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Relaxing non-photochemical quenching (NPQ) to improve photosynthesis in crops

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1 Introduction

The conversion of energy from the sun into biochemical energy by plants and other photosynthetically competent organisms drives and sustains life on the earth. While the ability to perform photosynthesis provides autotrophic growth, it can be a double-edged sword. Even for non-photosynthetic organisms, exposure to sunlight can be treacherous, as anyone who has spent a little too much time in the sun will know (the global market for after-sun products currently amounts to ~US$2 billion – Statista, 2021). However, in photosynthetic organisms, the light-harvesting antennae, which are used to collect and concentrate the radiant energy for photochemistry, aggravate the risk of overexposure, precisely because of their function.

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While low-intensity energy fluxes can be processed via photochemistry in a hazard-free manner, performing photosynthesis under high-intensity sunlight can be risky business. Given that plants depend on light to grow and survive, yet are liable to suffer damage to proteins, nucleic acids and membranes as a result of high light exposure, they have a clear need for high light avoidance strategies, as well as ways to deal with energy absorbed in excess. For example, light absorption can be reduced via paraheliotropic movements (Koller, 1990). These leaf movements position the leaf lamina in parallel to the solar beam, thereby reducing energy load. Alternatively, or in addition, some plants can modulate leaf reflectance, such as via trichome density on the leaf surface (Ehleringer, 1984), which helps to adjust the fraction of incident irradiance that gets absorbed by the leaf. However, such avoidance strategies are typically too slow to respond to rapid fluctuations in light intensity and thus come with a significant penalty to photosynthetic CO_2 fixation, while still allowing overexposure upon sharp increases in light. This explains why more dynamic mitigation strategies to safely dissipate excessively absorbed light energy are universally found across plants and other photosynthetic organisms. The focus in this chapter will be on the protective energy dissipation strategies at the level of the thylakoid, the chloroplast internal membrane system, which contains the proteins and pigments for light harvesting and photochemistry. At this level, non-photochemical quenching (NPQ) processes in the antennae of photosystem II can dynamically adjust light-harvesting efficiency in response to the relative excess of intercepted irradiance, thereby offering tailored photoprotection to the photochemical machinery downstream. In the following paragraphs, the mechanism of photoprotection via NPQ as well as the opportunities to improve photosynthetic efficiency and crop yield via adjustment of NPQ will be laid out.

2 Light harvesting and photochemistry

In higher plants, the photosynthetic light-dependent reactions are organised into two photosystems, which harvest light energy to drive redox chemistry in their reaction centres. The light-absorbing cross-section of these photosystems is greatly enlarged by pigment-binding antenna proteins surrounding the core. These antenna proteins are members of the light-harvesting complex superfamily (named a or b for photosystem I or II, respectively) and bind light-absorbing pigments in configurations that allow absorbed energy to efficiently flow from the site of absorption to the reaction centre in the core of each photosystem (Fig. 1).

In the reaction centres, a special pair of chlorophylls forms an energy trap to concentrate the energy flux from the surrounding antennae. This energy is used to start electron transfer. Photosystem II (PSII) forms a homodimeric

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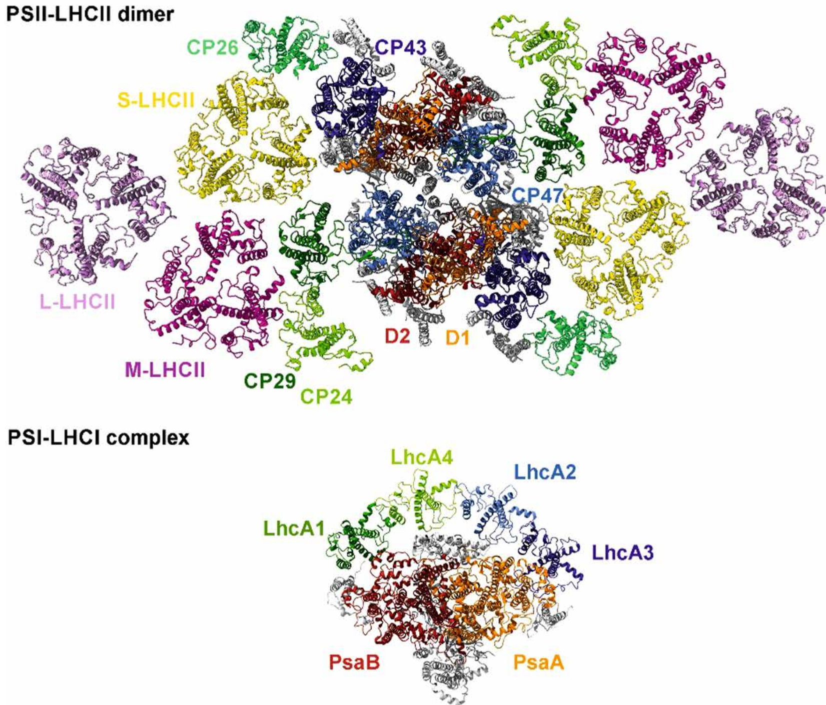

Figure 1 Pigment–protein supercomplexes PSII-LHCII and PSI-LHCI in photosynthetic thylakoid membranes of higher plants (top view). Trimeric light-harvesting complexes (LHC) are strongly (S-LHCII), moderately (M-LHCII) and loosely (L-LHCII) connected to the PSII reaction centre via the monomeric LHCII proteins CP29/CP24 and CP26 and the core proteins CP43 and CP47. Absorbed light energy is channelled from the periphery towards the PSII reaction centre, which is composed of the D1 and D2 proteins. The PSI-LHCI supercomplex contains the reaction centre proteins PsaA and PsaB, which are linked to two heterodimers, consisting of the LhcA1/A4 and LhcA2/A3 proteins. PDB entries 7OUI (PSII-LHCII from *Arabidopsis thaliana*) and 7DKZ (PSI-LHCI from *Pisum sativum*) were downloaded into ChimeraX and protein residues were coloured by amino acid chains. Grey subunits indicate PSII/PSI proteins adjacent to the reaction centres and core proteins.

complex and, moving from the reaction centre proteins D1/D2 outwards, consists of the CP47 and CP43 core proteins, Lhcb4, Lhcb5 and Lhcb6 (CP29, CP26 and CP24) minor distal antennae, and the major distal antennae Lhcb1, Lhcb2 and Lhcb3 (Jansson, 1994), which are involved in light harvesting. Whereas the core and minor antennae are present as monomers, the major antennae form homotrimers of Lhcb1 or heterotrimers of Lhcb1/2 and Lhcb1/3 (Jansson, 1999), which are classified as strongly (S), moderately (M) or loosely (L) bound, corresponding to specific binding positions around the PSII core. In contrast to PSII, photosystem I (PSI) in higher plants is formed by a monomeric core complex, which coordinates most chlorophyll and carotenoid pigments of

PSI. The absorption cross-section of PSI is further enhanced by four peripheral antenna pigment-protein complexes, Lhca1, Lhca2, Lhca3 and Lhca4, which are arranged in a half-moon shape of two heterodimers (Lhca1/4 and Lhca2/3) around the core (Qin et al., 2015). In addition, a mobile pool of LHCII trimers can flexibly (dis-)associate to either photosystem in a process called state transitions (Allen, 2003), which helps to balance the excitation energy across both photosystems upon changing light conditions.

The chlorophyll and carotenoid pigments used in light harvesting absorb light in the spectral region of 400–700 nm, which is termed the Photosynthetically Active Radiation (PAR) part of the solar spectrum. PSII absorbs at slightly different wavelengths than PSI, with the latter having a broader range of absorption into the far-red region. Absorption of the reaction centre pigments peak at approximately 680 nm (P680) and 700 nm (P700) for PSII and PSI, respectively. Whole-chain electron transport can be neatly summarised using the Z-scheme (Fig. 2, reviewed by Govindjee et al., 2017)

Electron transfer is initiated by charge separation in P680 and reduction of the first electron acceptor pheophytin. This very unstable $Pheo^-$ intermediate rapidly passes on the electron to the first stable electron acceptor plastoquinone (PQ) bound to the Q_A site in D2, leaving oxidised $P680^+$. $P680^+$ in the PSII reaction centre is reduced back to P680 via tyrosine 161 (Y_Z) in D1 by electrons liberated from water-splitting by the manganese cluster in the oxygen-evolving complex (OEC). For every four oxidation events, the OEC generates molecular oxygen (O_2) and four protons (H^+) from two water (H_2O) molecules in the thylakoid lumen. Electrons accepted by PQ_A are then passed on from Q_A to the

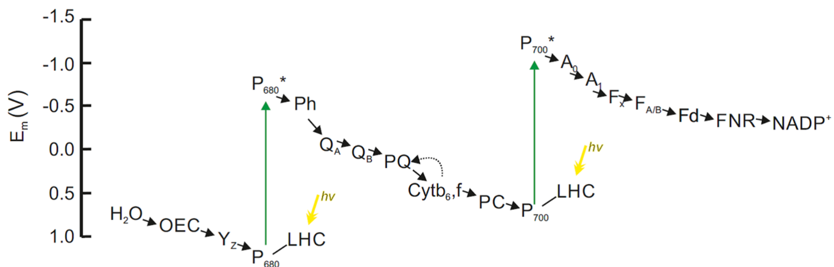

Figure 2 Linear electron transfer according to the Z-scheme. Black arrows indicate electron flow with electron carriers plotted according to their mid-point potential (E_m) at pH 7; green arrows indicate charge separation in PSII (P680) and PSI (P700) reaction centres and yellow arrows (hv) represent excitation energy. LHC, light-harvesting complex; OEC, oxygen-evolving complex; Yz, tyrosine 161; P680, PSII special chlorophyll pair; Ph, pheophytin; Q_A, quinone A; Q_B, quinone B; PQ, plastoquinone/quinol; $Cytb_6$,f, cytochrome b_6f complex; PC, plastocyanin; P700, PSI special chlorophyll pair; A_0, chlorophyll a; A_1, phylloquinone; $F_{x/A/B}$, iron-sulphur (Fe_4S_4) clusters; Fd, ferredoxin; FNR, ferredoxin-$NADP^+$ reductase; $NADP^+$, nicotinamide adenine dinucleotide phosphate.

quinone bound to Q_B on D1, which upon acceptance of two electrons recruits two protons from the stroma to form the mobile electron carrier plastoquinol (PQH_2). PQH_2 undocks from Q_B, gets replaced by PQ from the inner-membrane pool, and diffuses to the lumen side of the thylakoid to become oxidised at the cytochrome b_6,f complex. Here, the path of electron transfer bifurcates. Electrons are either donated to the Rieske iron–sulphur (Fe–S) complex, leading to linear electron flow towards cytochrome f and plastocyanin, or passed on to cytochromes bc_i, inducing the Q-cycle around cytochrome $b_{6,f}$. In this cyclic route, electrons are transferred back to the PQ pool via several cytochromes, which leads to the reduction of PQ to PQH_2 at the stromal side of the membrane. The resulting PQH_2 then diffuses back through the membrane to the *p*-side (electrochemically positive side; Baniulis et al., 2013) of cytochrome b_6,f at the lumenal side of the membrane. In summary, this PQ/PQH_2 shuttle displaces two protons for every two electrons from the stromal to the lumenal side of the thylakoid membrane for each turn. In the other (linear) pathway, electron flow from PQH_2 to the Rieske Fe–S protein and cytochrome f reduces a second mobile electron carrier, plastocyanin, which carries electrons through the lumen to reduce $P700^+$ to P700 in PSI, following charge separation in the P700 reaction centre. Charge separation in PSI generates electron flow from P700 via phylloquinone to a series of three Fe–S clusters: F_X, F_A and F_B. Electrons from F_B are accepted by ferredoxin, a stromal electron carrier which can donate electrons to reduce $NADP^+$ to NADPH via the ferredoxin-$NADP^+$-reductase (FNR). Although useful for visualisation, it is important to remember that the Z-scheme shown in Fig. 2 does not accurately reflect the stoichiometry or localisation of the photosynthetic machinery. Photosystem II is predominantly found in the appressed regions of the thylakoid membranes in so-called grana stacks, whereas photosystem I is more prominent in the lumen-exposed margins.

The accumulation of protons in the lumen in conjunction with the electron transfer pathways, either liberated by water-splitting or displaced from the stroma to the lumen via the Q cycle, leads to the establishment of an electro-chemical proton gradient across the thylakoid membrane. This gradient drives the formation of adenosine triphosphate (ATP) via the activity of the chloroplastic F_1F_o ATP synthase (Hahn et al., 2018). The cF_1F_o ATPase conducts protons from the thylakoid lumen to the stroma through two aqueous channels, each spanning half of the membrane (Hahn et al., 2018). The resulting proton motive force drives rotation of the central stalk of the ATPase leading to ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate in the stroma-positioned catalytic F_1-head. Both NADPH and ATP are essential energy carriers which are used for downstream metabolic processes, such as the Calvin–Benson–Bassham cycle for photosynthetic carbon fixation.

3 Non-photochemical quenching: dynamic regulation of light-harvesting efficiency in the PSII antennae

Under high-intensity light, the electrons liberated from water-splitting can become too plentiful for downstream biochemistry to keep up. As a result, the components of the photosynthetic electron transport chain, that is the photosystems and mobile electron carriers that form the light-dependent reactions in photosynthesis, get more reduced. This enhances the probability of electron donation to alternative, potentially less favourable acceptors. A reduced electron transfer chain blocks efficient electron flow at the acceptor side of PSII, which increases the lifetime of singlet excited chlorophyll. This in turn enhances the probability of triplet chlorophyll formation via inter-system crossing. Energy transfer from triplet chlorophyll to molecular oxygen (which has a triplet ground state) produces singlet oxygen, a highly reactive molecule which can create damage to proteins, membranes and nucleic acids (Khorobrykh et al., 2020). In addition to this reactive oxygen species (ROS), an imbalance between the photosynthetic light reactions and utilisation of NADPH downstream also increases the chances of electron transfer to molecular oxygen at the acceptor side of PSI, leading to the formation of superoxide radical anions, another type of ROS. Superoxide damages DNA indirectly via the production of hydroxyl radicals, as well as Fe–S clusters via direct oxidation.

As explained above, the large antenna complexes surrounding each photosystem help to enhance light absorption per reaction centre and thereby improve light use efficiency of the nitrogen-costly photosynthetic machinery downstream. When light energy is absorbed in the light-harvesting antennae, the energy contained by the chlorophyll excited state can be quenched via photochemistry. In order to drive photochemistry, the absorbed energy needs to be transferred to the reaction centre, which can be done at very high efficiency via resonance energy transfer due to the proximity and orientation of neighbouring light-harvesting pigments in the light-harvesting complexes. However, when light energy is in excess, these large antennae instead enhance the risk of photoinhibition, defined as the light-induced decline of photosynthetic efficiency. For most plants, the availability of light is not constant but instead varies across orders of magnitude on timescales ranging from seconds to seasons. Hence, dynamic adjustment is crucial to leverage the trade-off between damage and efficiency. For this reason, NPQ processes are ubiquitous to photosynthetic eukaryotes (Müller et al., 2001), where they form part of an elaborate feedback de-excitation mechanism to dissipate excess energy harmlessly as heat. NPQ induction is triggered by the acidification of the thylakoid lumen, whose pH was estimated to stay between pH of 6.5 and 5.8 under most conditions (Kramer et al., 1999). Accumulation of protons in the lumen, deriving from several steps of the light-dependent reactions, thus provides an early signal that harvested light

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energy is in excess. This stress signal is further amplified via slowing down of ATP synthesis to match demand, or via stimulation of cyclic electron flow around PSI, which occurs due to the decreased abundance of electron acceptors downstream of PSI (high NADPH/$NADP^+$ ratio). To alleviate photoinhibition of PSI, electrons from PSI are instead recycled back to the PQ pool via alternative pathways to reduce PQ to PQH_2, aggravate lumen acidification and enhance NPQ. NPQ prevents over-reduction of the electron transport chain by funnelling energy away as heat, which in turn decreases the probability of ROS formation and concomitant damage. However, because NPQ competes with photochemistry, it can lead to a reduced light use efficiency of photochemistry, which is especially detrimental when light levels are non-saturating. To avoid this, NPQ is dynamically controlled in response to prevailing light levels. When light levels are low, dissipation of energy via NPQ is kept low and photochemistry can take place at high efficiency. On the other hand, when light levels are high enough, such that NADPH and ATP synthesis exceed the demands of the reactions downstream, NPQ is induced.

4 Assessing non-photochemical quenching via fluorescence measurements

While photochemical and non-photochemical quenching can de-excite chlorophyll, excited chlorophylls can also be quenched via the re-emission of a red-shifted photon, termed fluorescence. Although the fluorescence pathway is not meaningful for photoprotection, it offers a very useful, non-destructive mode to measure the energy transfer through the photochemical and non-photochemical routes; namely, both pathways competitively reduce fluorescence yield. While fluorescence yield can be retrieved as an absolute emission intensity as a function of absorbed excitation intensity, a more commonly used technique is pulse amplitude modulated (PAM) fluorescence, whereby a weak modulated measuring pulse elicits modulated fluorescence emission. Since the amplitude of the measuring pulse remains constant, the amplitude of the fluorescence modulation provides a relative fluorescence yield proxy, which can be easily determined in the background of actinic light (reviewed in Baker, 2008). A typical fluorescence quenching analysis is shown in Fig. 3.

Using brief saturating flashes of actinic light, photochemical quenching can be temporarily overwhelmed, which allows resolving the PSII operating efficiency from the fluorescence yield during the flash (F_m, F_m') and immediately prior to the flash (F_o, F'). Measurements of maximal fluorescence yield after full dark-adaptation and prior to illumination (F_m) can be used together with the maximal fluorescence yields during subsequent flashes (F_m') to determine NPQ at any point during a time series of fluorescence measurements. In this way, induction and relaxation kinetics of NPQ can be determined from the time series of NPQ in response to moving from darkness to actinic illumination or vice versa.

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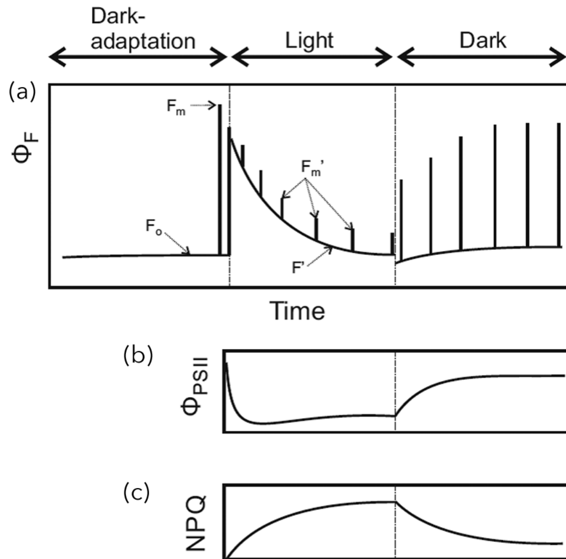

Figure 3 Typical PAM fluorescence experiment to estimate NPQ kinetics. (a) Fluorescence yield of minimal (F_o) and maximal (F_m) fluorescence after dark-adaptation are determined at the start of the protocol. Subsequently, actinic light is turned on at high intensity and saturating flashes are spaced out across the light period to measure F′ and F_m′ which are used to resolve induction kinetics. Finally, light is turned off again and recovery of maximal fluorescence yield over time is probed with saturating flashes to resolve relaxation kinetics. (b) Time series of PSII operating efficiency (Φ_{PSII}) corresponding to fluorescence trace in panel A. (c) Time series of NPQ corresponding to fluorescence trace in panel A.

5 PsbS and zeaxanthin: important factors controlling non-photochemical quenching formation and relaxation in higher plants

The identification of major molecular factors for NPQ induction has greatly benefited from the power of forward genetics. Using a fluorescence imaging screen on a population of mutagenised *A. thaliana* plants, Li et al. (2000) discovered a mutant with severely reduced levels of NPQ upon high light exposure. The corresponding mutation in this *npq4* mutant was found to be a complete deletion of the photosystem II subunit S (PsbS) sequence. PsbS is a member of the light-harvesting complex superfamily and contains four transmembrane helices, rather than the common three helices found in the major and minor LHCII antennae (Kim et al., 2020). PsbS was initially hypothesised to be the quenching site in LHCII (see review in Niyogi et al., 2005). PsbS is found in PSII preparations as a 22 kDa protein (Berthold et al., 1981) and immunoprecipitates with the 33 and 23 kDa subunits of the OEC

(Ljungberg et al., 1984), but also with several LHC proteins (Bergantino et al., 2003). This suggests that it associates both with the core and periphery of PSII, perhaps as a function of the prevailing conditions. If PsbS was the quenching site one would have expected to find a strong interaction with chlorophyll and carotenoid pigments. However, although purified PsbS was reported to bind exogenous zeaxanthin *in vitro* (Aspinall-O'Dea et al., 2002), the protein was stable in the absence of chlorophyll (Funk et al., 1995) and no clear evidence for chlorophyll-binding by PsbS could be found (reviewed in Niyogi et al., 2005). Instead, results indicated that the role of PsbS might be to sense the acidification of the lumen (ΔpH) in response to excess light and relay this signal to induce quenching in LHCII. In this context, protonation of two lumen-exposed glutamate-residues seems to be important for NPQ induction, as shown by site-directed mutational analysis (Li et al., 2002a). In addition, the effect of PsbS on NPQ induction could be restored in the *psbs* deficient *npq4* mutant, using the ΔpH enhancer diaminodurene (Johnson and Ruban, 2011). It is further proposed that upon sensing a decrease in lumen pH PsbS might interact with the LHCII components and initiate the dissociation of certain major antennae from the PSII core. Thus, the structural organisation of PSII-LHCII supercomplexes might be rearranged and the qE-dependent NPQ site activated in detached LHCII trimers (Miloslavina et al., 2011; Jahns and Holzwarth, 2012; Correa-Galvis et al., 2016).

Whereas PsbS seems to signal a decrease in lumen pH to induce NPQ, the reversible xanthophyll cycle also strongly affects NPQ. When the lumen acidifies, enhanced activity of violaxanthin de-epoxidase (VDE) drives the formation of de-epoxidated xanthophylls antheraxanthin and zeaxanthin (Yamamoto and Kamite, 1972). The process is reversed under darkness or low light via the action of zeaxanthin epoxidase (ZEP), which is expressed in the stroma. Early seminal work had already established an important role for the xanthophyll cycle (Demmig-Adams and Adams, 1996) and zeaxanthin especially (Demmig-Adams, 1990) in photoprotection of photosynthesis. Thus, it came as no surprise that fluorescence imaging screening for mutants with altered levels of NPQ (Niyogi et al., 1998) yielded several mutant alleles for violaxanthin de-epoxidase (*npq1*). The *npq1* plants are unable to accumulate antheraxanthin and zeaxanthin and exhibit severely reduced NPQ. In contrast, *npq2* mutants identified on the same screen display similar levels of NPQ compared to wildtype but respond differently to changes in light intensity. In the *npq2* mutants, the ZEP enzyme is deficient, resulting in overaccumulation of zeaxanthin and no detectable levels of antheraxanthin or violaxanthin. Although this mutation also creates significant deficiencies downstream, most notably in abscisic acid synthesis (*aba1* identified by Koornneef et al. (1982) is a ZEP mutant allele), it is clear that NPQ induction in the *npq2* plants is much more rapid and more sustained, taking significantly longer to switch off under

dark-adapted conditions. Although the role of zeaxanthin in thermal energy dissipation was initially met with scepticism (see the personal account of Demmig-Adams, 2003), it is now undisputed. But how and where zeaxanthin exerts its influence on NPQ and how it interacts with PsbS is still subject to debate. The lowest excited singlet states (S_1) of antheraxanthin and zeaxanthin lie just below that of singlet chlorophyll, permitting downhill excitation transfer from chlorophyll to these xanthophylls, but not to violaxanthin which has a slightly higher S_1 state, lying above that of chlorophyll (Frank et al., 1994). Additionally, NPQ may result from charge transfer from chlorophyll to zeaxanthin, via the transient formation of a radical zeaxanthin cation (Holt et al., 2005). In both cases, the reversible xanthophyll cycle would control the conversion between accessory light-harvesting violaxanthin pigments to energy-dissipating antheraxanthin and zeaxanthin pigments. Alternatively, the effect of zeaxanthin on NPQ may work via allosteric regulation, whereby binding of zeaxanthin to LHCs leads to a change in the pH sensitivity of NPQ induction towards higher pH values, effectively priming NPQ for repeat exposure (Nocter et al., 1991). In this case, the induction of NPQ is not a result of zeaxanthin as a quencher, but rather of the protonation and aggregation of LHCs, both of which are promoted by the presence of zeaxanthin (Horton et al., 1991; Ruban et al., 2012). Although these mechanisms are not necessarily specific about which LHC is involved, nor mutually exclusive, they all rely on zeaxanthin to interact and associate with LHCs. All LHCs (majors and minors) have a conserved structure with three transmembrane helices and a maximum of 14 chlorophylls per monomer. In addition, up to four carotenoid binding sites, L1, L2, N1 and V1, coordinate xanthophylls in each LHC (Jahns and Holzwarth, 2012). The N1 and V1 sites may not be present in all LHCs although their peripheral locations make this harder to assess, since any bound xanthophylls are easily removed during purification. From these four binding sites, experimental data seem to be most consistent with a putative role in NPQ for the xanthophylls bound to the V1 site in the LHCII trimers (Ruban et al., 1997, 1999) and the L2 site of the monomeric minor antennae (Dall'Osto et al., 2005; Ahn et al., 2008), although it is also possible that zeaxanthin interacts with chlorophyll at other peripheral positions between the protein-protein interface of adjacent LHCs (Xu et al., 2015).

Whereas PsbS and zeaxanthin are both required for the full induction of NPQ, PsbS seems to be specifically associated with energy-dependent quenching or qE (Wraight and Crofts, 1970), named to indicate a form of NPQ which directly depends on low lumen pH and thus readily switches off in 10-30 seconds in darkness. The accumulation of PsbS protein strongly determines the amplitude of qE (Li et al., 2002b) with saturation reached approximately 5 PsbS per D1 protein. In contrast to PsbS, zeaxanthin seems to affect multiple forms of NPQ. A threshold level of zeaxanthin seems to be required for the complete

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formation of qE (Niyogi et al., 1998) and when zeaxanthin levels are elevated, qE induces more rapidly and relaxes more slowly. In addition, zeaxanthin is also associated with a form of NPQ called qZ (Nilkens et al., 2010) which does not directly depend on the lumen pH, but instead depends on the turnover kinetics of the reversible xanthophyll cycle. Thus, through its involvement in qE and qZ, zeaxanthin provides a memory effect to NPQ, to maintain higher levels after repeated or prolonged exposure to high light.

6 Manipulating non-photochemical quenching to improve photosynthetic efficiency

The previous paragraphs have introduced NPQ as an important photoprotective mechanism to harmlessly dissipate excess energy. Exposure to light in the absence of adequate photoprotection leads to a decrease in the light use efficiency of photosynthesis, which is clearly observable in the shape of light response curves of electron transport rate or CO_2 fixation. These light response curves can be simplified as non-rectangular hyperbolae (NRH; Fig. 4).

Under low light, photosynthesis is determined by the initial slope, which represents the maximal efficiency with which light energy can be used to drive electron transfer ($\varphi PSII_{max}$) or CO_2 fixation ($\varphi CO_{2,max}$). In contrast, at high light, electron transport and CO_2 fixation rates are no longer limited by available light energy and plateau at their maximum capacities J_{max} and A_{sat}. The inflection from the initial slope towards the asymptote values is determined by a curvature factor θ, which ranges between zero and one for very gradual or sharp transitions, respectively. When photosynthesis is inhibited due to high light exposure, this can be seen in a decrease in the initial slope of the light response, as well as a decrease in θ and eventually in A_{sat} when the inhibition is particularly severe. Decreases in any of the NRH parameters thus signify that electron transport and CO_2 fixation rates fall short of the uninhibited rates in part or across the full light response.

Photoinhibition can either result from reversible downregulation of light-harvesting via NPQ processes as described in previous paragraphs or via photoinactivation, often involving photodamage of the reaction centre protein D1 (Aro et al., 1993; Keren and Krieger-Liszkay, 2011) which requires a dedicated repair cycle to recover uninhibited efficiency, taking hours or even longer. In contrast, photoinhibition due to NPQ is usually reversible in timescales ranging from seconds to minutes (qE) or minutes to hours (qZ) although some sustained forms of NPQ such as qH (Malnoë, 2018) can also take much longer. Using a modelling approach to simulate the decrease in maximal PSII quantum yield as a function of light history, Long et al. (1994) predicted the impact of transient photoinhibition due to reversible photoprotection on a clear-sky day in southern England. Based on their calculations, canopy CO_2

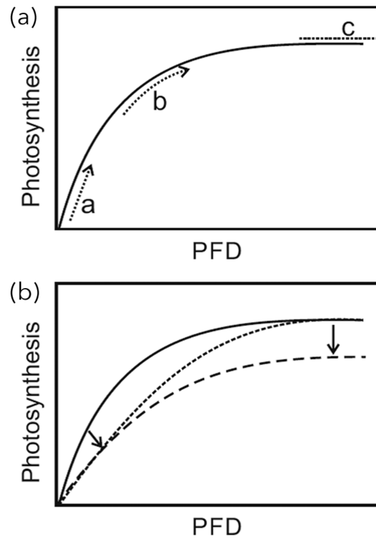

Figure 4 Non-rectangular hyperbolae to describe the light response of photosynthesis. Photosynthesis in non-stressed leaves (a) can be depicted by hyperbolic response to light intensity, which can be characterised by a non-rectangular hyperbole with parameters a (initial slope), b (curvature factor) and c (asymptote). (b) Photoprotective downregulation of light-harvesting efficiency or photoinhibition due to reaction centre damage both can lead to a decrease in the initial slope (dotted line), as well as the curvature factor and asymptote parameters (intermitted line) in more severe cases.

fixation of a hypothetical canopy with a leaf area index of 3 m^2 leaf/m^2 soil could be decreased by approximately 9%, with more pronounced losses at a higher leaf area index. Using a 3D model parameterised for Mediterranean oak, Werner et al. (2001) calculated a hypothetical loss of 6.1% carbon gain as a lower bound of photoinhibitory losses. In both cases, the light environment and concomitant loss of efficiency were predicted at hourly resolution. Realising that the averaging of light intensity profiles over hourly timesteps would remove the sharp transitions in light occurring within a plant canopy, Zhu et al. (2004) performed simulations with a ray-tracer algorithm at one second time resolution for a clear-sky spring day at 44°N at a range of temperatures. Reversible photoprotection was predicted to cause greater carbon loss in chilling-susceptible species (maize) compared to chilling-tolerant species (willow), ranging between 17–32% and 13–24%, respectively. The increase in predicted losses associated with the higher time resolution of the simulated light environment emphasised the importance of being able to recover more rapidly from the photoinhibited state.

Experiments in which PsbS was overexpressed to enhance qE were done for a range of plant species, such as the model plant *A. thaliana* (Li et al., 2002b), as well as the crop species rice (Hubbart et al., 2012) and tobacco

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(Głowacka et al., 2018). The effect on NPQ induction and recovery is shown in Fig. 5a. An increase in PsbS levels results in the enhancement of the level of qE, which potentially decreases the extent of photoinhibitory damage. If so, this could enhance the recovery rate of photosynthetic efficiency after high light exposure since recovery from qE is much more rapid than from photodamage. The effects on crop productivity were surprisingly different between rice and tobacco. PsbS overexpressing rice plants showed higher biomass accumulation and yield under greenhouse conditions (Hubbart et al., 2018), whereas field-grown tobacco plants overexpressing PsbS were instead slightly smaller in size and weight than the control plants (Głowacka et al., 2018). These differences may be explained by the differences in plant architecture between these two species, leading to vastly different light environments. In addition, the PsbS overexpressing tobacco plants were found to have a dampened stomatal opening response to light, which enhanced photosynthetic water use efficiency but may have impacted their growth characteristics.

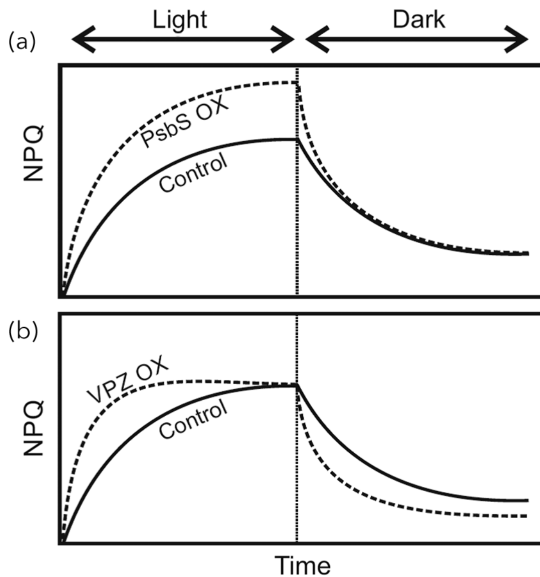

Figure 5 NPQ induction and recovery as a result of different genetic manipulations. Using the experimental protocol shown in Fig. 3, NPQ induction and relaxation can be quantified for genotypes with contrasting characteristics. The plots show example responses to illustrate the main effects associated with specific transgenic manipulations. (a) Overexpression of photosystem II subunit S (PsbS) increases the amplitude of qE and thereby total NPQ. Relaxation kinetics is similar to the control. (b) Combined overexpression of violaxanthin de-epoxidase (VDE), PsbS and zeaxanthin epoxidase (ZEP) increases induction and relaxation kinetics of NPQ, while maintaining the same amplitude of the NPQ response.

Since the overexpression of PsbS raises the amplitude of NPQ, this is unlikely to decrease the losses due to slowly reversible photoprotection, such as qZ, substantially. Experiments with plants overexpressing β-carotene hydroxylase 1 (Johnson et al., 2008), which have an enlarged xanthophyll cycle pool size, demonstrated that the cycle turnover rate is controlled by the VDE and ZEP enzyme abundance relative to the size of the pigment pool. Thus, in an attempt to speed up NPQ responses without affecting the amplitude of the response, Kromdijk et al. (2016) overexpressed the xanthophyll-converting enzymes VDE and ZEP in conjunction with PsbS in transgenic tobacco plants. This strategy aimed to speed up the xanthophyll cycle to decrease qZ amplitude and speed up qZ recovery, as well as complement the decrease in total NPQ levels specifically with qE, by overexpressing PsbS. The resulting VPZ overexpressing plants showed NPQ responses with similar amplitude to the control plants at high light, but a faster recovery rate leading to lower NPQ than the control plants at low light (Fig. 5b). Interestingly, these differences could only be observed under fluctuating light conditions, whereas under constant light, NPQ levels were indistinguishable from the control plants. In line with the model predictions by Zhu et al. (2004), this acceleration of NPQ responses improved photosynthetic efficiency, as well as biomass productivity under greenhouse and field conditions by 15%. However, when the same approach was used in *Arabidopsis*, productivity was negatively affected (Garcia-Molina and Leister, 2020). Thus, similar to the effects of overexpression of PsbS, the effects of VPZ overexpression on photosynthetic efficiency and productivity seem to be specific to species and growth conditions. In fact, the large impact of NPQ relaxation kinetics on photosynthetic efficiency is likely to be enhanced by an ongoing change in cultivation conditions. Namely, the trend in increasing plant density across many staple crops (e.g. Assefa et al., 2018) has led to an increasing proportion of leaves routinely subjected to sharp fluctuations in light intensity and may also make yield more dependent on photosynthetic efficiency (Sales et al., 2021). Adjusting the photosynthetic and photoprotective responses to these changing production conditions may offer opportunities to enhance photosynthetic efficiency and crop productivity.

7 Conclusion

Plants routinely absorb light energy strongly in excess of potential utilisation via photochemistry and have therefore developed elegant feedback mechanisms to harmlessly dissipate this energy. Modification of these NPQ mechanisms in the chloroplast thylakoid membrane system can have a profound effect on plant performance under fluctuating field conditions and could offer a trajectory towards enhanced photosynthetic efficiency and crop productivity.

8 Where to look for further information

The following articles provide a good overview of the subject:

- Murchie, E. H. and Niyogi, K. K. (2011). 'Manipulation of photoprotection to improve plant photosynthesis', *Plant Physiol*., 155(1), 86–92.
- Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K. and Long, S. P. (2016). 'Improving photosynthesis and crop productivity by accelerating recovery from photoprotection', *Science*, 354(6314), 857–861.
- Murchie, E. H. and Ruban, A. V. (2019). 'Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity', *Plant J.*, 101(4), 885–896.

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