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ORIGINAL ARTICLE

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Down-regulation of linear and activation of cyclic electron transport during drought

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Abstract The effects of short-term drought on the regulation of electron transport through photosystems I and II (PSI and PSII) have been studied in *Hordeum vulgare* L. cv. Chariot. Fluorescence measurements demonstrated that electron flow through PSII decreased in response to both drought and CO_2 limitation. This was due to regulation, as opposed to photoinhibition. We demonstrate that this regulation occurs between the two photosystems—in contrast to PSII, PSI became more oxidised and the rate constant for P700 re-reduction decreased under these conditions. Thus, when carbon fixation is inhibited, electron transport is down-regulated to match the reduced requirement for electrons and minimise reactive oxygen production. At the same time non-photochemical quenching (NPQ) increases, alleviating the excitation pressure placed on PSII. We observe an increase in the proportion of PSI centres that are 'active' (i.e. can be oxidised with a saturating flash and then rapidly re-reduced) under the conditions when NPQ is increased. We suggest that these additional centres are primarily involved in cyclic electron transport, which generates the ΔpH to support NPQ and protect PSII.

Keywords Cyclic electron transport · Drought · *Hordeum* (electron transport) · Non-photochemical quenching · ΔpH

Abbreviations A: assimilation rate · Ci: internal CO_2 concentration · ETC: electron transport chain · *g*: stomatal conductance · FR: far red · *k*: pseudo first-order rate constant for the reduction of oxidised P700 · NPQ: non-photochemical quenching · P700: primary electron donor of photosystem I · PSI, PSII: photosystem I, II · qP: proportion of open PSII centres · ROS: reactive oxygen species · ΔpH: pH gradient across the thylakoid membrane · ΦPSII: quantum yield of photosystem II

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Introduction

It is well established that drought inhibits photosynthetic carbon fixation, either through limiting entry of CO_2 into the leaf (stomatal limitation; Cornic 1994) or possibly through directly inhibiting metabolism (non-stomatal limitation; Ortiz-Lopez et al. 1991). This is liable to result in the plant absorbing more light energy than can be consumed through photosynthetic C fixation (for a review, see Smirnoff 1993). Although this excess may be partially dissipated through photorespiration (Haupt-Hertig and Fock 2002), drought still has the potential to cause over-reduction of the electron transport chain (ETC). Such over-reduction leads to an increase in oxidative stress (see Smirnoff 1993).

There are two principle routes for the light-driven production of reactive oxygen species (ROS)—the photoreduction of molecular oxygen by iron sulphur centres on the acceptor side of photosystem I (PSI; referred to as the Mehler reaction or Water Water cycle; Mehler 1951; Asada 1999); and the formation of singlet excited oxygen, through the interaction of molecular oxygen with triplet excited chlorophyll. The former reaction will be favoured under conditions where electron acceptors from PSI (especially FeS centres A, B and X and ferredoxin) are reduced, as will occur if the limiting step in photosynthesis is associated with the Calvin cycle. The latter reaction is particularly associated with the antenna of PSII and is favoured under conditions where the lifetime of excitation in the PSII antenna is increased (i.e. when there is no efficient sink for the excitation energy) or when inhibition of electron flow away from PSII results in a high probability of charge recombination in the PSII reaction centre.

Whilst there are a number of enzymatic processes that scavenge ROS (for a review, see Asada 1999), these require the synthesis of high concentrations of antioxidants and enzymes and so are highly energetically demanding. An alternative strategy, which will place less of a metabolic burden on the plant, would be to avoid the production of ROS in the first place. This might be achieved by regulating photosynthetic electron transport (Ott et al. 1999).

The responses of the ETC to drought are less well characterised than those of C fixation. Many workers have found the ETC to be highly resistant to drought (e.g. Cornic and Briantais 1991; Havaux 1992; Tourneux and Peltier 1995), as indicated, in particular, by the insensitivity of the fluorescence parameter Fv/Fm (Epron and Dreyer 1992; Havaux 1992). However, a number of studies have demonstrated a decrease in the quantum yield of PSII electron transport (ΦPSII; Genty et al. 1989) with increasing water stress (e.g. Giardi et al. 1996; Sanchez-Rodriguez et al. 1997; Loggini et al. 1999). This is suggested to result from a down-regulation of PSII electron transport capacity, as opposed to damage to the PSII reaction centres (Cornic 1994; Osmond 1994)—this decrease in ΦPSII under drought was shown to be fully restored to control levels upon re-watering (Loggini et al. 1999). Associated with decreased ΦPSII is an increase in non-photochemical quenching (NPQ—Osmond 1994), a protective process which dissipates energy as heat.

Ott and co-workers (1999) observed that the conductance of the ETC between PSII and PSI decreased under drought conditions, as indicated by a slowing down of the reduction of oxidised P700, the primary electron donor of photosystem I. They suggested that this regulation functions to limit superoxide formation under stress conditions. Down-regulation of the ETC has previously been observed under conditions of low CO_2 (Harbinson 1994). Although inhibiting electron flow to PSI limits electron flow to O_2, it will also limit the plant's ability to form a pH gradient across the thylakoid membrane (ΔpH) required for NPQ. As NPQ increases to protect PSII, some process must occur that maintains the pH gradient across the thylakoid membrane. Here we describe the effects of CO_2 and drought on electron transport through PSI and PSII in barley leaves. We present evidence that, under drought, cyclic electron transport increases to maintain the ΔpH.

Materials and methods

Plant growth and treatment

Barley (*Hordeum vulgare* L. cv. Chariot) seeds were supplied by PBI Cambridge (UK). Plants were grown in a controlled-environment cabinet (E.J. Stiell, Glasgow, UK) with a photon flux density (PFD) of 300 μmol m^{-2} s^{-1} provided by high-frequency fluorescent strip lights supplemented with tungsten lights, on a 12 h light/12 h dark cycle. The daytime temperature was 20 °C and the nighttime temperature, 16 °C.

Seeds were germinated by standing in moderate light for 5 days on water-saturated tissue paper in a sealed container. The seedlings were then grown hydroponically in phostrogen solution (Phostrogen, Denbighshire, UK; 10 ml phostrogen/5 l water) for a further 7 days. The phostrogen solution was changed 3 times a week. Plants were then placed in 0, 0.025, 0.15, 0.3, 0.5 or 1 M sorbitol (ICN, Basingstoke, UK) solution for 22 h in their normal growth environment. They were dark-adapted for 2 h prior to measurements being made.

Gas exchange, P700 oxidation and chlorophyll fluorescence measurements

Gas-exchange measurements were made using an infra-red gas analyser (IRGA) (CIRAS-1; PP Systems, Herts., UK). The first leaves of three individual plants were clamped into the IRGA cuvette chamber. This enabled the external CO_2 concentration supplied to the leaf to be controlled, whilst measuring assimilation rate, A (μmol m^2 s^{-1}), internal CO_2 concentration, Ci (μl l^{-1}), and stomatal conductance, g (mmol m^2 s^{-1}), calculated as described by von Caemmerer and Farquhar (1981).

Changes in absorbance at 830 nm were used to give a measure of the redox state of the PSI primary donor, P700. Measurements were made using a Walz PAM 101 fluorometer in combination with an ED-P700DW-E emittor–detector unit (Walz, Effeltrich, Germany). Actinic light (1,900 μmol m^{-2} s^{-1}) was supplied by a Volpi Intralux 6000 lamp (Volpi, Schlieren, Switzerland). Light from a Rank Aldis Tutor 2 lamp (Rank Aldis, Japan) was passed through an RG715 far-red (FR) filter (H.V. Skan, Solihull, UK) to provide FR light. All light sources were filtered with Calflex-X heat filters (Balzers, Lichtenstein) to prevent interference with the detector for 830 nm absorbance changes. The actinic light was shuttered using a Uniblitz 14-mm electronic shutter (Vincent Associates, Rochester, NY, USA), controlled by a custom-made shutter controller. Transient changes in absorbance on a millisecond timescale were captured using a data-acquisition board (1700 series; Keithley Metrabyte, Cleveland, OH, USA), fitted in an IBM-compatible computer running laboratory-written software.

Chlorophyll fluorescence measurements were made using a PAM 101 fluorometer in combination with a 101-ED emittor–detector unit (Walz). Saturating pulses of light were supplied by a Volpi Intralux 1500 lamp and were produced using a Uniblitz 14-mm electronic shutter in front of the lamp, controlled by a Model T132 shutter driver/timer (Vincent Associates). All fluorescence signals were recorded using a chart recorder.

Experimental protocol for measurements of fluorescence and absorbance

Calibration of maximum P700 signal size In order to establish the change in the absorbance signal corresponding to maximum oxidation of P700, dark-adapted leaves were illuminated for 10 s with FR light. To control whether this FR light was saturating, a 100-ms flash of white light (7,500 mol m^{-2} s^{-1}) was applied on top of the FR light. The absence of a transient rise in signal size as a result of this flash was taken to indicate that the signal was saturated by the FR light. Repeating this measurement after an experiment gave a signal size that did not differ significantly from the measurement made before illumination. Illuminating leaves at a range of CO_2 concentrations also did not alter the FR-induced signal. No variation in FR signal could be detected between different sorbitol treatments, over and above the normal inter-leaf variation.

Measurements of chlorophyll fluorescence After a 2-min recovery period following the FR illumination, measurements of fluorescence were begun. Measurements of Fo and Fm were made as described elsewhere (Maxwell and Johnson 2000). A 1.2-s pulse of white light, with a PFD of 7,500 μmol m^{-2} s^{-1}, was found to produce a maximal fluorescence signal under all the conditions

used. Following this, the actinic light was switched on and the plant was left for 45 min, to allow the leaf to reach a steady state. After recording the gas exchange, a further 1.2-s pulse of saturating light was given to estimate Fm′ in the light. To measure Fo′, the actinic light was switched off for approx. 10 s. Fluorescence parameters were calculated as described in Maxwell and Johnson (2000).

Measurements of P700^{+} To measure the extent of P700 oxidation induced by the actinic light, changes in absorbance during a 100-ms period of darkness were recorded. This 'dark pulse' was repeated, at 5-s intervals, to give an average signal of 10 accumulations. The signal change induced upon a dark pulse was normalised to the FR-induced signal for the same leaf, to estimate the proportion of P700 that was oxidised under steady-state conditions and could then be rapidly reduced following a transition to darkness. Decay of P700^{+} was found to approximate to a first-order kinetic (see Ott et al. 1999). Fitting data with a mono exponential decay curve yielded a pseudo-first order-rate constant (k). The product of the signal size and the rate constant for the decay was taken as an estimate of the rate of electron transport through PSI (see Fig. 6 and Discussion)

Measurements of active P700 pool and conductance of the electron transport chain In order to establish the relative concentration of "active" PSI centres (defined as centres that can be oxidised by light and are then rapidly re-reduced in a subsequent period of darkness), 100-ms flashes of 7,500 μmol m^{-2} s^{-1} light were applied in addition to the actinic light and the decay in absorbance then followed upon transition to darkness (Klughammer and Schreiber 1994). This intensity was found to be saturating across all conditions used. Under these conditions, application of a flash induced a rapid rise in the absorbance signal, with no decrease during the duration of the flash (not shown). This contrasts with observations at lower actinic irradiances where such flashes are liable to result in transient oxidation and then re-reduction of P700 (Klughammer and Schreiber 1994). The extent of the absorbance change was normalised to the FR-induced absorbance change for the same leaf. The absorbance decay curve under such conditions approximated closely to a first-order reaction and was fitted with a mono exponential curve as above, yielding a rate constant. This was taken as a measure of the conductance of the electron transport chain.

Following the first set of measurements, the CO_2 concentration supplied to the leaf was altered. The plant was then left for 12–15 min to reach a new steady state before subsequent measurements were made. All CO_2 concentrations were measured on each sample, with the CO_2 concentration being first lowered and then increased. No significant hysteresis was observed comparing measurements at the beginning and end of the experiment.

Water content

Plants were weighed at the end of the experiment, dried at 80 °C for 24 h and re-weighed. The water content of leaves was calculated as follows:

$$WC = \frac{(FW - DW)}{DW}$$

where FW = fresh weight (g) and DW = dry weight (g). Values given are plotted relative to the control value for water content, which was 9.67 g (water) g^{-1} (dry matter; Fig. 1).

Results

Subjecting barley to a range of sorbitol concentrations brought about a clear droughting response. Water content decreased across the range of concentrations used, falling by approximately half, per unit dry weight, between 0 and 0.5 M sorbitol (Fig. 1). Plants appeared visibly wilted at the highest sorbitol concentrations.

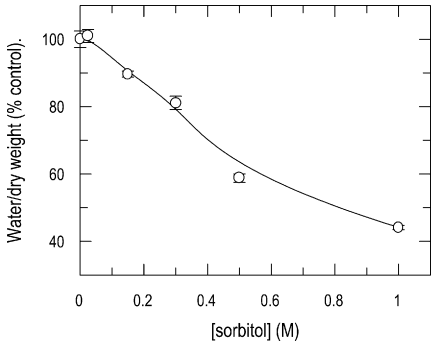

Fig. 1 Water content (expressed as water per unit DW, normalised to the control value of 9.67 g g^{-1}) of barley (*Hordeum vulgare*) plants following 24 h exposure to sorbitol. Means ± SE of at least 4 replicates

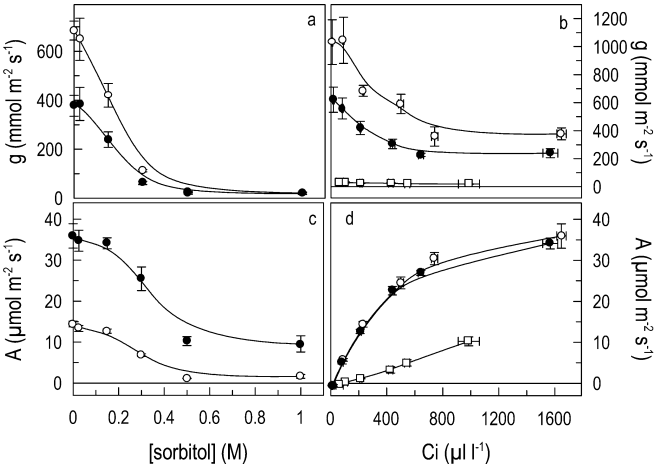

Fig. 2a–d Stomatal conductance (g; **a, b**) and assimilation rate (A; **c, d**) of barley plants subjected to varying degrees of drought and various CO_2 concentrations. **a, c** *Open circles* plants supplied with an external CO_2 concentration of 2,000 μl l^{-1}, *closed circles* 360 μl l^{-1}. **b, d** *Open circles* control plants, *closed circles* 0.15 M sorbitol, *open squares* 0.5 M sorbitol. Means ± SE of at least 4 replicates

Exposure of plants to sorbitol induced stomatal closure, as indicated by the decline in stomatal conductance (Fig. 2a). At the lower sorbitol concentrations used, the stomata were still sensitive to low CO_2 but stomatal conductance became largely independent of external CO_2 at higher concentrations. Below 0.15 M sorbitol, stomatal conductance (although lower than that of the control) increased at low Ci (Fig. 2b). At 0.5 M, sorbitol had the effect of reducing stomatal conductance to almost zero across the entire range of internal CO_2 concentrations.

Due to the limitation on CO_2 entry imposed by drought, the CO_2 concentration within the leaf decreased and CO_2 fixation was reduced (Fig. 2c). At 0.15 M sorbitol, the relationship between assimilation and the estimated internal CO_2 concentration (Ci) was unaffected compared to the control, despite the plants having considerably reduced stomatal conductance (Fig. 2b, d). This suggests that, at this concentration and

below, the only effects of sorbitol on the plant were due to the limitation of CO_2 entry into the leaf, i.e. stomatal-limitation. At higher sorbitol concentrations, however, (0.5 M) in addition to complete stomatal closure (Fig. 2b) the relationship between assimilation and Ci appeared altered. Carbon assimilation could not be restored to control levels by supplying the droughted plants with CO_2 concentrations up to 2,000 μl l^{-1}.

With increasing drought stress, electron transport through PSII was inhibited, as indicated by the drop in PSII quantum efficiency (ΦPSII) (Fig. 3a). Similar down-regulation also occurred in response to low Ci (Fig. 3b). However, in contrast to the effect of drought on CO_2 fixation, a sorbitol concentration of 0.3 M was required to bring about down-regulation of PSII and, even then, at this level of drought the effect was reversible by increasing CO_2 supply (Fig. 3a). At higher levels of drought, i.e. 0.5 M sorbitol, PSII electron transport was inhibited and independent of CO_2 concentration (Fig. 3b).

This inhibition of ΦPSII was partly due to a decrease in the proportion of open PSII centres (qP). The response of qP to both drought and low CO_2 corresponds very closely to that of ΦPSII.

The maximum efficiency of the open PSII centres in the light (Fv′/Fm′) decreased at higher levels of drought (Fig. 3e) and at low Ci (Fig. 3f), and as such contributed to the decrease in ΦPSII. However, dark level Fv/Fm remained constant across the range of drought conditions measured (not shown).

As a consequence of the decrease in demand for electrons by CO_2 fixation, it might be expected that the intersystem ETC would become fully reduced. However, measurements of the redox state of PSI (Fig. 4a, b) show that, with increasing drought and decreasing Ci, PSI became more oxidised. At 0.5 M sorbitol, PSI was considerably more oxidised than the control, although, at low Ci, all plants, regardless of drought, tend to converge to a maximum level of oxidation (Fig. 4b).

The increase in the proportion of oxidised PSI can be explained by measurements of the conductance of the electron transport chain. We have measured this conductance as the rate constant for the decay in absorbance at 830 nm, from a saturating flash to darkness (see Materials and methods). The conductance of the ETC decreased in response to drought (Fig. 4c). At the highest level of drought imposed, under ambient external CO_2, conductance decreased to 45% of the control value. Under moderate drought this down-regulation can be partially reversed by increasing CO_2 (Fig. 4b, c). However, at higher levels of drought, increasing CO_2 up to external concentrations of 2,000 μl l^{-1} was insufficient to reverse this effect.

In addition to an increase in the proportion of oxidised PSI, the proportion of PSI centres that are measured to be active (i.e. can be oxidised by a flash and are then re-reduced on a millisecond timescale) also increased in response to drought (Fig. 4e) and low Ci (Fig. 4f). Again, this increase was not brought about

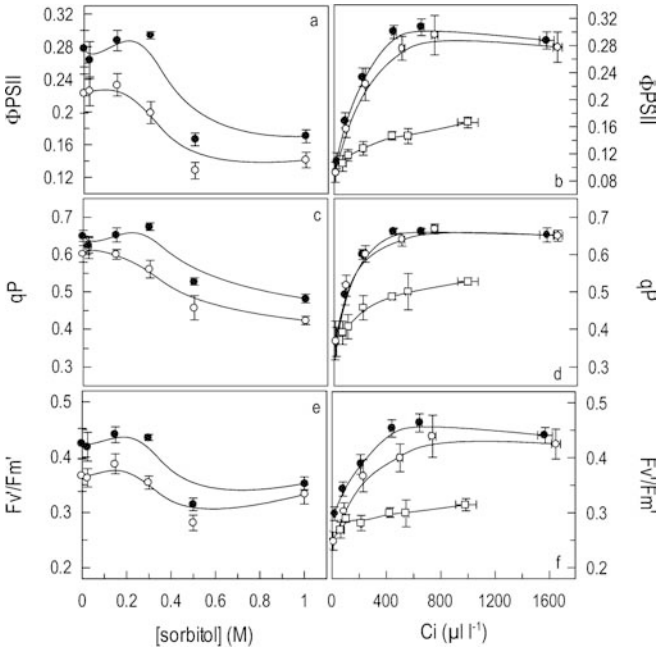

Fig. 3a–f Photochemical quenching parameters in barley plants subjected to varying degrees of drought (**a, c, e**) and various CO_2 concentrations (**b, d, f**). **a, c, e** *Open circles* plants supplied with an external CO_2 concentration of 2,000 μl l^{-1}, *closed circles* 360 μl l^{-1}. **b, d, f** *Open circles* control plants, *closed circles* 0.15 M sorbitol, *open squares* 0.5 M sorbitol. Means ± SE of at least 4 replicates

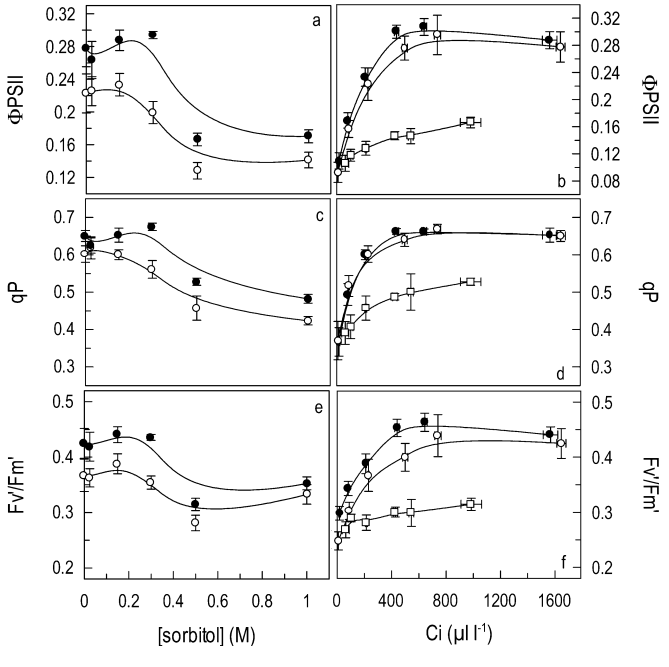

Fig. 4a–f PSI parameters in barley plants subjected to varying degrees of drought (**a, c, e**) and various CO_2 concentrations (**b, d, f**). **a, c, e** *Open circles* plants supplied with an external CO_2 concentration of 2,000 μl l^{-1}, *closed circles* 360 μl l^{-1}. **b, d, f** *Open circles* control plants, *closed circles* plants subjected to 0.15 M sorbitol, *open squares* 0.5 M sorbitol. Means ± SE of at least 4 replicates

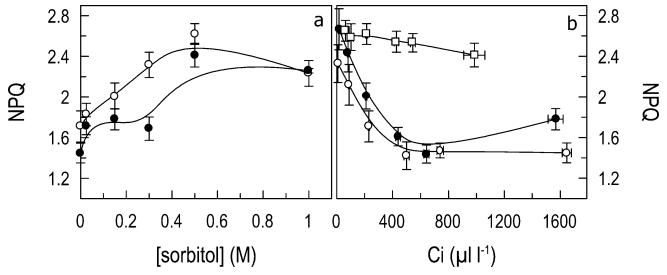

Fig. 5a, b Non-photochemical quenching in barley plants subjected to varying degrees of drought (**a**) and various CO_2 concentrations (**b**). **a** *Open circles* plants supplied with an external CO_2 concentration of 2,000 µl l^{-1}, *closed circles* 360 µl l^{-1}. **b** *Open circles* control plants, *closed circles* 0.15 M sorbitol, *open squares* 0.5 M sorbitol. Means ± SE of at least 4 replicates

until 0.3 M sorbitol and at this level of drought the effect was reversible by supplying saturating external CO_2 (Fig. 4e). At saturating CO_2, under control conditions, we measured only 68% of PSI centres as being active (Fig. 4f). At 0.5 M sorbitol this increases to 85%. At low Ci, all plants approach 100% active PSI centres.

With increasing drought and decreasing Ci, NPQ increased up to a maximum level of 2.6 (Fig. 5a, b). At 2,000 µl l^{-1} external CO_2, NPQ was considerably higher at 0.5 M sorbitol than in the control, being close to the maximum value obtained (Fig. 5b). Plants subjected to 0.15 M sorbitol tended towards the same maximum value as 0.5 M sorbitol at the lowest Ci. Control plants also increased NPQ at low Ci but to a lesser extent.

Discussion

Consistent with previous findings (e.g. Cornic 1994), PSII electron transport was observed to decrease under conditions of drought. This was due to a decrease in qP and down-regulation of PSII (reversible NPQ), rather than photoinhibition, as dark-level Fv/Fm was maintained. Whilst a number of studies have considered PSII, the effects of drought on PSI are less well characterised. In contrast to PSII, with increasing drought, the primary donor of PSI, P700, became progressively more oxidised. Decreasing the demand for reductant by the Calvin cycle might be expected to cause both PSII and PSI to become more reduced. Under such conditions there should be an 'excess' of electrons within the ETC, as these are not being fully consumed in carbon fixation. The observation that P700 becomes more oxidised indicates that regulation is occurring between PSII and PSI. We have demonstrated this to be the case—the conductance of the ETC decreased in response to low CO_2 and drought. The most likely point for this regulation is the cytochrome b_6f complex (Cyt b_6f), as this is generally accepted as the rate-limiting step in the chain (Stiehl and Witt 1969; Haehnel 1984; Heber et al. 1988). The supply of electrons to PSI is regulated to the point where, even though CO_2 fixation is inhibited, electrons are still being fed into the Calvin cycle at a rate that matches their arrival at PSI. Under moderate drought, this down-regulation can be reversed by increasing external CO_2 (Fig. 4b, c) and so is due to stomatal limitation. At higher levels of drought, increasing external CO_2 up to 2,000 µl l^{-1} had little effect. Estimates of Ci under such conditions are, however, liable to be inaccurate, due to errors in estimating stomatal conductance (Boyer et al. 1997) distorting the apparent relationship between assimilation and Ci.

The mechanism by which electron transport is down-regulated is unclear. Given that the decrease in conductance of the ETC correlates with an increase in NPQ, this could be assigned to "photosynthetic control", the inhibition of the cytochrome b_6f complex by low pH in the lumen. If this were occurring, however, the lumen pH would have to fall to a level where it directly inhibits PSII (see Discussion in Ott et al. 1999). An alternative suggestion is that regulation involves a system of redox sensing (Ott et al. 1999), possibly via a thiol-linked mechanism (Johnson 2003). Whichever of these mechanisms proves to be correct, the effect of drought on electron transport appears not to be direct but to operate via the inhibition of carbon metabolism—the relationship between gas exchange and electron transport was constant regardless of the severity of the droughting regime (not shown).

By down-regulating electron transport to match the demands of carbon fixation, electron transport to oxygen and thus ROS production, will be minimised. The effect of this is to increase the degree of reduction of the PSII acceptor side, increasing the risk of photoinhibition and singlet-oxygen formation. Consistent with previous studies, we observed that, under conditions when PSII is under extra pressure, there is an increase in NPQ (Fig. 5). This increase in NPQ can largely be attributed to high-energy-state quenching (qE), as shown by the lack of hysteresis in measurements of NPQ versus Ci and by the rapid recovery of Fv/Fm following illumination (not shown). Induction of NPQ at low Ci and under drought alleviates the excitation pressure on PSII. The question therefore arises as to what process generates the pH gradient needed to support this increase in NPQ.

Under the conditions where NPQ increased, we observed that there was an increase in the proportion of PSI centres that could be oxidised with a saturating flash. Therefore, at low Ci and under drought, there are a proportion of centres that appear to be 'switched on', changing from a state where they do not turnover rapidly to one where they do. Thus, although each individual PSI centre is turning over more slowly, the total pool of centres that are active is increased. We suggest that these 'additional' centres are involved in cyclic electron transport and that this process generates the ΔpH required to support NPQ. Whilst a number of studies have suggested that cyclic electron transport may be important in generating a high ΔpH under low CO_2 (e.g. Heber et al. 1992; Cornic et al. 2000), to our knowledge an increase in the active pool of PSI centres under such conditions has not previously been reported.

One of the major problems associated with examining PSI electron transport is the lack of a reliable technique to measure this. A number of studies have estimated relative PSI electron transport rate as the proportion of reduced centres multiplied by the light intensity (Harbinson and Woodward 1987; Weis et al. 1987). There are a number of limitations to this method. Firstly, it makes the assumption that PSI trapping efficiency is constant. Given the large changes that occur in the PSII trapping efficiency associated with NPQ, this could well be incorrect. Also, the assumption is made that the trapping efficiencies of oxidised and reduced P700 are identical, an assumption based on measurements of excitation lifetimes in the PSI antenna (Nuijs et al. 1987). However, lifetime measurements were made on isolated PSI centres in the presence of detergent and the lifetimes measured may well have been determined by processes other than quenching by P700. A further problem with this approach is that it assumes that all centres with reduced P700 are capable of charge separation. If the acceptor side of the PSI reaction centre is reduced, this may not be the case. Klughammer and Schreiber (1994) introduced a method that overcomes this problem, whereby the pool of 'active reduced' PSI centres is taken to be the difference between a flash-induced signal and the signal recorded following a light–dark transition.

If we accept that the proportion of reduced but oxidisable P700 gives an estimate of PSI quantum efficiency, comparison of this parameter with the quantum efficiency of PSII (ΦPSII) indicates that PSI efficiency is maintained relative to that of PSII (Fig. 6a)—in other words PSI turnover falls less than that of PSII, implying, that cyclic electron transport is occurring.

An alternative approach to measuring PSI turnover is to examine the re-reduction kinetics of P700. $P700^+$ decays, following an actinic light to dark transition, with a pseudo first-order kinetic. The product of the rate constant for this decay and the amount of oxidised P700 should give a measure of PSI turnover (Clarke and Johnson 2001). There are problems with this approach

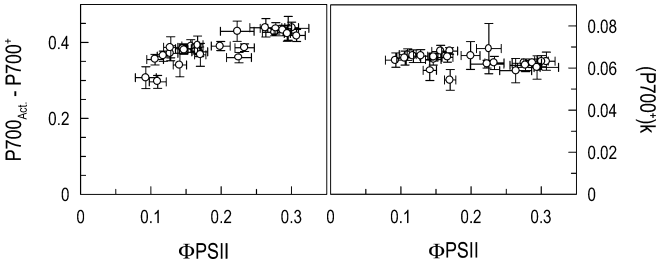

Fig. 6a, b Relationship between different estimates of PSI electron transport rate, and ΦPSII. $P700_{Act.}-P700^+$ represents the difference between the signal change following a transition from saturating flash to darkness and that following a transition from actinic light to darkness (**a**). $(P700^+)k$ represents the proportion of oxidised P700 in actinic light multiplied by the rate constant for re-reduction of $P700^+$ (**b**). Data points on both **a** and **b** represent the combination of all data points collected, i.e. all three sorbitol and both CO_2 concentrations. Means ± SE of at least 4 replicates

(Sacksteder and Kramer 2000), due to portioning of electrons within the high potential part of the ETC (i.e. from the Rieske FeS protein to P700). However, as is apparent from data in Sacksteder and Kramer (2000; Fig. 6) this is only a significant problem under conditions where the chain is largely reduced—at high irradiances, the reduction kinetics of cytochrome *f* cease to have a significant effect on the flux through the chain. Hence, under the conditions used here, such errors will be small. PSI turnover estimated in this way was also maintained under conditions when PSII turnover fell (Fig. 6b).

The observation of an increase in the proportion of PSI centres that are 'active' at low Ci and under drought raises the question as to the nature of these extra centres. Klughammer and Schreiber (1994) suggested that centres would be 'inactive' when their acceptor side was reduced. However, we observe that the total pool of active centres (i.e. centres that can be oxidised and then re-reduced) actually increases under conditions where we would expect the PSI acceptor side to be most vulnerable to over-reduction. It is possible that the 'inactive' centres we have measured appear as such because they do not have access to an efficient electron acceptor. As such, they may undergo charge separation but this would result in rapid recombination and they would not accumulate in the oxidised state. An alternative suggestion is that centres are measured as inactive if they are not readily re-reduced following a light–dark transition. In the dark, P700 will be reduced in all centres and FR illumination will induce the oxidation of all P700 (the FR-induced signal was found to be insensitive to drought and low CO_2, data not shown). Under optimal conditions, the pool of 'inactive' PSI centres will be oxidised by the light but will only slowly re-reduce (second time scale or longer). These will not be observed in our measurements, which are on a millisecond time scale. At low CO_2 and under drought, these centres become 'active', i.e. they gain access to an efficient electron donor. We propose that these centres are primarily or wholly involved in cyclic electron transport.

The organisation of photosynthetic complexes in the thylakoid membrane has received much attention (Anderson 1992; Albertsson 1995; Andreasson et al. 1988). Whilst PSII is primarily located in the appressed regions, PSI is found in the non-appressed part of the thylakoid (Andreasson et al. 1988) and cyt b_6f is spread throughout the membrane (Wollenberger et al. 1994). Anderson (1992) suggested that there are three membrane domains: appressed grana, grana margins and non-appressed stromal regions. Thus the pools of PSI and cyt b_6f complexes located at the grana margins (close to PSII complexes in the appressed grana) are primarily involved in linear electron flow, whereas those pools located in the non-appressed regions are involved in cyclic electron transport (Anderson 1992). According to the model proposed here, it seems likely that the PSI centres located in the non-appressed regions are inactive under optimal physiological conditions, but under

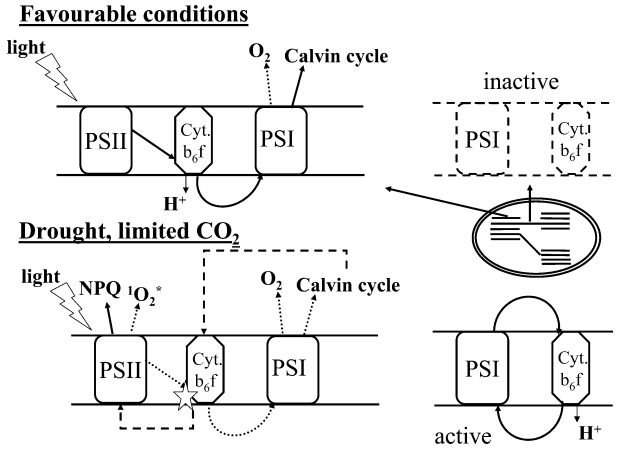

Fig. 7 Model of regulation of electron transport under drought and low CO_2. Under favourable conditions, electrons are passed through the ETC and are consumed in the Calvin cycle, thus minimising electron flow to O_2. Under conditions where the Calvin cycle is inhibited (i.e. drought and low CO_2), electron transport is down-regulated to minimise electron flow to O_2. At the same time, a separate pool of PSI centres involved in cyclic electron transport becomes active, generating a pH gradient to support NPQ, so limiting singlet-oxygen formation at PSII

conditions when electron transport is down-regulated (i.e. drought) they become active to generate a pH gradient to support NPQ and thus protect PSII. Alternatively, it is possible that rather than distinct separate pools, PSI might organise itself into supercomplexes in order for cyclic electron transport to occur, as suggested by Joliot and Joliot (2002).

Recently, an NPQ mutant has been identified that appears to be deficient in cyclic electron transport (Munekage et al. 2002). This observation, if supported by clearer data on the absence of cyclic electron transport, is consistent with the role of this process suggested here. An alternative model explaining the increase in NPQ at low CO_2 concentrations has also recently been published (Kanazawa and Kramer 2002), in which ΔpH is suggested to vary due to a change in the permeability of the thylakoid ATPase, possibly regulated by phosphate. Whilst these two models are clearly not mutually exclusive, there is at present no direct evidence for such regulation of the ATPase from in vitro studies. A slowing down in ATPase activity could equally be explained by substrate limitation (possibly of Pi), which would not per se lead to an increase in ΔpH.

In summary, our results indicate that plants are able to protect themselves from ROS production under drought conditions through regulation of the photosynthetic ETC (Fig. 7). Down-regulation of electron transport between PSII and PSI allows the production of reducing potential by PSI to be matched to the demands of C-fixation. At the same time, an activation of cyclic electron transport around PSI increases ΔpH, so activating NPQ and limiting ROS productions in the PSII reaction centres.

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