and consumption of oxygen studied with oxygen isotopes. *Arch Biochem Biophys* 38: 365–370
- Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo?. *Trends Plant Sci* 4: 130–135
- Mishra NP, Mishra RK and Singhal GS (1993) Changes in the activities of antioxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of different protein synthesis inhibitors. *Plant Physiol* 102: 867–880
- Mishra NP, Fatma T and Singhal GS (1995) Development of antioxidative defense system of wheat seedlings in response to high light. *Physiol Plant* 95: 77–82
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7: 405–410
- Mittler R and Zilinskas BA (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant J* 5: 397–405
- Miyake C and Asada K (1992) Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant Cell Physiol* 33: 541–553
- Miyake C and Yokota A (2000) Determination of the rate of photoreduction of O₂ in the Water–Water cycle in watermelon leaves and enhancement of the rate by limitation of photosynthesis. *Plant Cell Physiol* 41: 335–343
- Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV and Aparicio-Tejo P (1994) Drought induces oxidative stress in pea plants. *Planta* 194: 346–352
- Murgia I, Tarantino D, Vannini C, Bracale M, Carravieri S and Soave C (2004) *Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to Paraquat-induced photooxidative stress and to nitric oxide-induced cell death. *Plant J* 38: 940–953
- Neubauer C and Yamamoto HY (1992) Mehler-peroxidase reaction mediates zeaxanthin formation and zeaxanthin-

- related fluorescence quenching in intact chloroplasts. *Plant Physiol* 99: 1354–1361
- Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. *Annu Rev Plant Physiol Plant Mol Biol* 50: 333–359
- Noctor G, Veljovic-Jovanovic and Foyer CH (2000) Peroxide processing in photosynthesis: antioxidant coupling and redox signaling. *Phil Trans Royal Soc Lond, Ser B* 355: 1465–1475
- Ort DR and Baker NR (2002) A photoprotective role for O₂ as an alternative electron sink in photosynthesis? *Curr Opin Plant Biol* 5: 193–198
- Osmond CB and Grace SC (1995) Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? *J Exp Bot* 46: 1351–1362
- Payton P, Allen RD, Trolinder N and Holaday AS (1997) Overexpression of chloroplast-targeted Mn superoxide dismutase in cotton (*Gossypium hirsutum* L., cv. Coker 312) does not alter the reduction of photosynthesis after short exposures to low temperature and high light intensity. *Photosynth Res* 52: 233–244
- Payton P, Webb R, Korniyev D, Allen R and Holaday S (2001) Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. *J Exp Bot* 52: 2345–2354
- Peltier JB, Emanuelsson O, Kalume DE, Ytterberg J, Friso G, Rudella A, Liberles DA, Soderberg L, Roepstorff P, von Heijne G and van Wijk KJ (2002) Central functions of the lumenal and peripheral thylakoid proteome of Arabidopsis determined by experimentation and genome-wide prediction. *Plant Cell* 14: 211–236
- Pfannschmidt T and Liere K (2005) redox regulation and modification of proteins controlling chloroplast gene expression. *Antioxidants Redox Signal* 7: 607–618
- Pitcher LH, Brennan E, Hurley A, Dunsmuir P, Tepperman JM and Zilinskas BM (1991) Overproduction of petunia chloroplastic copper/zinc superoxide dismutase does not confer ozone tolerance in transgenic tobacco. *Plant Physiol* 97: 452–455
- Polle A (2001) Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. *Plant Physiol* 126: 445–462
- Prasil O, Adir N and Ohad I (1992) Dynamics of Photosystem II: mechanisms of photoinhibition and recovery processes. In: Barber J (ed) *Topics in Photosynthesis, Vol. 11, The Photosystems: Structure, Function and Molecular Biology*, pp 295–348. Elsevier Science Publishers, Amsterdam
- Richter M, Rühle W and Wild A (1990) Studies on of Photosystem II photoinhibition. II. The involvement of toxic oxygen species. *Photosynth Res* 24: 237–243
- Rubio MC, Gonzalez EM, Minchin FR, Webb KJ, Arrese-Igor C, Ramos J and Becana M (1997) Effects of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa overexpressing superoxide dismutase. *Physiol Plant* 115: 531–540
- Ruuska SA, Badger MR, Andrews TJ and von Caemmerer S (2000a) Photosynthetic electron sinks in transgenic tobacco with reduced amounts of rubisco: little evidence for significant Mehler reaction. *J Exp Bot* 51: 357–368
- Ruuska SA, von Caemmerer S, Badger MR, Andrews TJ, Price GD and Robinson SA (2000b) Xanthophyll cycle, light energy dissipation and electron transport in transgenic tobacco with reduced carbon assimilation capacity. *Aust J Plant Physiol* 27: 289–300
- Schöner S and Krause GH (1990) Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta* 180: 383–389
- Schwanz P and Polle A (2001) Differential stress responses on antioxidative systems to drought in pendunculate oak (*Quercus robur*) and maritime pine (*Pinus pinaster*) grown under high CO₂ concentrations. *J Exp Bot* 52: 133–143
- Sen Gupta A, Heinen JL, Holaday AS, Burke JJ and Allen RD (1993a) Increased tolerance to oxidative stress in transgenic plants that overexpress chloroplastic Cu Zn superoxide dismutase. *Proc Natl Acad Sci USA* 90: 1629–1633
- Sen Gupta A, Webb PR, Holaday AS and Allen RD (1993b) Overexpression of superoxide dismutase protects plants from oxidative stress: induction of ascorbate peroxidase in superoxide dismutase-overproducing plants. *Plant Physiol* 103: 1067–1073
- Slooten L, Capiou K, Van Camp W, Van Montagu M, Sybesma C and Inze D (1995) Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts. *Plant Physiol* 107: 737–750
- Smith IK, Vierheller TL and Thorne CA (1989) Properties and functions of glutathione reductase in plants. *Physiol Plant* 77: 449–456
- Stevens RG, Creissen GP and Mullineaux PM (2000) Characterisation of pea cytosolic glutathione reductase expressed in transgenic tobacco. *Planta* 211: 537–545
- Strohm M, Eiblmeier M, Langebartels C, Jouanin L, Polle A, Sandermann H and Rennenberg H (1999) Responses of transgenic poplar (*Populus tremula* × *P. alba*) overexpressing glutathione synthetase or glutathione reductase to acute ozone stress: visible injury and leaf gas exchange. *J Exp Bot* 50: 363–372
- Tepperman JM and Dunsmuir P (1990) Transformed plants with elevated level of chloroplastic SOD are not more resistant to superoxide toxicity. *Plant Mol Biol* 14: 501–510
- Terashima I, Noguchi K, Itoh-Nemoto T, Park Y-M, Kubo A and Tanaka K (1998) The cause of PS I photoinhibition at low temperatures in leaves of *Cucumis sativus*, a chilling sensitive plant. *Physiol Plant* 103: 295–303
- Tschiersch H and Ohmann E (1993) Photoinhibition in *Euglena gracilis*: involvement of reactive oxygen species. *Planta* 191: 316–323
- Torsethaugen G, Pitcher LH, Zilinskas BA and Pell EJ (1997) Overproduction of ascorbate peroxidase in the tobacco chloroplast does not provide protection against ozone. *Plant Physiol* 114: 529–537
- Tyystjärvi E, Riikonen M, Arisi A-CM, Kettunen R, Jouanin L and Foyer CH (1999) Photoinhibition of Photosystem II in tobacco plants overexpressing glutathione reductase and poplars overexpressing superoxide dismutase. *Physiol Plant* 105: 409–416
- Van Camp W, Capiou K, Van Montagu M, Inze D and Slooten L (1996) Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. *Plant Physiol* 112: 1703–1714
- Wang J, Zhang H and Allen RD (1999) Overexpression of an Arabidopsis peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. *Plant Cell Physiol* 40: 725–732

- Wang Y, Wisniewski M, Meilan R, Cui M, Webb R and Fuchigami L (2005) Overexpression of cytosolic ascorbate peroxidase in tomato confers tolerance to chilling and salt stress. *J Am Soc Hort Sci* 130: 167–173
- Winkler BS, Orselli SM and Rex TS (1994) The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radicals Biol Med* 17: 333–349
- Wise RR (1995) Chilling-enhanced photooxidation. The production, action and study of reactive oxygen species produced during chilling in the light. *Photosynth Res* 45: 79–97
- Wu J, Neimanis S and Heber U (1991) Photorespiration is more effective than the Mehler reaction in protecting the photosynthetic apparatus against photoinhibition. *Bot Acta* 104: 283–291
- Yabuta Y, Motoki T, Yoshimura K, Takeda T, Ishikawa T and Shigeoka S (2002) Thylakoid membrane-bound ascorbate peroxidase is a limiting factor of antioxidative systems under photo-oxidative stress. *Plant J* 32: 915–925
- Yan J, Wang J, Tissue D, Holaday AS, Allen R and Zhang H (2003) Photosynthesis and seed production under water-deficit conditions in transgenic tobacco plants that overexpress an Arabidopsis ascorbate peroxidase gene. *Crop Sci* 43: 1477–1483
- Yamasaki H and Grace SC (1998) EPR detection of phyto-phenoxy radicals stabilized by zinc ions: evidence for the redox-coupling of plant phenolics with ascorbate in the H₂O₂-peroxidase system. *FEBS Lett* 422: 377–380
- Yoshimura K, Yabuta Y, Ishikawa T and Shigeoka S (2000) Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. *Plant Physiol* 123: 223–233
- Zhang J and Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol* 35: 785–791