CHAPTER 8

Modes of electron transport chain function during stress: Does alternative oxidase respiration aid in balancing cellular energy metabolism during drought stress and recovery?

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Introduction

Photosynthesis and respiration comprise the core pathways of primary carbon and energy metabolism in plants, providing the ATP, reducing power [NAD(P)H] and carbon intermediates essential for growth and development. Photosynthesis in the chloroplast harvests light energy and transforms it to usable chemical energy in the form of ATP and NADPH. These are used by the Calvin cycle to produce carbohydrate via the assimilation of atmospheric CO_2 (Stitt *et al.*, 2010; Rochaix, 2011; Foyer *et al.*, 2012). Mitochondrial respiration converts the chemical energy stored in carbohydrate back to ATP and NAD(P)H, thus providing these usable forms of energy for numerous other growth and maintenance processes (Fernie *et al.*, 2004; McDonald and Vanlerberghe, 2006; Plaxton and Podestá, 2006; Millar *et al.*, 2011; Tcherkez *et al.*, 2012).

A defining feature of both chloroplast and mitochondrial metabolism is the presence of specialized membrane systems that are largely responsible for the above energy transformations. These membranes house electron transport chain (ETC) components that allow for step-wise electron transfer reactions. In the case of the thylakoid membrane system of the chloroplast, this step-wise process ultimately transfers electrons from H_2O to $NADP^+$, producing O_2 and NADPH. In the case of the inner mitochondrial membrane, this step-wise process transfers

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electrons from NAD(P)H to O_2, producing H_2O and $NAD(P)^+$. Further, electron transport in each organelle is coupled to proton translocation across the respective membrane. In each case, this generates a proton motive force used by a membrane-localized ATP synthase to generate ATP from ADP and P_i. It should be emphasized that continued electron transport in either membrane system is therefore dependent upon both the availability of a terminal electron acceptor (principally $NADP^+$ and O_2 in the chloroplast and mitochondrion, respectively) and upon the availability of ADP and P_i.

Imbalances in energy metabolism

Both photosynthetic and respiratory metabolism can experience energy imbalances, when there is a mismatch between rates of synthesis and rates of utilization of ATP and/or NAD(P)H. Such imbalances can have broad consequences for plant productivity and performance (De Block and Van Lijsebettens, 2011; Kramer and Evans, 2011). In the chloroplast such an imbalance is perhaps most likely to occur when the use of ATP and NADPH by the Calvin cycle does not keep pace with the harvesting of light energy by the thylakoid membranes. This can result in excess 'excitation energy' that can damage photosynthetic components, such as through the generation of reactive oxygen species (ROS). Similarly, in the mitochondrion, an imbalance could arise when the rate of ATP turnover for growth and maintenance processes is not keeping pace with the metabolism of carbohydrate and oxidation of NAD(P)H. Plants are perhaps most susceptible to imbalances in energy metabolism during periods of abiotic stress such as drought, salinity, nutrient deficiency and temperature extremes (Baena-González and Sheen, 2008; Hüner *et al.*, 2012; Suzuki *et al.*, 2012). First, such stresses can perturb metabolism such as by the disruption of enzymes and membrane processes. Such disruption can differentially impact energy-producing and energy-consuming steps within metabolism. Second, such stresses often dramatically slow growth, a major energy sink in some tissues, while at the same time eliciting cell- and tissue-specific acclimation responses that may be quite energy-intensive.

Both chloroplasts and mitochondria have the potential to experience energy imbalances and for these imbalances to be manifest at the level of their ETC. In this case, individual electron carriers may become overly reduced or oxidized, depending upon the rate of upstream and downstream processes. An important consequence of over-reduction of ETC components is that it can increase the rate of side reactions that result in the generation of excessive ROS (Møller, 2001; Apel and Hirt, 2004; Murphy, 2009; Vass, 2012). Specific components of the ETC are most susceptible to such side reactions. In the chloroplast, single electron leak to O_2 at the acceptor side of photosystem I (PSI) produces superoxide (O_2^-), while in the mitochondrion Complexes I and III are the most likely sites of O_2^- formation. Each organelle contains superoxide dismutase (SOD) isoforms

(FeSOD and CuZnSOD in the chloroplast; MnSOD in the mitochondrion) able to convert the O_2^- to another ROS, H_2O_2. If these ROS are not effectively scavenged, they can give rise to a more damaging ROS, the hydroxyl radical. The chloroplast ETC can also give rise to singlet excited oxygen due to over-reduction at photosystem II (PSII) (Vass, 2012). Since ROS have the potential to damage macromolecules and other cell components, it is important to minimize their generation by preventing over-reduction of the ETC, and also by maintaining effective ROS-scavenging systems throughout the cell (Møller, 2001; Apel and Hirt, 2004; Møller *et al.*, 2007; Foyer and Noctor, 2009).

Some ROS species, particularly H_2O_2, are important signal molecules in the control of diverse cell processes (Apel and Hirt, 2004; Foyer and Noctor, 2009; Miller *et al.*, 2010; Cvetkovska *et al.*, 2013). This may include a role as a retrograde signal from organelle to nucleus, acting to control the expression of nuclear genes encoding organelle proteins. Hence, some minimal level of ROS generation by chloroplast and mitochondrial ETC's is likely important to retain this signalling role. Therefore an over-oxidation of key ETC components may be as detrimental as over-reduction. This has led to the concept of physiological redox poising in which specific ETC components likely have optimal reduction states that support both their metabolic and signalling functions (Foyer and Shigeoka, 2011; Juszczuk *et al.*, 2012; Pfalz *et al.*, 2012; Scheibe and Dietz, 2012; Schwarzländer and Finkemeier, 2013). Examples exist in which genetic manipulation of ETC components has been shown to have a relatively minor impact on overall energy metabolism, but is nonetheless found to dramatically alter gene expression, development and/or growth (Noctor *et al.*, 2004; Giraud *et al.*, 2008; Liu *et al.*, 2009; Yoshida *et al.*, 2011). It is possible that changes in the redox poise of particular ETC components, while not greatly perturbing energy metabolism, is nonetheless acting as a signal in the regulation of these higher level processes. This signalling function may act via changes in ROS generation at the ETC or by some other unknown mechanism deriving from the change in ETC composition.

More recently, the generation of reactive nitrogen species (RNS) has been linked to mitochondria (Modolo *et al.*, 2005; Poyton *et al.*, 2009; Gupta *et al.*, 2010). These include nitric oxide (NO) and peroxynitrite, the product of a reaction between O_2^- and NO. The generation of NO by the plant ETC is poorly understood but likely involves single electron leak from complex III and/or IV to nitrite (Poyton *et al.*, 2009; Cvetkovska and Vanlerberghe, 2012). Like ROS, RNS such as NO have been shown to act as signalling molecules in numerous plant processes, and may act in conjunction with ROS (Baudouin, 2011; Molassiotis and Fotopoulos, 2011; Signorelli *et al.*, 2013).

Drought is an excellent example of a common and widespread abiotic stress that has dramatic impacts on carbon and energy metabolism (Lawlor and Tezara, 2009; Pinheiro and Chaves, 2011), as well as on the production and scavenging of ROS (Cruz de Carvalho, 2008; Miller *et al.*, 2010). Leaves respond to drought

by closing their stomata, a means to reduce transpirational water loss. However, stomatal closure also restricts CO_2 diffusion into the leaf, which can result in steep declines in CO_2 assimilation. Under these conditions, a strong imbalance will develop between light energy absorption by the thylakoid membranes and metabolic energy utilization by the stromal Calvin cycle enzymes. Exacerbating this imbalance will be a strong curtailing of growth (an early response to water deficit), a major consumer of metabolic energy.

Chloroplasts and mitochondria appear to have a range of processes to buffer against the development of energy imbalances during stress. The next two sections below provide brief descriptions of some of these strategies, particularly at the ETC level. These sections also discuss current knowledge regarding the strategies that may be most prevalent during drought.

Strategies to combat energy imbalances in the chloroplast electron transport chain

To buffer against energy imbalances, chloroplasts have a number of means by which the electrons derived from water-splitting and resulting in the release of O_2 may be transferred back to O_2. First, the thylakoid membrane includes a protein termed the plastid terminal oxidase (PTOX) that directly catalyses the oxidation of plastoquinol and reduction of O_2 to H_2O (McDonald *et al*., 2011). In general, the amount and maximum activity of PTOX appear quite low relative to overall rates of photosynthetic electron flow. Nonetheless, a number of studies have shown that the protein is induced under stress conditions, suggesting that it may represent a significant alternate electron sink in some circumstances (Stepien and Johnson, 2009; Ivanov *et al*., 2012; Laureau *et al*., 2013). To our knowledge, the significance of PTOX as an electron sink during drought stress has not been critically evaluated, although it has been reported that tobacco PTOX transcript increased under severe drought (Wang and Vanlerberghe, 2013) as did a measure of maximal PTOX activity in isolated thylakoids from *Hibiscus rosa-sinensis* and from *Rosa meillandina* (Muñoz and Quiles, 2013; Paredes and Quiles, 2013).

A second means to transfer electrons in the chloroplast ETC back to O_2 and producing H_2O is via the so-called Mehler reaction, which is in fact a process with ROS as intermediates (Asada, 1999). The Mehler reaction is initiated by the leak of single electrons from PSI to O_2 producing O_2^-. The O_2^- is then converted by SOD to H_2O_2 which is then reduced to H_2O by ascorbate peroxidase, using ascorbate as electron source. The oxidized ascorbate is then converted back to its reduced form by NADPH or ferredoxin. While the Mehler reaction might be considered simply a ROS-scavenging pathway to deal with electron leak at PSI, it is possible that the reaction can be advantageous in terms of balancing energy needs since it not only acts as a sink for PSII-derived electrons, but also allows

for the generation of extra ATP relative to NADPH. This is similarly the case with PTOX which, while acting as an electron sink, also supports the generation of ATP. There remains uncertainty whether the Mehler reaction is a significant or minor electron sink during drought (Biehler and Fock, 1996; Badger *et al*., 2000). A recent study suggests that the capacity of the Mehler reaction (and PTOX) to act as alternate electron sinks may be greater in gymnosperms than angiosperms (Shirao *et al*., 2013), while most studies of these pathways during drought have examined angiosperms.

A third means to consume O_2 in the chloroplast during photosynthesis is via photorespiration, initiated when Rubisco oxygenates (rather than carboxylating) ribulose bisphosphate. This generates 3-phosphoglycerate and 2-phosphoglycolate, the latter of which is metabolized in the chloroplast and peroxisome (with glycolate and glyoxylate as intermediates) to produce glycine (Foyer *et al*., 2009; Bauwe *et al*., 2012). The glycine is then metabolized in the mitochondrion to serine, which is then transferred to the peroxisome and converted to glycerate, with hydroxypyruvate as an intermediate, and with consumption of NADH. Glycerate is then converted to 3-phosphoglycerate in the chloroplast, for use by the Calvin cycle. Conversion of glycine to serine in the mitochondrion involves glycine decarboxylase (GDC), in a reaction that also produces CO_2, NH_3 and NADH (thus balancing the NADH requirement in the peroxisome). Refixation of the CO_2 and NH_3 by the chloroplast requires the consumption of ATP and NADPH, and thus photorespiration acts as a net energy sink. During drought, stomatal closure decreases the ratio of CO_2 to O_2 at Rubisco, favouring the oxygenase reaction. For this reason, it is well accepted that the rate of photorespiration relative to that of CO_2 assimilation increases under drought. However, the absolute rate of photorespiration under drought is more controversial and is likely dependent upon species and drought severity (Biehler and Fock, 1996; Cornic and Fresneau, 2002; Noctor *et al*., 2002; Guan and Gu, 2009; Abogadallah, 2011). This rate may be slightly increased, unchanged or even declined relative to that seen in well-watered plants, suggesting that the path, while certainly active during drought, may not represent much greater an absolute energy sink than under well-watered conditions, particularly as drought severity increases (Lawlor and Tezara, 2009).

As outlined earlier, there remains uncertainty regarding absolute rates of PTOX, the Mehler reaction and photorespiration as electron sinks during drought. Undoubtedly this is due in part to the technical challenges associated with distinguishing between these O_2-consuming processes in the light. What is clear is that these paths collectively become of increased proportional significance during drought, relative to CO_2 assimilation. In addition to these alternate paths of O_2 consumption, chloroplasts may also utilize other related strategies to combat energy imbalances in their ETC during drought. Four will be briefly highlighted here: cyclic electron transport (CET), down-regulation of linear electron transport (LET), non-photochemical quenching (NPQ), and metabolite shuttles.

Electron flow from H_2O to $NADP^+$ in the thylakoid membrane system is referred to as LET. However, another route(s) of electron transport, referred to as CET, is also possible. While different specific routes of CET have been described, their defining feature is that electrons beyond PSI are cycled back to the plastoquinone pool for transport again through cytochrome (cyt) b_6f (Johnson, 2011). This electron flow generates additional proton motive force for ATP synthesis, but without concomitant generation of NADPH. In this way, CEt alters the stoichiometry between ATP and NADPH synthesis. Changes in the rate of CET could buffer against energy imbalances developing in either or both of these metabolic pools. Nonetheless, this strategy is constrained by the fact that changes in the rate of CET can only have opposing impacts on rates of ATP and NADPH synthesis. By promoting the generation of the pH gradient across the thylakoid membrane, CEt also supports the activation of NPQ (Miyake *et al.*, 2004), another mechanism to combat energy imbalance in the chloroplast (see later). Partitioning of electrons between LET and CET appears to be controlled by the reduction state of the chloroplast pyridine nucleotide pool, with increased NADPH favouring CET, perhaps by promoting the formation of a CET complex (Joliot and Johnson, 2011). Interestingly, a study has shown that the slow growth phenotype of mutant plants defective in CET can be alleviated by mutation of PTOX (Okegawa *et al.*, 2010). This may indicate that the redox poise of the plastoquinone pool, as determined by an interplay of these pathways is critical to plant growth and development. There is strong evidence that CET becomes more prevalent during drought (Golding and Johnson, 2003; Kohzuma *et al.*, 2009), an indication that drought does increase the reduction state of the chloroplast stroma.

Beside the up-regulation of CET during drought, there is strong evidence that LET between PSII and PSI is actively down-regulated during drought (Golding and Johnson, 2003; Kohzuma *et al.*, 2009). The details of this down-regulation are not well understood but likely occur at the level of the cyt b_6f complex, which is usually regarded as the rate-limiting step in photosynthetic electron flow. Generally, there is evidence that regulation of cyt b_6f occurs in response to a low pH of the thylakoid lumen and/or a high stromal NADPH (Hald *et al.*, 2008; Rott *et al.*, 2011). This down-regulation of LET is likely important in preventing over-reduction at PSI.

Another major mechanism available to the chloroplast to achieve energy balance is to directly dissipate excess light energy absorbed at PSII in the form of heat. This heat dissipation is referred to as NPQ and the main mechanisms to increase NPQ occur in response to low lumen pH (de Bianchi *et al.*, 2010; Ruban *et al.*, 2012). Low lumen pH promotes the synthesis of the carotenoid zeaxanthin, as well as the protonation of the PSII-related protein PsbS. While all of the molecular details regarding how these changes lead to increased energy dissipation are still being elucidated, the key factor is that these changes result in a re-organization of the supercomplex consisting of PSII and light-harvesting

complex II, resulting in an increased dissipation of the absorbed light energy as heat. Numerous studies have shown that drought stress increases NPQ as a central mechanism of photoprotection (Golding and Johnson, 2003; Lawlor and Tezara, 2009). This increase in NPQ under drought may be supported by increased CET (see earlier).

Chloroplasts have effective metabolite shuttles for the transfer of excess reducing power to the cytosol (Taniguchi and Miyake, 2012). During drought, when Calvin cycle activity is declined, reductant balance in the organelle could be at least partially achieved by the export of reducing power, for consumption by extra-chloroplastic processes, including mitochondrial electron transport. The two metabolite shuttles capable of reductant export are the malate/oxaloacetate (OAA) shuttle, also known as the malate valve, and the triose phosphate/3-phosphoglycerate shuttle. However, the triose phosphate/3-phosphoglycerate shuttle is likely not active under conditions of low Calvin cycle activity because it is dependent upon Calvin cycle intermediates. Hence, the malate valve is likely the key shuttle system that may contribute to reductant balance under drought stress. The components of the malate valve include a malate/OAA exchanger in the inner chloroplast membrane, a NADP-malate dehydrogenase (MDH) in the stroma and a NAD-MDH in the cytosol. Reduction of OAA to malate in the stroma consumes NADPH. Malate is then delivered to the cytosol in exchange for cytosolic OAA, and malate oxidation back to OAA in the cytosol produces NADH. To our knowledge, plants altered in malate valve activity (Hebbelmann *et al.*, 2012) have not yet been used to directly evaluate the role of this pathway during drought stress and little other information appears available regarding the malate valve during drought.

Strategies to combat energy imbalances in the mitochondrial electron transport chain

Plant mitochondria also have several potential mechanisms by which they could balance energy metabolism at the ETC level during drought. Two of these mechanisms, the uncoupling proteins (UCPs) and the alternate dehydrogenases, will only be briefly described here since their potential role during drought stress has not yet been extensively examined. A third mechanism, involving the alternative oxidase (AOX) will be discussed in more detail, discussing its potential role in buffering against energy imbalances, and evaluating the current evidence for its role in combating drought stress.

As is the case in animals, plants contain a family of mitochondrial UCPs that are members of a larger family of anion carriers. UCPs are integral proteins of the inner membrane that can facilitate the conductance of protons down their electrochemical gradient from inner membrane space to matrix (Vercesi *et al.*, 2006). This proton flow across the membrane occurs at the expense of proton

translocation through ATP synthase and coupled with ATP generation. Hence, UCPs represent an effective means to uncouple carbon metabolism and electron transport from ATP turnover. The proton conductance activity of UCP can be activated by matrix O_2^- in the presence of fatty acids (Considine *et al.*, 2003; Smith *et al.*, 2004). Specifically, O_2^- catalyzes the generation of the lipid peroxidation product 4-hydroxy-2-nonenal, which then activates the proton conductance. This mode of biochemical control appears well-suited to UCP acting as a means to dampen O_2^- generation by the ETC. Over-reduction of the ETC due to high proton motive force would stimulate O_2^- generation, leading to UCP activation. This in turn would reduce the proton gradient and over-reduction of the ETC, thus lowering the rate of ROS generation.

Recently, Begcy *et al.* (2011) showed that overexpression of an *Arabidopsis* UCP in tobacco reduced leaf amounts of H_2O_2 compared to wild-type plants, particularly under drought (actually watering with mannitol) or high salt conditions. This suggests an ability of UCP to dampen ROS generation, particularly during stress. Significantly, the transgenic plants displayed a pronounced increase in stomatal conductance, which allowed them to maintain higher rates of CO_2 assimilation under stress, and improving their ability to recover from the stresses. These findings suggest that an important link may exist between mitochondrial function (perhaps mitochondrial ROS) and the signal paths controlling stomatal function. In another study, it was shown that knockdown of UCP1 in *Arabidopsis* hampered photorespiration, although this was not specifically examined during drought (Sweetlove *et al.*, 2006). The oxidation of glycine in the mitochondrion, which generates NADH, was restricted as shown by a reduction in the metabolism of ^{13}C-labelled glycine to serine in the *ucp1* mutant. Photosynthesis was also impeded in these plants (Sweetlove *et al.*, 2006), likely since a reduction in photorespiration can feedback and inhibit photosynthesis (Timm *et al.*, 2012). Overall, these results suggest that mitochondrial UCP supports photorespiratory function during drought, either by supporting glycine metabolism and/or by influencing stomatal function.

In addition to complex I, which oxidizes matrix NADH, plants have a series of 'alternate dehydrogenases' embedded on either the inner or outer face of the inner mitochondrial membrane. Unlike complex I, these dehydrogenases are not proton pumping and hence relax the coupling between carbon metabolism, electron transport and ATP turnover (Rasmusson *et al.*, 2004). In *Arabidopsis*, there appear to be seven alternate dehydrogenases (Rasmusson *et al.*, 2008). Three of these, the internal alternate dehydrogenases, are on the matrix side of the membrane and are collectively able to oxidize both NADH and NADPH generated in the matrix. Four others, the external alternate dehydrogenases, are on the external side of the membrane and are collectively able to oxidize NADH and NADPH deriving from the cytosol. The external dehydrogenases appear to require high Ca^{2+} for activity, suggesting that they may become engaged in response to stress. Genetic manipulation of one of the external dehydrogenases

altered stem NADPH/$NADP^+$ ratio, which then impacted stem bolting (Liu *et al.*, 2009). The regulation of at least some alternate dehydrogenase genes by light (Escobar *et al.*, 2004) suggests they may function in support of photosynthesis, although more direct evidence for this is still required. To our knowledge, the potential role of the alternate dehydrogenases during drought stress has not been reported. This represents an important area for future study, as these dehydrogenases could facilitate the turnover of excess reductant by relaxing its coupling to ATP synthesis.

Another defining feature of the plant mitochondrial ETC is the presence of two terminal oxidases, the usual energy-conserving cyt oxidase (complex IV) and another termed AOX (Finnegan *et al.*, 2004; Vanlerberghe, 2013). The ETC is essentially bifurcated, such that electrons in the ubiquinone pool are partitioned between the cyt pathway (consisting of complex III, cyt *c* and complex IV) and AOX. AOX directly couples the oxidation of ubiquinol with the reduction of O_2 to H_2O. AOX activity dramatically reduces the energy yield of respiration since it is not proton pumping and since electrons flowing to AOX bypass the proton pumping complexes III and IV. Further, in combination with an alternate dehydrogenase to bypass proton pumping complex I, AOX activity could allow for a completely uncoupled route of electron transport from matrix or cytosolic NAD(P)H to O_2. AOX is an interfacial membrane protein, oriented toward the matrix side of the inner mitochondrial membrane.

The *maximum* possible flux of electrons to AOX is often termed AOX capacity, is typically a reflection of AOX protein abundance, and can be measured in isolated mitochondria or *in vivo* by making use of pathway-specific inhibitors such as the complex IV inhibitor CN and the AOX inhibitor salicylhydroxamic acid (SHAM). The *actual* flux of electrons to AOX *in vivo* is termed AOX activity and is dependent upon the true partitioning of electrons between AOX and complex III. This partitioning of electrons is disrupted by inhibitors, so determination of AOX activity requires a more sophisticated approach. The oxygen isotope discrimination method to measure AOX activity is based on the fact that AOX and cyt oxidase discriminate to different extents against heavy O_2 ($^{18}O^{16}O$) (Guy *et al.*, 1989). In photosynthetic tissues, such measurements must be performed in the dark (due to the opposing gas exchange characteristics of photosynthesis and respiration), thus precluding the determination of AOX activity during photosynthesis.

AOX is encoded by a small gene family. Dicotyledons contain members of two distinct subfamilies, *AOX1 and AOX2,* while monocotyledons contain only *AOX1* genes (Considine *et al.*, 2002). *AOX2* genes show specific developmental and tissue expression, while the expression of *AOX1* genes is highly induced by abiotic and biotic stresses (Clifton *et al.*, 2006; Chai *et al.*, 2010). It has also been established that the stress-inducible AOX1a isoforms in tobacco and *Arabidopsis* are subject to sophisticated biochemical control (Vanlerberghe *et al.*, 1995; Rhoads *et al.*, 1998). It is this biochemical control, rather than simply

AOX protein abundance, that controls AOX activity *in vivo* (Guy and Vanlerberghe, 2005). Through covalent modification and allosteric mechanisms, AOX activity is modulated by upstream respiratory metabolism. Activation of AOX occurs in response to a high reduction state of matrix NAD(P)H, combined with high levels of pyruvate. These are conditions that might be expected to occur when there is an imbalance between the rate of upstream respiratory metabolism and downstream electron transport to O_2. Hence, the biochemical properties that govern AOX activity make it well suited as a mechanism to prevent the energy imbalances that lead to ETC over-reduction. In keeping with this, it was recently shown that transgenic tobacco leaves lacking AOX have increased concentrations of mitochondrial-localized O_2^- and NO, the products that can arise when over-reduced ETC components results in electron leak to O_2 or nitrite (Cvetkovska and Vanlerberghe, 2012). This interpretation is corroborated by experiments with the complex III inhibitor antimycin A. In wild-type plants, antimycin A increased both mitochondrial O_2^- and NO since restriction of electron flow leads to an over-reduction of ETC components. However, in plants over-expressing AOX, O_2^- and NO did not increase in response to antimycin A since these plants are able to maintain high rates of electron flow to O_2, even with the sudden and complete loss of complex III activity (Cvetkovska and Vanlerberghe, 2013).

It was reported that AOX activity may be essential to support mitochondrial glycine oxidation during photorespiration, the pathway which most directly links the mitochondrion to photosynthetic metabolism (Igamberdiev *et al*., 1997, 2001). However, several studies have examined photosynthesis in *aox1a* mutant *Arabidopsis* plants and, to our knowledge, clear evidence that AOX supports photorespiration has not emerged from these studies (Florez-Sarasa *et al*., 2011; Yoshida *et al*., 2011; Gandin *et al*., 2012). In particular, there is no evidence reported whether glycine metabolism is restricted in *aox1a.* This is unlike the case with the *ucp1 Arabidopsis* mutant, in which glycine metabolism is clearly restricted (Sweetlove *et al*., 2006, see earlier). Interestingly, this study found that, in the absence of UCP, AOX amount also declined. On the one hand, this response is counter to what one might expect if AOX could step in – at least in the absence of UCP – and support glycine oxidation. On the other hand, it does introduce an uncertainty whether the restriction in glycine metabolism observed in *ucp1* was due to the absence of UCP1 or due to the accompanying decline in AOX. Lack of AOX, with its concomitant increase in NO (Cvetkovska and Vanlerberghe, 2012), could perhaps inactivate GDC, as a mechanism for NO inactivation of GDC has been described (Palmieri *et al*., 2010).

In recent years, studies have investigated the role of AOX in numerous stress conditions, including drought, and have made use of tools such as oxygen isotope discrimination and plants with manipulated AOX amount (Vanlerberghe, 2013). The next section provides further background regarding plant respiration under drought, as well as providing a comprehensive analysis of studies which have specifically examined the role of AOX during drought stress and recovery.

Plant respiration and alternative oxidase during drought stress

A defining feature of drought stress is that it results in a dramatic decline in the rate of carbon assimilation, by the gradual imposition of a combination of stomatal and biochemical limitations of photosynthesis (Lawlor and Tezara, 2009). Given the declines in carbon assimilation, it might be assumed that another defining feature of drought stress would be a drop in plant carbon status, followed by a decline in respiration rate due to substrate limitation. However, this does not appear to be a typical scenario. First, recent studies suggest that the carbon status of plants during drought stress is relatively robust, particularly compared with the decline in photosynthesis (Muller *et al.*, 2011; Pinheiro and Chaves, 2011). This is likely primarily because overall growth declines relatively more than photosynthesis during drought, thus buffering against a decrease in carbon status (Muller *et al.*, 2011). Second, based on studies to date, there is no clear expectation as to the rate of respiration during drought. In some cases, drought has been reported to have little or no impact on total respiration rate (Ribas-Carbo *et al.*, 2005; Giraud *et al.*, 2008; Gimeno *et al.*, 2010), while other studies have reported decreases (Haupt-Herting *et al.*, 2001; Haupt-Herting and Fock, 2002; Taylor *et al.*, 2005; Vassileva *et al.*, 2009; Galle *et al.*, 2010) or even increases (Bartoli *et al.*, 2005; Feng *et al.*, 2008; Hummel *et al.*, 2010; Begcy *et al.*, 2011). It has also been reported that respiration can decrease in response to mild water deficit but then increase with more severe stress (Wang and Vanlerberghe, 2013).

Despite the variable response of respiration rate to drought, a general conclusion that can be drawn from the literature is that, in most instances, drought causes a substantial increase in the ratio of respiration rate to photosynthetic rate (Flexas *et al.*, 2006; Atkin and Macherel, 2009). In this case, the question of how respiration responds to drought takes on added significance in terms of the overall energy and carbon budget of the plant. Recent studies suggest that enzymes and metabolites in respiratory metabolism stay high or even increase under drought (Vasquez-Robinet *et al.*, 2008; Hummel *et al.*, 2010; Acevedo *et al.*, 2013). Interestingly, Bartoli *et al.* (2004) found that wheat mitochondria suffered relatively more oxidative damage (estimated by protein carbonyl content) in response to drought than did either chloroplasts or peroxisomes. It has also been shown that the expression of MnSOD is drought-inducible in wheat (Wu *et al.*, 1999). Such findings are consistent with the view that respiration remains active during drought and that it may indeed take on additional functional roles and significance in support of acclimation to drought and recovery from drought. The observations also suggest that mitochondrial ROS may be prevalent, perhaps the result of an energy imbalance in this organelle during drought.

As outlined earlier, chloroplast metabolism responds to drought – and the decline in the Calvin cycle as a major energy consumer – with the engagement of multiple mechanisms that likely act in parallel to buffer against energy imbalances. Given the prominent role of respiratory metabolism during drought, interest has turned to whether specific mitochondrial mechanisms able to buffer against energy imbalances are also being engaged during drought. In particular, Table 8.1 provides a summary of studies that have focused on AOX respiration in the response of plants to drought stress. Following are some observations and discussion based on the insights gained from these studies:

1 In several species, including both monocots and dicots, drought has been shown to increase the transcript amount of gene(s) encoding AOX (Table 8.1). Similarly, increases in AOX protein and capacity have often been reported. One possibility is that increased AOX expression during drought is due to changes in abscisic acid (ABA) signalling, although this possibility has not been directly evaluated. Increased ABA is a common response to drought, as this hormone is responsible for important acclimation responses such as stomatal closure (Neill *et al.*, 2008; Kim *et al.*, 2010; Daszkowska-Golec and Szarejko, 2013). In *Arabidopsis,* a molecular link has been made between ABA signalling and the regulation of AOX expression. Functional characterization of the promoter of *Arabidopsis AOX1a* identified a repressor element that was shown to bind the transcription factor abscisic acid insensitive 4 (ABI4) (Giraud *et al.*, 2009). ABI4 is an ABA signalling responsive transcription factor. These results hint that increased ABA during drought could act to de-repress *AOX1a* transcription. Supporting this idea, studies have shown that exogenous ABA treatment of *Arabidopsis* increases *AOX1a* transcript amount (Ghassemian *et al.*, 2008; Giraud *et al.*, 2009; Liu *et al.*, 2010; He *et al.*, 2012; Miura *et al.*, 2013). Interestingly, ABA also increased the transcript amount of several genes encoding alternate dehydrogenases, indicating that the components for a completely non-energy conserving path of mitochondrial electron transport can be induced by ABA (Ghassemian *et al.*, 2008; He *et al.*, 2012).

While most studies examining AOX amount in response to drought have reported increased AOX, there are some exemptions (Table 1). In particular, soybean was shown to dramatically increase AOX activity in response to drought (see below) but without any increase in AOX protein amount (Ribas-Carbo *et al.*, 2005). Also, in some species such as tobacco it was shown that a relatively severe drought stress was required before substantial increases in AOX expression and protein amount were evident (Wang and Vanlerberghe, 2013). The variability between species may relate to their 'non-stress' constitutive level of AOX. For example, soybean is known to have relatively high constitutive amounts of AOX, meaning that an increase in AOX amount in response to drought may not be necessary to allow increased AOX activity. For example, Bartoli *et al.*, (2005) provide evidence that, in wheat, drought stress was associated with an increased conversion of AOX protein from its oxidized

Table 8.1 Studies examining the role of AOX during drought stress.

Plant species	Treatment(s)	Major findings	Reference
Arabidopsis thaliana (WT plants and *aox1a* knockout plants)	A moderate combined stress treatment (increased irradiance and drought) that had no impact on leaf RWC of WT plants but reduced the RWC of knockout plants by approximately 10%.	Compared to WT, mutant had reduced root growth that may have been responsible for its compromised RWC under stress. Compared to WT, mutant leaves under stress accumulated more anthocyanins, displayed some reduction in photosynthetic efficiency, had elevated levels of whole leaf O_2^-, and had generally increased amounts of sugars and decreased amounts of amino and organic acids.	Giraud *et al.,* 2008
Arabidopsis thaliana (WT plants, plants overexpressing *AOX1a* and *aox1a* knockout plants)	Mild osmotic stress (mannitol). Drought.	Under non-stress conditions, growth rate was compromised in over-expressing plants and increased in knockout plants, relative to WT. However, under stress conditions, growth rate was improved in over-expressing plants, while knockout plants were similar to WT. In WT plants, AOX expression was induced by stress, but only in young leaves with predominantly dividing cells, suggesting an important role of AOX in proliferating cells under stress.	Skirycz *et al.,* 2010
Nicotiana tabacum (WT and *aox1a* knockdown plants)	Drought resulting in a progressive decline in leaf RWC. Severe drought combined with increased irradiance. Re-watering.	Mild to moderate drought resulted in a progressive and modest increase in AOX protein amount while severe stress (particularly when combined with increased irradiance) strongly increased AOX. All plant lines displayed similar declines in leaf RWC with increasing stress severity. Under severe stress, knockdown lines exhibited more cellular and oxidative damage than WT, and were found to down-regulate rather than up-regulate the transcript level of several important ROS-scavenging components. Compared to WT, knockdown lines were strongly compromised in their ability to recover from severe stress after re-watering.	Wang and Vanlerberghe, 2013
Nicotiana sylvestris (WT plants and CMSII plants lacking complex I)	Drought resulting in an approximately 15% decline in leaf RWC.	In WT plants, AOX protein increased under drought. Isotope discrimination experiments showed that drought decreased electron flow through the cyt pathway, while electron flow to AOX was maintained.	Galle *et al.,* 2010

(*Continued*)

Table 8.1 (*Continued*)

Plant species	Treatment(s)	Major findings	Reference
Glycine max	Drought resulting in a 3% (mild stress) to 15% (severe stress) decline in leaf RWC.	No change in leaf AOX protein level under drought. Isotope discrimination experiments showed that, in response to severe drought, about 40% of total electron flow occurred through AOX, compared to just 10–12% in well-watered plants or plants experiencing mild drought.	Ribas-Carbo *et al.*, 2005
Triticum aestivum	Drought resulting in a 22% decline in leaf RWC. Some plants treated with 1 mM SHAM to inhibit AOX.	Drought increased the total amount of AOX protein and shifted more of the protein toward its reduced (active) form. SHAM treatment of drought-stressed plants reduced photosynthetic performance, decreasing photochemical quenching and increasing NPQ.	Bartoli *et al.*, 2005
Triticum aestivum	Drought resulting in leaf RWC of approximately 78%.	Drought increased AOX transcript and approximately doubled the AOX capacity of leaves. SHAM treatment of drought-stressed leaves increased H_2O_2 amount.	Feng *et al.*, 2008
Triticum aestivum (several varieties)	Moderate drought stress. Re-watering.	Drought approximately doubled the AOX capacity measured in isolated mitochondria, and remained high three days after re-watering.	Vassileva *et al.*, 2009
Nothofagus solandri and Nothofagus menziesii (beech tree species)	Mild to severe drought. Re-watering.	AOX protein amount increased (relative to a cyt pathway protein) under severe drought and this pattern persisted after re-watering. However, isotope discrimination experiments suggested little change in electron partitioning between AOX and the cyt pathway in response to drought or re-watering.	Sanhueza *et al.*, 2013
Pisum sativum	Drought.	Leaf AOX protein amount increased 2.5-fold by drought.	Taylor *et al.*, 2005
Oryza sativa	Drought resulting in approximately 20% decline in leaf RWC.	Leaf AOX transcript amount increased in response to drought.	Feng *et al.*, 2009
Medicago trunculata	Drought. Re-watering.	AOX transcript levels declined in the leaf and increased in the root in response to drought.	Fillippou *et al.*, 2011

(inactive) to reduced (active) form, indicating a biochemical control of AOX activity due to the prevailing metabolic conditions present during drought. It is worth noting that the majority of drought studies to date have examined AOX amount in leaf, so little is yet known about how root AOX may respond to drought. It is also not known whether changes in AOX amount or activity occur in guard cells, an important ABA target during drought stress. A previous study has reported that respiration rates in pea are several-fold higher in guard cells than mesophyll cells (Vani and Raghavendra, 1994). However, little else is known about respiration in guard cells and, in particular, what role the cyt and AOX pathways may have in terms of stomatal function.

Interestingly, a number of recent studies are suggestive of a link between mitochondrial ROS, ABA signalling and stomatal function. In one case, altered expression of an *Arabidopsis* mitochondrial glutathione peroxidase was shown to disrupt H_2O_2 amount in guard cells and to disrupt ABA-mediated stomatal closure in response to drought (Miao *et al.*, 2006). In another example, mutation of a DEXH box RNA helicase that disrupted complex I resulted in higher levels of mitochondrial O_2^-, which in turn reduced stomatal aperture and improved drought tolerance (He *et al.*, 2012). Similarly, a mitochondrial RNA editing mutant defective in complex I accumulated more H_2O_2 in guard cells after ABA treatment and displayed enhanced drought tolerance (Yuan and Liu, 2012). Finally, the complex I mutant of tobacco (CMSII) is also reported to display reduced stomatal aperture in response to drought, perhaps again through changes in ROS (Djebbar *et al.*, 2012). These studies suggest that ABA control of stomatal aperture may be mediated, at least in part, through changes in mitochondrial ROS amounts. Salicylic acid (SA) can also influence stomatal aperture. Several mutants with increased SA displayed reduced stomatal aperture due to increased ROS amount (Miura *et al.*, 2013), which may have been mitochondrial in origin given the ability of SA to disrupt mitochondrial metabolism (Norman *et al.*, 2004). The study by Miura *et al.* (2013) also found high levels of AOX transcript in guard cells and – through cluster analysis of several microarray datasets – identified AOX as a 'gene of interest' in the regulation of stomatal movement by SA, ROS and drought. Despite the interest of these studies, a unifying model of how AOX and mitochondrial ROS may function in the regulation of stomatal aperture by drought, ABA and/or SA is not yet reported, and will require further study at the guard cell level using plants with modified AOX expression.

2 While increases in AOX transcript, protein and capacity in response to drought suggest that AOX activity may be increased under drought, this can only be directly evaluated using the oxygen isotope discrimination technique. To our knowledge, only three such drought studies involving four plant species (*Glycine max, Nicotiana sylvestris* and two *Nothofagus* tree species) has been reported (Table 8.1). Of these species, soybean showed the most dramatic changes in AOX activity under drought. In well-watered soybean, AOX

activity accounted for 10–12% of total electron flow. Drought stress saw both a decline in absolute cyt pathway activity and an increase in absolute AOX activity such that, during drought, total respiration rate was similar to well-watered plants but with 40% of total electron flow occurring via AOX (Ribas-Carbo *et al.*, 2005). The increase in AOX activity under drought may be facilitated by a high energy charge restricting cyt pathway flow and/or by an abundance of reducing equivalents supplying electrons to the ubiquinone pool via complex I and the alternate dehydrogenases. The fact that the increase in AOX activity was combined with a decrease in cyt pathway activity favours high energy change being responsible for the change in electron partitioning. If high energy charge was not being experienced, but only an abundance of electrons in the ubiquinone pool, one might expect the activity of both AOX and the cyt pathway to increase, but this was not the case. Nonetheless, energy charge was not directly measured in this study, so other possibilities for the decline in cyt pathway activity and the increase in AOX activity are also possible. For example, drought might directly inhibit the cyt pathway by another unknown mechanism. Interestingly, the study with *N. sylvestris* also reported that drought decreased cyt pathway activity (Galle *et al.*, 2010). In this case, AOX activity remained unchanged in response to drought; however, due to the decline in cyt pathway respiration, AOX did represent a higher percentage of total respiration under drought than under well-watered conditions. Finally, a study on two *Nothofagus* species suggested no change in electron partitioning between AOX and the cyt pathway due to drought, although this study was hampered because the end-points for discrimination against $^{18}O_2$ by each pathway could not be determined (Sanhueza *et al.*, 2013). In sum, the available evidence indicates that drought can strongly impact the activity of both cyt and AOX respiration under drought and is suggestive that the ratio of AOX to cyt pathway respiration increases under drought. This is consistent with a need for AOX to dissipate excess energy under drought, more so than under well-watered conditions. In this respect, it is worth noting that energy imbalances during drought would be expected to be greater in the light than dark. Hence, the partitioning of electrons to AOX in the light might be even greater than those estimated in the dark by isotope discrimination. Nonetheless, it is obvious that still too little isotope discrimination data overall is available to conclude that increased AOX is a defining feature of respiratory metabolism under drought.

Given our speculation above that high energy charge may be responsible for the shift in electron partitioning toward AOX during drought, it is worth emphasizing some other studies which suggest that the biochemical impairment of photosynthesis during drought is primarily due to a disabling or down-regulation of the chloroplast ATP synthase (Tezara *et al.*, 1999; Kohzuma *et al.*, 2009; Lawlor and Tezara, 2009). This likely should decrease rather than

increase ATP amounts during drought, as some studies have demonstrated (Tezara *et al*., 2008; Lawlor and Tezara, 2009).

3 There is some evidence that AOX respiration is important to maintain respiratory carbon flow under drought (Table 8.1). This is based primarily upon a study comparing wild-type (WT) *Arabidopsis* with T-DNA mutants lacking AOX1a, and involved a stress that combined mild drought with a shift to higher irradiance (Giraud *et al*., 2008). A survey of metabolites found that, under stress, mutant plants maintained generally higher levels of carbohydrate and lower levels of amino and organic acids than WT. These differences between lines were not seen under the normal growth condition. The results are consistent with a restriction of respiratory carbon flow through glycolysis and the TCA cycle in the plants lacking AOX. On the other hand, no differences were seen in oxygen uptake by the plants suggesting that, while carbon flow appeared restricted by the lack of AOX, the total rate of electron flow through the ETC to oxygen was normal. It is difficult to reconcile these two findings.

4 In both wheat and *Arabidopsis,* there is some evidence that AOX activity under drought acts in support of photosynthetic metabolism (Table 8.1). In wheat, this is primarily based upon experiments comparing the photosynthetic characteristics of well-watered and drought-stressed plants, in the presence or absence of the AOX inhibitor SHAM (Bartoli *et al*., 2005). It was found that SHAM had no impact on photosynthesis in well-watered plants. In drought-stressed plants, however, SHAM significantly reduced the efficiency of PSII, while increasing NPQ and decreasing photochemical quenching, compared to drought-stressed plants without SHAM treatment. These effects of SHAM were particularly evident at higher irradiances, consistent with an energy imbalance in the chloroplast in the absence of AOX activity. The authors suggest that the positive impact of AOX may be due to it acting both as a sink for reductant (such as generated by glycine oxidation) and by providing increased respiratory CO_2 release for reassimilation by the Calvin cycle. While it was shown that SHAM itself had no apparent direct effect on photosynthesis in isolated chloroplasts, experiments utilizing SHAM should nonetheless be interpreted with caution due to the potential side-effects of this inhibitor. The impact of AOX on photosynthesis during drought was also investigated in the *Arabidopsis aox1a* mutant subjected to drought combined with a shift to higher irradiance (see above, Giraud *et al*., 2008). Similar to the studies in wheat, lack of AOX during stress decreased PSII efficiency and increased non-photochemical energy dissipation. This study also reported increased whole leaf levels of O_2^- which was suggested to arise in the chloroplast due to the disrupted photosynthetic metabolism. Consistent with this, there was a strong similarity between the transcriptome changes of the *aox1a* mutant under drought and transcriptome changes previously reported to occur in response to chloroplast-generated ROS (Giraud *et al*., 2008).

5 Theoretically, AOX activity could negatively impact plant productivity since it reduces the respiratory yield of ATP, an important general requirement for biosynthesis and growth. A study with *Arabidopsis* suggests that AOX amount can influence growth under drought stress (Skirycz *et al.*, 2010) (Table 8.1). This study compared the relative growth rate of WT plants with that of plants either lacking AOX or overexpressing AOX. Under optimal growth conditions all the plants displayed similar relative growth rates. However, under drought stress, plants overexpressing AOX displayed higher relative growth rate than WT. This suggests, paradoxically, that the non-energy conserving nature of AOX can positively impact growth under drought stress. While the specific mechanisms responsible for this growth response still need to be elucidated, one possibility is that higher AOX activity improved energy balance, with positive impacts on metabolism and/or signalling processes.

Interestingly, the study by Giraud *et al.* (2008) also reported a growth phenotype in *Arabidopsis aox1a* mutants. Root growth in vertical agar plates was reduced in the mutant by about 10% compared to WT. This study also found that, after the combined drought/irradiance stress (see earlier), the leaf relative water content (RWC) of the mutant plants had declined by about 10%, while no decline occurred in the WT. It seems possible that the root growth defect could account for the greater leaf water deficit being experienced by the mutant plants. It might also provide an explanation for the decline in photosynthetic performance of the mutant, compared to WT (see earlier). If the mutant plants are experiencing a greater water deficit than the WT, as the RWC measurements suggest, they might also experience a greater stomatal limitation of photosynthesis. Hence, there remains some uncertainly whether lack of AOX in these plants was directly impairing photosynthesis, such as by impairing oxidation of excess chloroplast reductant, or indirectly, by impairing the capacity for water uptake due to reduced root growth. As discussed in the study, another explanation is also possible. The *aox1a* mutant plants display a marked reduced expression of the ABI4 transcription factor that is a negative regulator of *AOX1a* expression, presumably an attempt by the plants to increase AOX1a levels (Giraud *et al.*, 2008, 2009). Given that ABI4 is a central stress responsive transcription factor involved in ABA responses as well as chloroplast retrograde responses (León *et al.*, 2013; Wind *et al.*, 2013), its altered amount in *aox1a* might also contribute to the changes in photosynthetic metabolism during stress.

6 There is some evidence that AOX can protect against oxidative and cellular damage during severe drought stress (Table 8.1). Knockdown of AOX in transgenic tobacco had little impact on the amount of oxidative damage (lipid peroxidation) or cellular damage (electrolyte leakage) during mild to moderate drought. However, in response to severe drought combined with a shift to higher irradiance, the knockdown plants exhibited small but significant increases in both oxidative and cellular damage relative to WT plants with

similar RWC (Wang and Vanlerberghe, 2013). Further, *aox1a Arabidopsis* mutants were unable to survive a stress combination in which drought-stressed plants were subsequently subjected to both increased irradiance and elevated temperature (35 °C) (Giraud *et al.*, 2008). Finally, inhibition of AOX by SHAM during drought stress was shown to increase leaf levels of H_2O_2 in wheat (Feng *et al.*, 2008).

7 There is some evidence that the presence of AOX may be important in the recovery phase from drought stress (Table 8.1). In particular, the tobacco study noted earlier showed that plants lacking AOX were strongly compromised in their ability to recover from severe drought stress when re-watered (Wang and Vanlerberghe, 2013). While all WT plants showed rapid evidence of recovery, the knockdown plants were either significantly delayed in their recovery or did not recover at all during the study period. At present, however, it is difficult to untangle whether the compromised ability of these plants to recover is due to an essential role for AOX during the recovery period itself or whether it is due to the slight increased oxidative and cellular damage experienced by the knockdown plants during the severe drought (see earlier, Wang and Vanlerberghe, 2013). Further, the late stages of severe stress were characterized by a down-regulation of expression of several ROS-scavenging components in the knockdowns, while these were increasing in the WT. This may indicate a re-programming of knockdown plants (perhaps a programmed death or senescence program?), which may have also contributed to their susceptibility during the subsequent recovery period. It would be interesting to examine AOX activity using isotope discrimination in tobacco plants during a recovery period from severe drought to examine whether the pathway is highly engaged under such conditions. The study of Galle *et al.* (2010) reported little impact of re-watering on AOX activity, while cyt pathway activity increased. However, this re-watering followed a much less severe drought treatment than reported by Wang and Vanlerberghe (2013). Finally, in the study with *Nothofagus*, drought increased the ratio of AOX protein to that of a cyt oxidase protein and this increased ratio persisted – or even increased further – following re-watering (Sanhueza *et al.*, 2013). There is evidence that re-watering can actually enhance the oxidative stress being experienced by drought-stressed plants (Mittler and Zilinskas, 1994; Flexas *et al.*, 2006). If this is the case, it could provide some explanation for the high AOX after re-watering.

Conclusions

Drought is a widespread abiotic stress that can have strong negative impacts on plant growth, productivity and survival. There is overwhelming evidence from photosynthesis studies that this stress acts to exacerbate energy imbalances in the chloroplast. Given the connectivity of primary energy metabolism between different cellular

compartments and given that mitochondrial components such as AOX may be ideally suited to combat cellular energy imbalances, it is clear that more effort should be directed toward the study of mitochondrial and respiratory metabolism during drought and recovery from drought, and in relation to photosynthetic metabolism (Flexas *et al*., 2006; Atkin and Macharel 2009; Lawlor and Tezara, 2009). Beside the potential metabolic roles of respiration during drought, the potential signalling roles of the mitochondrion in processes such as stomatal function or cell survival during and following severe stress are also of considerable interest.

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Abbreviations

ABA, abscisic acid; ABI4, abscisic acid insensitive 4; AOX, alternative oxidase; CET, cyclic electron transport; cyt, cytochrome; ETC, electron transport chain; GDC, glycine decarboxylase; MDH, malate dehydrogenase; NO, nitric oxide; LET, linear electron transport; NPQ, non-photochemical quenching; OAA, oxaloacetate; PSI, photosystem I; PSII, photosystem II; PTOX, plastid terminal oxidase; O_2^-, superoxide; RNS, reactive nitrogen species; ROS, reactive oxygen species; RWC, relative water content; SA, salicylic acid; SHAM, salicylhydroxamic acid; SOD, superoxide dismutase; UCP, uncoupling protein; WT, wild-type

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