

# Is electron transport to oxygen an important mechanism in photoprotection? Contrasting responses from Antarctic vascular plants

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Photoreduction of oxygen by the photosynthetic electron transport chain has been suggested to be an important process in protecting leaves from excess light under conditions of stress; however, there is little evidence that this process occurs significantly except when plants are exposed to conditions outside their normal tolerance range. We have examined the oxygen dependency of photosynthetic electron transport in the two vascular plants found growing in Antarctica – *Colobanthus quitensis* and *Deschampsia antarctica*. Photosynthetic electron transport in *C. quitensis* is insensitive to changes in oxygen concentration under non-photorespiratory conditions, indicating that electron transport to oxygen is negligible; however, it has a substantial capacity for non-photochemical quenching (NPQ) of chlorophyll fluorescence. In contrast, *D. antarctica* has up to 30% of its photosynthetic electron transport being linked to oxygen, but has a substantially lower capacity for NPQ. Thus, these plants rely on contrasting photoprotective mechanisms to cope with the Antarctic environment. Both plants seem to use cyclic electron flow associated with PSI, however, this is activated at a lower irradiance in *C. quitensis* than in *D. antarctica*.

## Introduction

Photosynthetic electron transport, occurring in the chloroplast thylakoid membrane, is a process that is uniquely sensitive to environmental stress. Such stress occurs when the environmental conditions to which a plant is exposed lead to an imbalance between the absorption of light energy and the assimilation of that energy through carbon fixation and other metabolic processes, e.g. as a result of drought stress or extreme high or low temperatures (Biehler and Fock 1996, Foyer et al. 2002, Havaux 1994). Under such conditions, the electron transport chain can produce reactive oxygen species (ROS) through one of the two major routes – photore-

duction of oxygen to form superoxide ( $O_2^-$ ; the Mehler reaction) or formation of singlet-excited oxygen ( $^1O_2^*$ ) via the interaction of molecular  $O_2$  with triplet-excited chlorophyll (Asada 1999, 2000, Foyer and Noctor 2000). The former of these reactions is thought to occur primarily at the iron sulphur centres associated with PSI (Asada 1999) although there is growing evidence that this can also occur around PSII (Cleland and Grace 1999, Khorobrykh and Ivanov 2002, Khorobrykh et al. 2004). The latter is mainly related to the antenna associated with PSII (Foyer and Noctor 2000, Owens 1996). Under conditions where metabolism is inhibited, absorbed light energy will continue to drive the production of reducing

**Abbreviations** – ETR, electron transport rate;  $g_{ETC}$ , conductance of the electron transport chain; NPQ, non-photochemical quenching; PQH<sub>2</sub>, plastoquinol; PTOX, plastid terminal oxidase; qL, PSII acceptor redox state; Q<sub>A</sub>, quinone A; ROS, reactive oxygen species.

equivalents; however, these will not be consumed at a normal rate. Instead, they will tend to reduce oxygen, a reaction that increases under stress. At the same time, the absence of an efficient sink for excitation energy will increase the lifetime of singlet-excited chlorophyll in the PSII antenna, increasing the probability of intersystem crossing giving rise to triplet chlorophyll and so to singlet oxygen.

Once ROS are formed within the chloroplast, they are liable to cause widespread and unspecific oxidative damage, including lipid peroxidation and DNA damage. Among the forms of damage that occur, there is a relatively specific lesion in the PSII reaction centre, whereby singlet oxygen produced through charge recombination reactions causes cleavage of the D1 polypeptide (Aro et al. 2005, Nixon et al. 2005). This cleavage gives rise to an altered form, the reaction centre that is incapable of normal photochemistry but is able to dissipate absorbed light energy as heat. Increased damage to PSII results in photoinhibition, a process that has been widely studied over many years and currently known with molecular detail (Aro et al. 2005, Nixon et al. 2005). In vivo photoinhibition appears to be a consequence of over-excitation of the reaction centre, especially under conditions where sinks for the reductant produced are limited. Different routes seem to be available to plants to minimize the extent of photoinhibition. Light energy can be dissipated as heat in the PSII antenna before the excitation energy reacts with the reaction centre – this primarily occurs through the pH-dependent process of high-energy-state quenching, which involves changes in the PSII antenna proteins, including the synthesis of the carotenoid zeaxanthin from violaxanthin (Horton and Ruban 2005, Niyogi et al. 2005). However, it has been reported that photoinactivated PSII reaction centres are able to dissipate energy in vivo by reaction centre quenching (Finazzi et al. 2004, Ivanov et al. 2006, Matsubara and Chow 2004). Alternatively, photoinhibition can be minimized by optimizing the flow of electrons away from the reaction centre. For example, the process of photorespiration, whereby the enzyme Rubisco can react with oxygen in place of CO<sub>2</sub> has long been thought of as a wasteful side reaction of photosynthesis. However, it has been increasingly recognized that this also provides a sink for excitation energy under stress conditions and helps to protect plants against photoinhibition (Wingler et al. 2000).

Among the alternative sinks for the reducing potential produced in photosynthesis, the direct reduction of oxygen to form superoxide plays an enigmatic role and has been widely debated in recent years (Asada 1999, Heber 2002, Ort and Baker 2002, Polle 1996). First, it is thought to provide a sink for reductant, taking electrons away from the electron transport chain and is also

expected to generate a  $\Delta$ pH across the thylakoid membrane, promoting protective high-energy-state quenching (Asada 1999). However, direct reduction of oxygen involves the uncontrolled production of not only superoxide but, through subsequent reactions, hydrogen peroxide, hydroxyl radicals and other ROS. The production of these might be contained through the specific association of superoxide dismutase and ascorbate peroxidase with the PSI acceptor side; however, there is still liable to be leakage from this pathway, causing widespread damage to the chloroplast. Indeed, a number of authors have argued that the damaged reaction centres, which tend to accumulate unrepaired under stress conditions, might represent a form of defence in their own right. They are unable to perform charge separation, thereby limiting the rate of production of reducing equivalents and, at the same time, they dissipate absorbed energy as heat, lowering singlet oxygen production (Öquist et al. 1992).

Much of the debate concerning the role of photoreduction of oxygen has centred on observations of the extent of this reaction in a variety of plants as well as on the experimental approaches adopted to measure it. Studies in C3 plants have tended to conclude that under no stressful conditions, including at high light, the proportion of electron flow to have tended to find electron flow to oxygen constitutes only a small proportion of total electron flux, generally less than 10% of total flux (Badger et al. 2000, Clarke and Johnson 2001, Flexas and Medrano 2002, Ruuska et al. 2000). Higher levels are generally seen only when plants are exposed to extreme stress, e.g. severe drought (Biehler and Fock 1996), high temperature (Clarke and Johnson 2001) and salt stress (Chen et al. 2004). For example, a report by Biehler and Fock (1996) suggested that in wheat subjected to drought, up to 30% of electron flow might be directly to oxygen, although this figure has since been questioned (Heber 2002). Clarke and Johnson (2001), using the same experimental approach as that adopted here, recorded similar oxygen dependence of electron transport, but only at high temperatures (>30°C), which also induced other signs of stress in the plants. In C4 plants, most studies have concluded that electron transport to oxygen (including any photorespiration) is lower than in C3 plants; however, a study by Siebke et al. (2003) concluded that gross oxygen uptake represents up to 18% of gross O<sub>2</sub> about, being two to seven times greater than dark respiration. Additionally, light-induced O<sub>2</sub> uptake was suggested to primarily be the result of photoreduction of oxygen, although the possibility of light modulating the rate of dark respiration cannot be ruled out.

Although a great deal can be learnt about the responses of plants to stress by studying how sensitive species react when exposed to that stress, it can be argued that a true understanding of how plants can defend themselves can

only be achieved by examining those species that are tolerant to stress. At the same time, comparative studies using a single set of experimental protocols can sometimes avoid ambiguity introduced when data using different methods from different experimental systems are compared. The Antarctic represents one of the most extreme environments existing on earth. In this region, temperatures usually range between 0 and 6°C and irradiance can reach up to 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in summer (Edwards and Smith 1988, Schroeter et al. 1995). Only two species of vascular plants are found in the Antarctic – a grass, *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae). Both plants show a high degree of adaptation to their environment, being able to maintain approximately 30% of their maximum photosynthetic rates at 0°C and are not photoinhibited at high light or low temperature (Xiong et al. 1999). The mechanisms adopted by these species in response to stress are currently being studied in detail, with preliminary indications that they adopt contrasting strategies. *D. antarctica* has been seen to contain very high levels of antioxidants, while *C. quitensis* has levels comparable to other plant species (Perez-Torres et al. 2004a, 2004b). In the present study, we have investigated the responses of these two species to high light and low temperature. Although both adapted to the same, very extreme environment, the strategies adopted by these species seem to differ markedly. In particular, the use of oxygen as a sink for reductant is dominant in *D. antarctica*, but largely absent in *C. quitensis*.

## Materials and methods

### Plant material and growth conditions

*C. quitensis* (Kunth) Bartl. (Caryophyllaceae) was collected on King George Island, Maritime Antarctic (62°14'S; 58°48'W) and *D. antarctica* Desv. (Poaceae) was collected on the Coppermine Peninsula on Robert Island, Maritime Antarctic (62°22'S; 59°43'W). Plants were propagated vegetatively in plastic pots using a soil:peat mixture (3:1) and were fertilized with 0.12 g l<sup>-1</sup> Phostrogen (Solaris, Buckinghamshire, UK) once a week. Growth conditions were a photosynthetic photon flux density of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (cool-white fluorescent tubes F40CW; General Electric, Charlotte, NC) at 15°C (100/15) with an 18-h photoperiod. Photoperiod 18/6 is very close to the environmental conditions found in the Antarctic during the growing season of these plants and 15°C is close to their optimum photosynthetic temperature (Xiong et al. 2000). High light treatments were as high as 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  considering that in the Antarctic environment during the summer, these plants are exposed

to high light episodes of up to 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Schroeter et al. 1995).

### Measurements of photosynthetic parameters

The redox state of P700 was measured as an absorbance change at 830 nm using a Walz PAM 101 fluorometer system in combination with an ED-P700DW emitter-detector unit (Walz, Effeltrich, Germany). Fluorescence was measured using a separate PAM 101, with measurements being made in parallel on the same plant material. Experimental set up was as described previously (Clarke and Johnson 2001, Golding and Johnson 2003).

Fluorescence parameters were calculated as described in Maxwell and Johnson (2000) except for PSII acceptor redox state (qL), which was calculated as described in Kramer et al. (2004). Absorbance data were analysed as described by Golding and Johnson (2003).

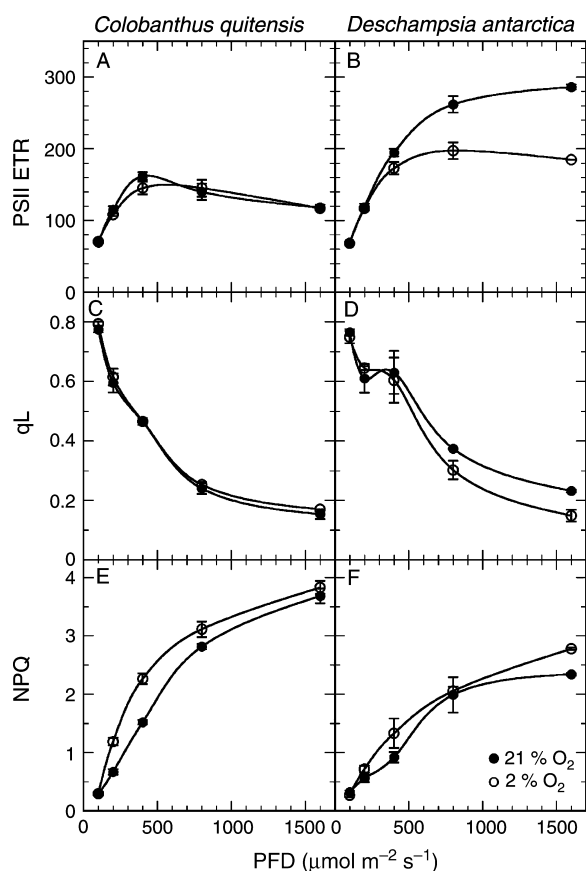
To determine the contribution of Mehler reaction as a photoprotective pathway, all parameters were measured in 2000 ppm CO<sub>2</sub>, which was found to be saturating for CO<sub>2</sub> fixation across all conditions measured. Measurements using 5% CO<sub>2</sub> gave essentially the same results. Under these conditions, differences observed between 21 and 2% O<sub>2</sub> treatments can be assigned to the contribution of O<sub>2</sub> as an alternative electron acceptor (Clarke and Johnson 2001). A 2000 ppm CO<sub>2</sub> atmosphere was generated using a CIRAS-1 Infra Red Gas Analyzer (PP Systems, Hitchin, UK). 2% O<sub>2</sub> gas was supplied from a cylinder of 2% O<sub>2</sub>/balance N<sub>2</sub> (BOC Gases, Guilford, UK). Measurements were made in a modified Hansatech LD2/2 leaf chamber (Hansatech, King's Lynn, UK). Chamber temperature was controlled using a cooling water bath and monitored continuously during measurements using a thermocouple located just below the leaf lower surface. All leaves used were removed from well-watered plants that had been dark adapted overnight prior to use as recommended by Maxwell and Johnson (2000).

CO<sub>2</sub> assimilation vs PSII electron transport rate (ETR) plot were by simultaneous measurements of CO<sub>2</sub> assimilation with an IRGA (CIRAS-2; PP Systems) and chlorophyll fluorescence (Portable Modulated Fluorimeter; Hansatech). Photosynthetic light response was registered every 5 min at 15°C between 0 and 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and measurements were made at 2000 ppm (in order to inhibit photorespiration) at 21 and 2% O<sub>2</sub> (AGA, Concepción, Chile).

## Results

When plants of *C. quitensis* and *D. antarctica* were exposed to a range of irradiances at 15°C in the presence

of saturating CO<sub>2</sub> and ambient O<sub>2</sub>, the relative rate of PSII photochemistry, estimated as the product of PSII efficiency ( $\Phi$ PSII) and irradiance, rose following the characteristic pattern of a typical light response curve (Fig. 1A, B). At 21% O<sub>2</sub>, PSII electron transport saturated at an irradiance of approximately 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in *C. quitensis*, meanwhile continued to rise to approximately 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in *D. antarctica*. When these measurements were repeated in the presence of a lowered O<sub>2</sub> concentration (2%), electron transport in *C. quitensis* was unaffected across all irradiances (Fig. 1A). By contrast, in *D. antarctica*, there was a marked effect of lowering O<sub>2</sub>. At the lowest irradiances (<400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), 2% oxygen did not alter electron transport, however, at higher light levels, it was markedly inhibited up to approximately 30% of total electron transport (Fig. 1B). In order to check that PSII ETR behaviour was not an artefact of low oxygen conditions, gas exchange measurements

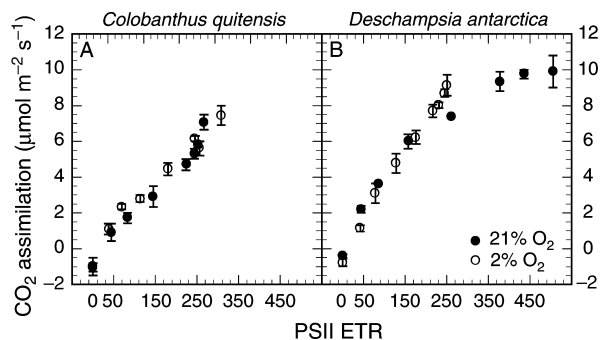


**Fig. 1.** Irradiance response of relative PSII ETR (A, B), PSII qL (C, D) NPQ (E, F) in *C. quitensis* (A, C, E) and *D. antarctica* (B, D, F) measured at 15°C in the presence of 2000 ppm CO<sub>2</sub> and either 21% (closed symbols) or 2% (open symbols) O<sub>2</sub>. Measurements represent the mean of at least three replicates with standard error bars shown.

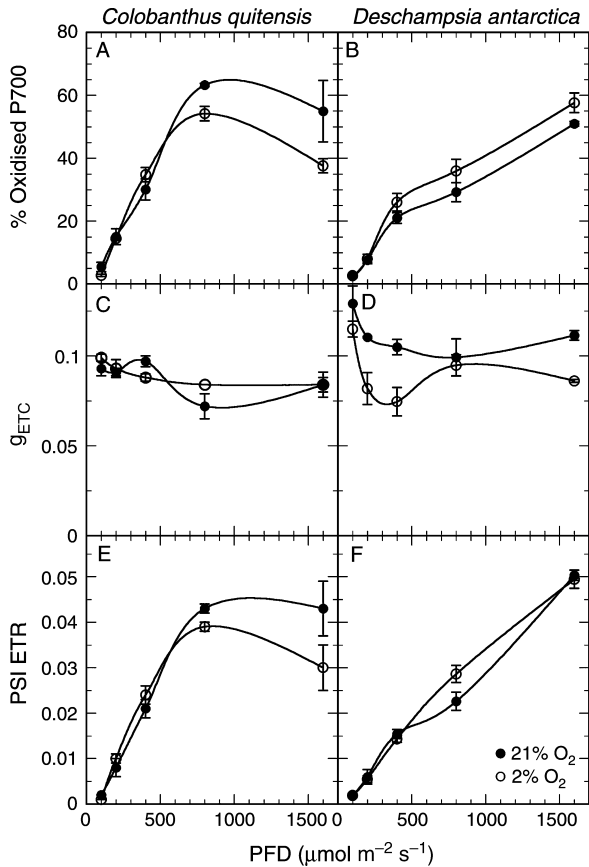
were carried out simultaneously with modulated fluorescence and showed a linear relationship between CO<sub>2</sub> assimilation and PSII ETR for *C. quitensis* at 21 and 2% O<sub>2</sub> and at 2% O<sub>2</sub> for *D. antarctica* (Fig. 2). The relative redox state of PSII was estimated using the parameter qL, which has been shown to be a better indicator of PSII redox state than the more familiar parameter qP (Kramer et al. 2004) (Fig. 1C, D). In *C. quitensis*, qL was found to be insensitive to O<sub>2</sub> at all irradiances, meanwhile in *D. antarctica* 2% O<sub>2</sub> resulted in qL being lower at high irradiances when compared with 21% O<sub>2</sub> implying that the primary acceptor, quinone A (Q<sub>A</sub>), was more reduced at low O<sub>2</sub>.

The process of non-photochemical quenching (NPQ) is known to be induced in plants under conditions of stress to dissipate excess light energy, protecting the plant from light damage (Horton et al. 2000). Estimates of NPQ at different irradiances showed that both species studied had a substantial capacity for this process, however, this was somewhat greater in *C. quitensis* than in *D. antarctica* (Fig. 1E, F). Lowering O<sub>2</sub> concentration had essentially the same effect on NPQ in both species – it marginally increased the level of this across the entire irradiance range.

Simultaneous to the above measurements, estimates were made of the redox state and turnover of the PSI primary electron donor P700, using absorbance in the near infrared. With increasing irradiance, P700 became progressively more oxidized in both species and was only marginally affected by altering the oxygen concentration (Fig. 3A, B). At the highest irradiances, in *C. quitensis*, P700 was slightly more reduced at low O<sub>2</sub>, this corresponding to a slight reduction in the pool of active PSI (not shown). In *D. antarctica*, P700 tended to be slightly more oxidized at low O<sub>2</sub> across most irradiances. In the same experiment, conductance of the electron transport chain ( $g_{\text{ETC}}$ ) was estimated as the rate constant for the



**Fig. 2.** Relationship between CO<sub>2</sub> assimilation and PSII ETR at 15°C in Antarctic vascular plants exposed to 21 and 2% O<sub>2</sub>. Measurements represent the mean of at least three replicates with standard error bars shown.



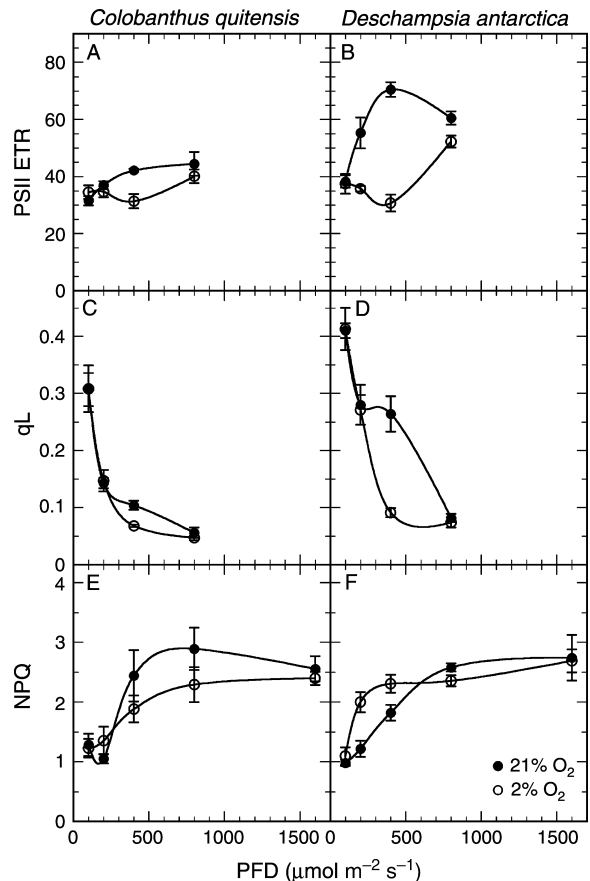
**Fig. 3.** Irradiance response of P700<sup>+</sup> (A, B),  $g_{ETC}$  (C, D) and PSI ETR (E, F) in *C. quitensis* (A, C, E) and *D. antarctica* (B, D, F) measured at 15°C in the presence of 2000 ppm CO<sub>2</sub> and either 21% (closed symbols) or 2% (open symbols) O<sub>2</sub>. Measurements represent the mean of at least three replicates with standard error bars shown. PFD, photon flux density.

decay of P700<sup>+</sup> following transition to darkness from a 100-ms flash (Golding and Johnson 2003). In *C. quitensis*, this parameter was found to be largely insensitive to both irradiance and oxygen concentration, being approximately  $0.9 \text{ ms}^{-1}$  across all conditions (Fig. 3C). In contrast, in *D. antarctica*, the  $g_{ETC}$  was found to be sensitive irradiance and oxygen levels, tending to fall with increasing irradiance, and being lower at low oxygen (Fig. 3D).

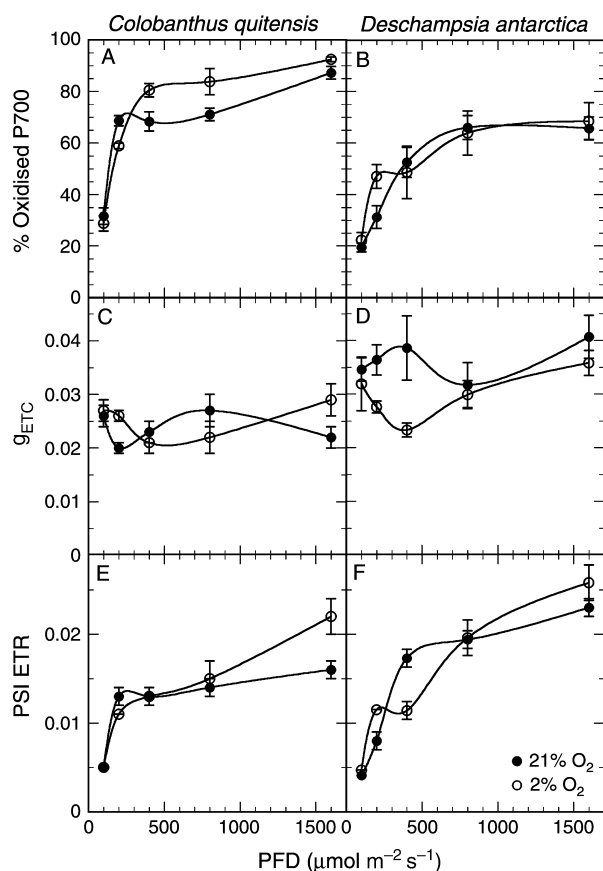
Fig. 3E, F shows the response of PSI turnover to irradiance and oxygen in the two species using the approach of Clarke and Johnson (2001). The light response of PSI turnover differed in a way that reflects the difference in light saturation of PSII – *C. quitensis* saturated at a lower irradiance than *D. antarctica*. In *C. quitensis*, PSII saturated at approximately  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whereas PSI saturated at  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ . However, in *D. antarctica*, PSII saturated between 400 and  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,

whereas PSI turnover was not saturated within the irradiance range considered. In both cases, PSI electron transport saturated at an irradiance that was higher than that required to saturate PSII turnover. When the redox state of P700 was used to estimate P700 turnover, the same overall conclusions were obtained (not shown).

The above measurements were repeated on plants exposed to 4°C (Figs 4 and 5). As expected, photosynthesis in both species was substantially inhibited at this temperature, making measurements of PSII electron transport at higher irradiances inaccurate. Nevertheless, the same general trends could be observed as at 15°C. In *C. quitensis*, PSII electron transport was already saturated at the lowest irradiance considered and was substantially lower than at 15°C (Figs 4A and 1A) and there was no clear indication that PSII turnover was inhibited by lowering the oxygen concentration. Measurements of qL indicated that the PSII primary acceptor Q<sub>A</sub> were



**Fig. 4.** Irradiance response of relative PSII ETR (A, B), PSII qL (C, D) and NPQ (E, F) in *C. quitensis* (A, C, E) and *D. antarctica* (B, D, F) measured at 4°C in the presence of 2000 ppm CO<sub>2</sub> and either 21% (closed symbols) or 2% (open symbols) O<sub>2</sub>. Measurements represent the mean of at least three replicates with standard error bars shown. PFD, photon flux density.



**Fig. 5.** Irradiance response of P700<sup>+</sup> (A, B),  $g_{ETC}$  (C, D) and PSII ETR (E, F) in *C. quitensis* (A, C, E) and *D. antarctica* (B, D, F) measured at 15°C in the presence of 2000 ppm CO<sub>2</sub> and either 21% (closed symbols) or 2% (open symbols) O<sub>2</sub>. Measurements represent the mean of at least three replicates with standard error bars shown. PFD, photon flux density.

reduced with increasing irradiance, however, this was again not substantially affected by O<sub>2</sub> concentration (Fig. 4C). In *D. antarctica*, measurements of PSII turnover at irradiances up to 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  mirrored those over the full irradiance range at 15°C, with the rate of electron transport rising to a higher level with increasing light in the presence of ambient O<sub>2</sub> and being already saturated at 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in low O<sub>2</sub> (Fig. 4B). (Measurements of PSII ETR at higher irradiances are probably erroneous, due to the high degree of saturation of PSII and were not included.) At 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $Q_A$  was substantially more saturated at low than at ambient O<sub>2</sub>.

NPQ saturated for both species within the irradiance range considered, regardless of O<sub>2</sub> concentration in the two species at 4°C (Fig. 4E, F). The irradiance response of this parameter did however tend to be sensitive to O<sub>2</sub>. In *C. quitensis*, NPQ tended to be lower at low oxygen, however this effect was not significant (Fig. 4E). In *D. antarctica*, NPQ saturated at a much lower irradiance

in the presence of low O<sub>2</sub> than in ambient (Fig. 4F). For *C. quitensis*, the maximum for NPQ was somewhat lower at 4°C than at 15°C. This was not true of *D. antarctica*.

At 4°C, P700 was more oxidized under any given set of conditions than at 15°C, implying a slowing of electron transport prior to PSI (Fig. 5A, B). This was supported by measurements of the  $g_{ETC}$ , which was considerably lower at 4°C than at 15°C (Figs 5C, D and 3C, D). Oxidation of P700 reached a maximum level in *C. quitensis* at a lower irradiance than was the case for *D. antarctica* (Fig. 5A, B). In both cases, P700 oxidation rose to be close to the active pool, measured as the signal following a flash-dark transition (not shown). In *D. antarctica*, only approximately 80% of PSI could be measured as active in this way (measured relative to the far-red-induced signal in dark adapted leaves), even at the highest irradiances (not shown). In both species, PSI electron transport was substantially lower at 4°C than at 15°C and saturated at a lower irradiance, although again at a higher irradiance than was the case for PSII turnover (Figs 5E, F and 3E, F). PSII turnover saturated at a higher irradiance in *D. antarctica* than in *C. quitensis* (Fig. 5E, F) and was not sensitive to O<sub>2</sub> concentration at 4°C in either species.

## Discussion

In *C. quitensis*, altering the oxygen concentration did not have any significant effect on electron transport through either PSII or PSI. The only substantial response was that at 15°C (although not clearly at 4°C) the irradiance response of NPQ was shifted, with this being induced at a lower irradiance at low O<sub>2</sub>. By contrast, in *D. antarctica*, electron transport through PSII at high irradiance was substantially inhibited when O<sub>2</sub> concentration was lowered. This was seen at 15 and 4°C and implies that under the non-photorespiratory conditions used here, O<sub>2</sub> was acting as a sink for electrons coming from PSII. Side effects as a result of exposure to low oxygen levels are highly improbable considering the short time period involved in the analysis and the successful application of similar experimental approaches elsewhere (Clarke and Johnson 2001, Streb et al. 2005). Thus, it can be suggested that, while *C. quitensis* is probably regulating its electron transport pathway in a way that avoids reduction of oxygen, *D. antarctica* may be actively promoting this reaction to protect PSII from excess light.

A consequence of using oxygen as an electron acceptor is that ROS are liable to be generated. This could be avoided, if the reduction was mediated by an alternative oxidase, such as the plastid terminal oxidase (PTOX), as proposed by Streb et al. (2005). Such an enzyme is known to exist in some plant chloroplasts (Cournac et al. 2000, Moore et al. 2002). However, Rosso et al. (2006)

recently showed strong evidence that does not support the role of PTOX in photoprotection. The susceptibility of PSII ETR to low oxygen levels in non-photorespiratory conditions and the very high concentrations antioxidant enzymes (superoxide dismutase, ascorbate peroxidase and glutathione reductase) found in *D. antarctica* (Perez-Torres et al. 2004b) suggest the use of direct reduction of O<sub>2</sub> and the water–water cycle in *D. antarctica* as an electron sink pathway. We propose that in *D. antarctica*, oxygen would act as a direct electron sink, with the resulting formation of superoxide and hydrogen peroxide. Alongside, these active oxygen species would be scavenged by an efficient antioxidant system, constituting a safe alternative to avoid the over-reduction of the electron transport chain under stressful conditions.

Based on measurements presented here, as well as earlier observations by Clarke and Johnson (2001), it is unclear whether photoreduction of oxygen occurs after or before PSI. Classically, it is always supposed that photoreduction of oxygen occurs on the acceptor side of PSI, with reduced iron sulphur centres being oxidized by O<sub>2</sub> (Asada 1999). However, in measurements presented here, as in the data of Clarke and Johnson (2001), there is no indication that altering oxygen concentration alters flux through PSI. Clarke and Johnson (2001) considered two possible explanations for this observation: either photoreduction of oxygen occurs in competition with cyclic electron flow, and thus the latter increases to maintain the same PSI turnover when O<sub>2</sub> is lowered, or photoreduction of O<sub>2</sub> occurs in the electron transport chain before PSI and therefore its inhibition has no effect on PSI turnover. We are at present not able to distinguish between these two possibilities, however, there is growing support from in vivo experiments that photoreduction of O<sub>2</sub> can occur in association with PSII – either directly from the PSII acceptors (Q<sub>A</sub><sup>-</sup> and quinone B<sup>-</sup>) (Cleland and Grace 1999) or from the PQH<sub>2</sub> pool (Khorobrykh and Ivanov 2002, Khorobrykh et al. 2004).

In addition to acting as a sink for excitation energy, it has been discussed that electron flow to O<sub>2</sub> will contribute to the protection of PSII indirectly by generating a pH gradient across the thylakoid membrane which will in turn drive protective NPQ (Ort and Baker 2002, Polle 1996). From our data, there is no evidence that electron flow to O<sub>2</sub> is a net contributor to ΔpH, indeed, if anything, the ΔpH is higher at low rather than ambient oxygen, indicating that electron flow to oxygen seems to be an alternative to, rather than a supporter of non-photochemical photoprotection. This might be explained if O<sub>2</sub> photoreduction is occurring in competition with cyclic electron flow because one ΔpH generating reaction would be replaced by another. If O<sub>2</sub> photoreduction is occurring prior to PSI, it

implies that the reaction does not result in the net generation of a ΔpH. This might be the case if, e.g. the activity of an alternative oxidase involved uptake of protons from the lumen, or if reduction of O<sub>2</sub> occurred, e.g. at the quinone oxidizing site on the cytochrome *b<sub>6</sub>f* complex and dismutation of superoxide and subsequent reactions were occurring primarily in the thylakoid lumen.

The two species studied show differences in their light saturation responses. All plants were grown under the same conditions; however, in the presence of ambient oxygen electron transport was saturated at a lower irradiance in *C. quitensis* than in *D. antarctica*. This was not the case at low oxygen where at 15°C both plants saturated at approximately 400 μmol m<sup>-2</sup> s<sup>-1</sup> and below 100 μmol m<sup>-2</sup> s<sup>-1</sup> at 4°C. This implies that O<sub>2</sub> is a sink for excitation energy at high light intensity in *D. antarctica* allowing higher PSII ETR, whereas in *C. quitensis* electron transport is saturated. However, *C. quitensis* seems to adopt NPQ as a more important protective mechanism under conditions of high light. Further measurements of NPQ indicated that even at the highest irradiances used, only a small proportion of the quenching was because of photoinhibition (qI) and most of it corresponded to qE (not shown). NPQ did not rise above approximately 2.5 in *D. antarctica*, and reached values of approximately 3.5 in *C. quitensis*. The extent of NPQ might be regulated by levels of zeaxanthin present (an operative xanthophyll cycle has been previously reported for *D. antarctica* by Perez-Torres et al. 2004b), expression of PsbS or the extent of the ΔpH that can be formed. It is noted that *C. quitensis* shows a lower extent of NPQ at low temperature, which suggests that under those conditions it is less able to generate a high ΔpH, contrasting with observations in barley (Clarke and Johnson 2001).

Recently, there has been some discussion as to the method used to estimate the turnover of PSI. Early measurements by Harbinson and Woodward (1987) used the redox state of P700 as an estimate of the quantum yield of PSI. This approach was modified by Klughammer and Schreiber (1994), who introduced an approach using a saturating flash to estimate the proportion of PSI that was active, i.e. had a reduced electron donor and an oxidized acceptor. Oja et al. (2003) have suggested that P700 redox state is not a good indicator of PSI turnover. An alternative method was adopted by Clarke and Johnson (2001), which examined the kinetics of re-reduction of P700 following a light–dark transition. It has been pointed out that this method might be inaccurate because of the partitioning of electrons within the electron transport chain (Sacksteder and Kramer 2000); however, recent data from Golding et al. (2005) have shown that this is only likely to be a problem when the chain is substantially reduced.

Comparison of the light response of PSI and PSII electron transport show that, in both species and all conditions, PSII is saturated at a lower irradiance than PSI. This conclusion is independent of the method used to estimate PSI electron flow. From this we can conclude that in both species, cyclic electron flow operates at high light. The light response of NPQ in both species was found to parallel the rise in PSI photochemistry, with NPQ continuing to rise after PSII turnover has saturated. This is consistent with the idea that cyclic flow around PSI is responsible for the generation of  $\Delta\text{pH}$  to support NPQ.

Regulation of the  $g_{\text{ETC}}$ , probably through regulation of the activity of the cytochrome  $b_6f$  complex, has been discussed as a mechanism that controls the redox poise of the chloroplast stroma and so limits photoreduction of  $\text{O}_2$  on the PSI acceptor side. Both species studied here appear to show some such regulation, although this is most pronounced in *D. antarctica*. In this plant, there is a clear effect of irradiance, with  $g_{\text{ETC}}$  falling with rising irradiance, as has previously been seen in *Silene dioica* (Ott et al. 1999). A lowering of oxygen concentration increased this effect, although this is not reflected in overall flux through the reaction centre. Two models have been discussed that explain the regulation of electron flow. A low lumen pH is known to slow oxidation of PQH2 by the cytochrome  $b_6f$  complex and this may regulate overall electron flow; however, based on measurements of NPQ, there is no indication that lowering oxygen concentration alters  $\Delta\text{pH}$ . Alternatively, Johnson (2003) presented evidence that regulation of electron transport might be

regulated by the stromal redox poise, specifically the extent of reduction of ferredoxin, mediated via thioredoxin. The lower  $g_{\text{ETC}}$  at low oxygen is consistent with this model.

Previous research indicates that both plants rely on a robust  $\text{CO}_2$  assimilation machinery, including high activation state of Rubisco and stromal fructose-1,6-bisphosphatase throughout high light and low temperature conditions (Perez-Torres et al. 2006). Consequently, positive  $\text{CO}_2$  assimilation rates (e.g. 30% of maximum photosynthetic activity at  $0^\circ\text{C}$ ) are observed in Antarctic vascular plants under conditions that are lethal to other plants. Thus,  $\text{CO}_2$  continues to be an important electron sink even under the extreme environmental conditions of the Maritime Antarctic. However, photorespiration rates, which are very similar in both plants, are very susceptible to low temperature, suggesting a contribution as a photoprotective mechanism mainly at moderate temperatures (not shown). Altogether, we present a model of the photoprotective mechanisms involved in the survival of *C. quitensis* and *D. antarctica* in the Antarctica under high light, contrasting the use of the water–water cycle and the extent of NPQ between both vascular plants (Fig. 6).

Data presented here provide a clear indication of diversity in terms of the strategy used by plants to cope with excess light conditions, even when those plants are adapted to very similar environments. The use of oxygen as a sink for excitation energy is clearly a viable strategy; however, this appears to come at the cost of having to invest heavily in antioxidant systems.

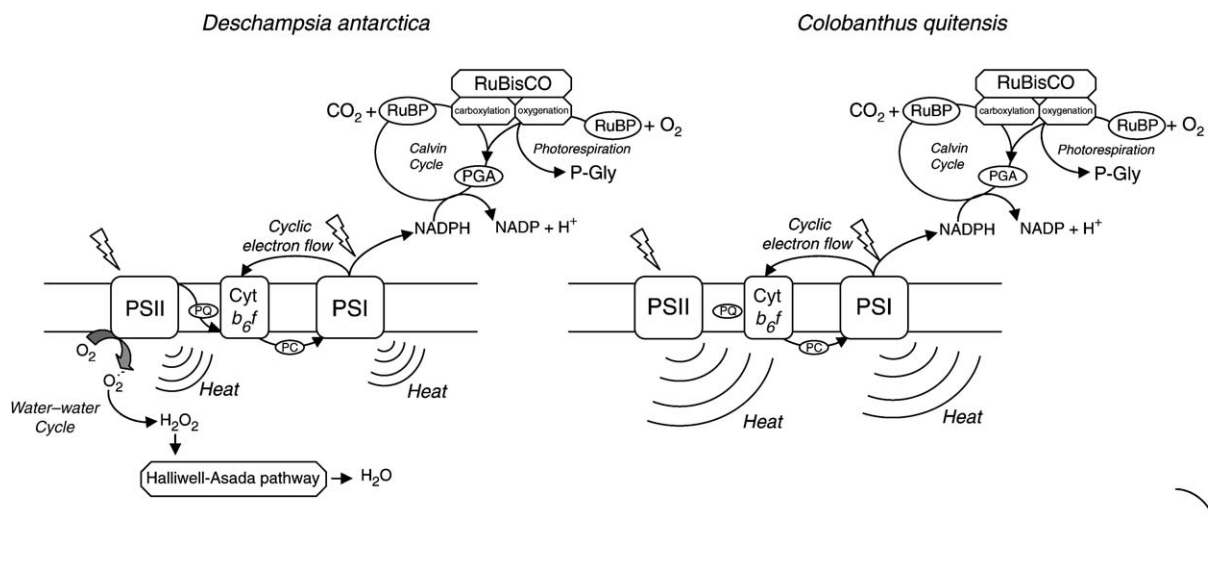


Fig. 6. Proposed model for the contrasting photoprotection mechanisms in Antarctic vascular plants under high light.



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## References

- Aro EM, Suorsa M, Rokka A, Allahverdiyeva Y, Paakkarinen V, Saleem A, Battchikova N, Rintamaki E (2005) Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. *J Exp Bot* 56: 347–356
- 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
- Asada K (2000) The water-water cycle as alternative photon and electron sinks. *Philos Trans R Soc Lond B Biol Sci* 355: 1419–1430
- Badger MR, von Caemmerer S, Ruuska S, Nakano H (2000) Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. *Philos Trans R Soc Lond B Biol Sci* 355: 1433–1445
- Biehler K, Fock H (1996) Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiol* 112: 265–272
- Chen HX, An SZ, Li WJ, Gao HY, Zou Q (2004) Enhancement of the Mehler-peroxidase reaction in salt-stressed *Rumex K-1* leaves. *Acta Bot Sin* 46: 811–818
- Clarke JE, Johnson GN (2001) In vivo temperature dependence of cyclic and pseudocyclic electron transport in barley. *Planta* 212: 808–816
- Cleland RE, Grace SC (1999) Voltammetric detection of superoxide production by photosystem II. *FEBS Lett* 457: 348–352
- Cournac L, Josse EM, Joët T, Rumeau D, Redding K, Kuntz M, Peltier G (2000) Flexibility in photosynthetic electron transport: a newly identified chloroplast oxidase involved in chlororespiration. *Philos Trans R Soc Lond B Biol Sci* 355: 1447–1453
- Edwards JA, Smith RIL (1988) Photosynthesis and respiration of *Colobanthus-quitensis* and *Deschampsia-antarctica* from the maritime Antarctic. *Br Antarct Surv Bull* 81: 43–63
- Finazzi G, Johnson GN, Dall'Osto L, Joliot P, Wollman FA, Bassi R (2004) A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. *Proc Natl Acad Sci USA* 101: 12375–12380
- Flexas J, Medrano H (2002) Drought-inhibition of photosynthesis in C-3 plants: stomatal and non-stomatal limitations revisited. *Ann Bot* 89: 183–189
- Foyer CH, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. *New Phytol* 146: 359–388
- Foyer CH, Vanacker H, Gomez LD, Harbinson J (2002) Regulation of photosynthesis and antioxidant metabolism in maize leaves at optimal and chilling temperatures: review. *Plant Physiol Biochem* 40: 659–668
- Golding AJ, Johnson GN (2003) Down-regulation of linear and activation of cyclic electron transport during drought. *Planta* 218: 107–114
- Golding AJ, Joliot P, Johnson GN (2005) Equilibration between cytochrome f and P700 in intact leaves. *Biochim Biophys Acta-Bioenerg* 1706: 105–109
- Harbinson J, Woodward FI (1987) The use of light-induced absorbency changes at 820 nm to monitor the oxidation-state of P-700 in leaves. *Plant Cell Environ* 10: 131–140
- Havaux M (1994) Temperature-dependent modulation of the photoinhibition-sensitivity of photosystem-II in *Solanum-tuberosum* leaves. *Plant Cell Physiol* 35: 757–766
- Heber U (2002) Irrungen, Wirungen? The Mehler reaction in relation to cyclic electron transport in C3 plants. *Photosynth Res* 73: 223–231
- Horton P, Ruban A (2005) Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. *J Exp Bot* 56: 365–373
- Horton P, Ruban AV, Wentworth M (2000) Allosteric regulation of the light-harvesting system of photosystem II. *Philos Trans R Soc Lond B Biol Sci* 355: 1361–1370
- Ivanov AG, Sane PV, Krol M, Gray GR, Balseris A, Savitch LV, Öquist G, Hüner NPA (2006) Acclimation to temperature and irradiance modulates PSII charge recombination. *FEBS Lett* 580: 2797–2802
- Johnson GN (2003) Thiol regulation of the thylakoid electron transport chain – a missing link in the regulation of photosynthesis? *Biochemistry* 42: 3040–3044
- Khorobrykh SA, Ivanov BN (2002) Oxygen reduction in a plastoquinone pool of isolated pea thylakoids. *Photosynth Res* 71: 209–219
- Khorobrykh S, Mubarakshina M, Ivanov B (2004) Photosystem I is not solely responsible for oxygen reduction in isolated thylakoids. *Biochim Biophys Acta-Bioenerg* 1657: 164–167
- Klughammer C, Schreiber U (1994) An improved method, using saturating light-pulses, for the determination of photosystem-I quantum yield via P700+-absorbency changes at 830 nm. *Planta* 192: 261–268
- Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of Q(A) redox state and excitation energy fluxes. *Photosynth Res* 79: 209–218

- Matsubara S, Chow WS (2004) Populations of photoinactivated photosystem II reaction centers characterized by chlorophyll a fluorescence lifetime in vivo. *Proc Natl Acad Sci USA* 101: 18234–18239
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *J Exp Bot* 51: 659–668
- Moore AL, Albury MS, Crichton PG, Affourtit C (2002) Function of the alternative oxidase: is it still a scavenger? *Trends Plant Sci* 7: 478–481
- Nixon PJ, Barker M, Boehm M, de Vries R, Komenda J (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. *J Exp Bot* 56: 357–363
- Niyogi KK, Li XP, Rosenberg V, Jung HS (2005) Is PsbS the site of non-photochemical quenching in photosynthesis? *J Exp Bot* 56: 375–382
- Oja V, Eichelmann H, Peterson RB, Rasulov B, Laisk A (2003) Deciphering the 820 nm signal: redox state of donor side and quantum yield of photosystem I in leaves. *Photosynth Res* 78: 1–15
- Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. *Planta* 186: 450–460
- Ort DR, Baker NR (2002) A photoprotective role for O<sub>2</sub> as an alternative electron sink in photosynthesis? *Curr Opin Plant Biol* 5: 193–198
- Ott T, Clarke J, Birks K, Johnson GN (1999) Regulation of the photosynthetic electron transport chain. *Planta* 209: 250–258
- Owens TG (1996) Processing of excitation energy by antenna pigments. In: Baker NR (ed) *Photosynthesis and the Environment*. Kluwer Academic Publishers, Amsterdam, the Netherlands, pp 1–23
- Perez-Torres E, Dinamarca J, Bravo LA, Corcuera LJ (2004a) Responses of *Colobanthus quitensis* (Kunth) Bartl. to high light and low temperature. *Polar Biol* 27: 183–189
- Perez-Torres E, Garcia A, Dinamarca J, Alberdi M, Gutierrez A, Gidekel M, Ivanov AG, Huner NPA, Corcuera LJ, Bravo LA (2004b) The role of photochemical quenching and antioxidants in photoprotection of *Deschampsia antarctica*. *Funct Plant Biol* 31: 731–741
- Perez-Torres E, Bascuñan L, Sierra A, Bravo LA, Corcuera LJ (2006) Robustness of activity of Calvin cycle enzymes after high light and low temperature conditions in Antarctic vascular plants. *Polar Biol* 29: 909–916. DOI 10.1007/s00300-006-0131-8
- Polle A (1996) Mehler reaction: friend or foe in photosynthesis? *Bot Acta* 109: 84–89
- Rosso D, Ivanov AG, Fu A, Geisler-Lee J, Hendrickson L, Geisler M, Stewart G, Krol M, Hurry V, Rodermeier SR, Maxwell DP, Huner NP (2006) IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of *Arabidopsis* during steady-state photosynthesis. *Plant Physiol* 142: 574–585
- Ruuska SA, Badger MR, Andrews TJ, von Caemmerer S (2000) Photosynthetic electron sinks in transgenic tobacco with reduced amounts of Rubisco: little evidence for significant Mehler reaction. *J Exp Bot* 51: 357–368
- Sacksteder CA, Kramer DM (2000) Dark-interval relaxation kinetics (DIRK) of absorbance changes as a quantitative probe of steady-state electron transfer. *Photosynth Res* 66: 145–158
- Schroeter B, Olech M, Kappen L, Heitland W (1995) Ecophysiological investigations of *Usnea-antarctica* in the maritime Antarctic. 1. Annual microclimatic conditions and potential primary production. *Antarct Sci* 7: 251–260
- Siebek K, Ghannoum O, Conroy JP, Badger MR, von Caemmerer S (2003) Photosynthetic oxygen exchange in C-4 grasses: the role of oxygen as electron acceptor. *Plant Cell Environ* 26: 1963–1972
- Streb P, Josse E-M, Gallouët E, Baptist F, Kuntz M, Cornic G (2005) Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis*. *Plant Cell Environ* 28: 1123–1135
- Wingler A, Lea PJ, Quick WP, Leegood RC (2000) Photorespiration: metabolic pathways and their role in stress protection. *Philos Trans R Soc Lond B Biol Sci* 355: 1517–1529
- Xiong FS, Ruhland CT, Day TA (1999) Photosynthetic temperature response of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*. *Physiol Plant* 106: 276–286
- Xiong FS, Mueller EC, Day TA (2000) Photosynthetic and respiratory acclimation and growth response of Antarctic vascular plants to contrasting temperature regimes. *Am J Bot* 87: 700–710