Natural variation in phosphorylation of photosystem II proteins in Arabidopsis thaliana: is it caused by genetic variation in the STN kinases?

Philosophical Transactions of the Royal Society of London. Biological Sciences (ISSN: 0962-8436)

Citation for the published paper:
Flood, P. ; Yin, L. ; Herdean, A. (2014) "Natural variation in phosphorylation of photosystem II proteins in Arabidopsis thaliana: is it caused by genetic variation in the STN kinases?". Philosophical Transactions of the Royal Society of London. Biological Sciences, vol. 369(1640), pp. 20130499.

http://dx.doi.org/10.1098/rstb.2013.0499

Research paper in Phil. Trans. R. Soc. B 2014 **369**, 20130499, published 3 March *2014*
Published version available to download at rstb.royalsocietypublishing.org

Natural variation in phosphorylation of photosystem II proteins in *Arabidopsis thaliana* – is it caused by genetic variation in the STN kinases?

Pádraic J. Flood[1,2,*], Lan Yin[3,*], Andrei Herdean[3], Jeremy Harbinson[2], Mark G.M. Aarts[1] and Cornelia Spetea[3,**]

[1] Laboratory of Genetics and [2]Horticulture Production Chains Group, Wageningen University, 6708 PB Wageningen, The Netherlands; [3] Department of Biological and Environmental Sciences, University of Gothenburg, 40530 Gothenburg, Sweden.

* These authors equally contributed to the work
**Author for correspondence
(cornelia.spetea.wiklund@bioenv.gu.se).

Reversible phosphorylation of photosystem II (PSII) proteins is an important regulatory mechanism that can protect plants from changes in ambient light intensity and quality. We hypothesized that there is natural variation in this process in *Arabidopsis* (*Arabidopsis thaliana*), and that this results from genetic variation in the *STN7* and *STN8* kinase genes. To test this, *Arabidopsis* accessions of diverse geographic origins were exposed to two light regimes, and the levels of phospho-D1 and phospho-LHCII proteins were quantified by western blotting with anti-phosphothreonine antibodies. Accessions were classified as having high, moderate or low phosphorylation relative to Col-0. This variation could not be explained by the abundance of the substrates in thylakoid membranes. In genotypes with atrazine-resistant forms of the D1 protein, low D1 and LHCII protein phosphorylation was observed, which may be due to low PSII efficiency resulting in reduced activation of the STN kinases. In the remaining genotypes, phospho-D1 levels correlated with STN8 protein abundance in high light conditions. In growth light, D1 and LHCII phosphorylation correlated with longitude and in the case of LHCII phosphorylation also with temperature variability. This suggests a possible role of natural variation in PSII protein phosphorylation in the adaptation of *Arabidopsis* to diverse environments.

Keywords: *Arabidopsis thaliana*; natural variation; phosphorylation; photosystem II; STN kinase; temperature seasonality.

1. INTRODUCTION

Plants require light energy to drive photosynthesis. Their photosynthetic machinery is profoundly affected by changes in irradiance, which can be in both the intensity and in the spectral quality of light, and can occur across a range of temporal scales, from seconds (light flecks) to months (seasonal changes). Plants have developed diverse response mechanisms to adjust and protect their photosynthetic machinery in the face of such fluctuations (for recent review, see [1]). Short-term responses involve a dynamic reorganization of photosynthetic complexes, whereas long-term responses involve changes in the chloroplast and nuclear gene expression resulting in altered levels of the photosynthetic machinery to optimize and sustain photosynthesis. Both types of responses are induced by changes in the redox state of the photosynthetic electron transport chain, and are mediated through a complex network of reactions involving protein kinases and phosphatases [1]. Plants which have a compromised ability to respond to both short and long term light fluctuations show fitness costs in nature [2, 3]. In this study we aimed to quantify the natural genetic variation in these responses, both to document the extent of this variation, and to gain some initial insights into the selective forces, which may be acting on these processes.

Photosystem II (PSII) and PSI are connected in series through the electron transport chain, which includes the plastoquinone (PQ) pool, the cytochrome b_6f ($Cytb_6f$) complex and plastocyanin. Changes in the light environment may lead to more reduction or oxidation of the PQ pool initiating signaling processes that drive changes in the organization and composition of the photosynthetic machinery. The process of state transitions is used by algae and also plants, to correct the redox state of the PQ pool. In case of a reduced PQ pool, plastoquinol (PQH_2) docks to the Q_o site of $Cytb_6f$ [4]. This event leads to the activation of a protein kinase that phosphorylates several proteins of the light-harvesting complex II (LHCII). Upon phosphorylation, the mobile part of LHCII is displaced from PSII to PSI, thus re-equilibrating the cross-sections of the antennae of PSII and PSI and their respective light excitation. The process is reversible, as over-excitation of PSI causes the oxidation of the PQ pool, deactivation of the kinase, dephosphorylation of LHCII proteins by a phosphatase and the return of LHCII to PSII. Using molecular genetic approaches the LHCII kinase was identified in *Arabidopsis* and named STN7 [5].

Of the two photosystems, PSII is more susceptible to photoinactivation, and undergoes a repair cycle to replace its reaction centre D1 protein [6]. In the plant thylakoid membrane, PSII is mostly present as PSII-LHCII dimeric supercomplexes located in the appressed (grana) membranes. However, the repair of photoinactivated PSII complexes and the assembly of new ones occur through the monomeric form of PSII in the nonappressed (stroma) thylakoid membranes. PSII core protein phosphorylation in general, and D1 phosphorylation in particular, has been suggested to facilitate disassembly of photoinactivated PSII complexes and is thought to play a role in the regulation of PSII repair [7]. The kinase involved in PSII core protein phosphorylation was identified in *Arabidopsis* and named STN8 [8, 9].

To elucidate the substrate specificity of STN7 and STN8 kinases, thylakoid protein phosphorylation patterns of wild-type *Arabidopsis* plants, *stn7* and *stn8* mutant lines have been monitored by different approaches, including Western blot analyses with different anti-phosphothreonine antibodies, and mass spectrometric analyses. The STN7 kinase is involved in phosphorylation of LHCII, CP29, CP26 and TSP9 proteins, whereas the STN8 kinase phosphorylates PSII core D1, D2, PsbH and to some extent CP43 proteins (for reviews, see [10, 11]). Besides the PSII core proteins, STN8 has additional targets, including the chloroplast calcium-sensing protein CAS [12], and a protein involved in cyclic electron

transport (PGRL1) [13], which is a part of the PSI-LHCI-LHCII supercomplex [14]. Analysis of the protein phosphorylation profiles of the *stn7* and *stn8* mutants of *Arabidopsis* showed residual phosphorylation of the LHCII and PSII core proteins, respectively. However, this residual phosphorylation was undetectable in the *stn7* × *stn8* double mutant, indicating some substrate overlap between the STN7 and STN8 kinases [8, 15].

The STN7 kinase appears to have a broader role than state transitions, and is also required for adaptation to light fluctuations [16]. For example, by subjecting *Arabidopsis* plants to alternative periods of low light and high light (HL), LHCII is phosphorylated during the low light and dephosphorylated during the high-light periods [17]. The loss of STN7 in plants subjected to this fluctuating light regime leads to a severe decrease in growth, indicating that STN7 has an important role in response to environmental changes [5, 18]. The loss of STN8 resulted in slower growth in rice [19] but not in *Arabidopsis* [9]. In both species, the mutation leads to increased susceptibility of PSII to high light due to suppressed mobility of inactivated complexes during repair. A high level of PSII core protein phosphorylation is required for the adjustment of macroscopic folding of the thylakoid membrane, which modulates protein mobility in this membrane [15]. Significant enhancement in the thylakoid grana size in the *stn8* mutant slows down the movement of PSII from the grana to the stromal region during repair cycle, indicating that PSII core protein phosphorylation is involved in this process. Since the *stn8* mutant also displays reduced cyclic electron transport, the possibility has been raised that STN8 kinase activity may be important for fine-tuning of the photosynthetic machinery to fulfill the NADPH/ATP demands of chloroplast metabolism [13].

Following the identification of the two kinases, their substrates and functions, an important remaining question concerns their mode of regulation. Previously, it was thought that light activates LHCII as a phosphorylation substrate by increasing the exposure of its N-terminal domain, containing the phosphorylation site, to the enzyme [20]. Another report provided evidence in support of a light-induced exposure of the phosphorylation site of the CP43 subunit of the PSII core complex [21]. The activation of the STN7 kinase was proposed to be strictly regulated by the redox state of PQ and the $Cytb_6f$ complex [5, 22]. In support of this, following a transfer from darkness to growth light (GL), an increase in the amount of the STN7 kinase was reported [23]. The protein abundance of STN7 is regulated in a post-translational manner involving proteolysis and autophosphorylation [24]. Most recently, it was shown that accumulation of the STN7 protein is controlled at the transcript level, in a light- and redox-dependent manner [25]. While low-light conditions increase STN7 kinase activity *in vivo*, HL levels bring about its inhibition. This inhibition appears to be in response to the increasing degree of reduction of the stroma and to be mediated by thioredoxin. This possible thioredoxin-linked inactivation of the kinase depends on, and is therefore subordinated to, the prior activation of the kinase by the redox state of PQ and the $Cytb_6f$ complex [22].

Compared to what is known about the regulation of STN7, much less is known about the determinants of STN8 activity. Upon transfer from the dark to GL, an increase in D1 phosphorylation was reported [23]. STN8 is even more active under HL conditions as the further increase in D1 phosphorylation in HL shows. The increased activity correlated with an increase of STN8 upon transfer from darkness to HL [23]. Another report found no change in STN8 level upon transfer from low to high light conditions despite increase in D1 protein phosphorylation [26], indicating that the regulation of kinase activity in response to changes in light intensity may be more important than its changing abundance. No interdependence seems to exist between the STN kinases at protein levels [25]. Nevertheless, it has been proposed that the activity of STN8 may be regulated by STN7 in *Arabidopsis*, since in the

green alga *Chlamydomonas reinhardtii* the activity of STN8 homologue Stl1 is regulated by its phosphorylation in an Stt7-dependent manner [27].

As outlined above, both the STN7 and STN8 kinases play essential roles in the response to changes in ambient light, by influencing LHCII distribution between PSII and PSI and facilitating protein repair, respectively. A recent review compares such responses across a wide phylogenetic spectrum [28], however very little is known about the intraspecific variation in these processes [29]. Such within-species variation drives natural selection and may represent different adaptive strategies to photosynthetic regulation within a species. *Arabidopsis* has a wide geographic distribution, ranging from Tanzania to Norway and from Portugal to Japan. As such it occupies a wide range of light environments it may have adopted different strategies to cope with this environmental diversity. Here we screen 16 *Arabidopsis* genotypes comprised of 13 diverse accessions from throughout the natural range of *Arabidopsis*, as well as a backcross, the *stn8* mutant and a hybrid between two of these accessions. We have analyzed thylakoid protein extracts by western blotting with anti-phosphothreonine antibodies and we used the levels in the standard accession Col-0 as reference. Furthermore, we have investigated whether variation in the protein or transcript level of the respective kinase, as well as the geographic and climatic origin of the genotype correlate with the observed variation in PSII protein phosphorylation.

2. MATERIAL AND METHODS

(a) *Plant growth and light treatment*

Arabidopsis thaliana plants were grown for 34 days in a chamber at 100 μmol photons m^{-2} s^{-1} (GL) using a 10/14hr day/night cycle. After 14 h of darkness, plants were exposed for 3 h to GL and subsequently transferred for 3 h to high light (HL, 600 μmol photons m^{-2} s^{-1}). Leaf material corresponding to 2-3 g bulked from 5-6 plants was harvested, frozen immediately in liquid N_2 and stored at -80° C until thylakoid isolation.

A total of 16 *Arabidopsis* genotypes were included in this study (table 1). We used 13 naturally occurring accessions from a range of geographic locations. In addition, a previously characterized *stn8* mutant line lacking a functional STN8 kinase [15] was used. One F1 hybrid was included (Tsu-0 × Ws-4) to test for the presence of a dominant phenotype and because pilot experiments identified both parental accessions as extremes. The atrazine-resistant Ely accession was included because it has compromised PSII functioning due to a mutation in the chloroplast-encoded D1 protein in the Q_B-binding pocket (Ser264Gly) [30]. Cytoplasm from the Ely genotype was introduced into the L*er*-1 nuclear background by six rounds of back crossing (genotype (Ely × L*er*) × L*er* BC6 (ELB)). In addition to altered PSII efficiency, ELB allowed us some preliminary insights into the role of cytoplasmic variation versus nuclear variation on PSII protein phosphorylation.

(b) *Thylakoid isolation and protein analysis*

Thylakoid membranes were isolated from frozen leaves as previously described [31] with the modification that 10 mM NaF (a general phosphatase inhibitor) was included in all isolation buffers. Chlorophyll (Chl) was extracted in 80% (v/v) acetone and the concentration was determined according to [32]. Thylakoid proteins were separated by SDS-PAGE in 14% (w/v) acrylamide gels containing 6 M urea followed by electrotransfer and immunoblotting with various antibodies. The following antibodies were used: anti-D1, anti-Lhcb2, anti-STN8 and anti-STN7 from Agrisera (Umeå, Sweden), anti-phosphothreonine antibodies from Cell Signalling (New England BioLabs, U.K.) and Zymed (Invitrogen, Carlsbad, USA), and anti-CP43 from our laboratory. Western blots were analysed using a Fusion FX-7 imager (Vilbert

Lourmat, France) and quantified using Multi Gauge software. Col-0 was used as internal standard, making it possible to compare different Western blots.

As shown by representative blots for Col-0, both anti-phospho-Thr antibodies recognized PSII phosphoproteins, but with different affinities (figure 1*a*). The Zymed antibody recognized the phospho-D1 protein best, whereas the Cell Signaling antibody was found most suitable for quantification of phospho-LHCII proteins. Two Chl loadings are shown to indicate the linearity of the immunodetected signal. Any possible variation in phosphorylation could be caused by variation in the amount of substrate, kinase involved or other factors. Western blots with anti-D1 and anti-Lhcb2 antibodies indicated the level of corresponding substrate for the phosphorylation reaction (figure 1*a*). The levels of the STN8 and STN7 kinases were assessed in samples from thylakoid preparations using specific antibodies, and control western blots with CP43 protein were used to correct the amount of protein loaded (figure 1*b*). The *stn7* × *stn8* double mutant [33] was used to verify the identity of the corresponding cross-reacting bands with the STN8 and STN7 antibodies. Two Chl loadings are shown to indicate the linearity of the immunodetected signal for the three antibodies.

The selected accessions were analyzed by the assay described above using conditions optimized for Col-0. The levels of various immunodetected proteins were determined and expressed relative to those in Col-0 (Supplementary tables S1 and S2). The genotypes were classified as displaying high (80-120%), moderate (40-80%) or low (<40%) levels of immunodetected proteins.

(c) ***RNA isolation and transcript analysis***

RNA was isolated from frozen leaves as described in [34]. One fully expanded leaf was taken from three plants after 3 h of GL or HL treatment. The RNA concentration was measured using a NanoDrop 2000 and the volume adjusted with Millipore water to obtain a final concentration of 0.25 µg/µl. 1 µg of RNA was used for cDNA synthesis. Equal volumes of cDNA were used in all subsequent qPCRs. Eight reference genes [35, 36] were tested on all samples (Supplementary table S3) and seven of them were found stable enough for use in further analysis. Primer sequences are listed in Supplementary table S3. A normalisation factor was calculated from the seven reference genes, which was used to calculate relative transcription levels.

(d) ***Statistical analysis***

Scatterplots were created in GraphPad Software (La Jola, CA). Best-fit lines were applied and the correlation coefficient (r^2) and its significance (two-tailed p value) were calculated with the same software. Correlations were considered significant at $p \leq 0.05$.

3. RESULTS

(a) ***D1 protein phosphorylation in growth and high light conditions***

Large differences in D1 protein phosphorylation were observed in GL in the 16 genotypes ranging between 4-100% of the phosphorylation level found in Col-0 (figure 2*a*). D1 protein level in the studied accessions ranged between 70 and 120% of Col-0 (Supplementary table S1), and cannot explain the large variation observed in phosphorylation. Notably, the relative level of the STN8 kinase varied largely among accessions (78-192%) (figure 2*a*). Col-0 had the highest phosphorylation level, which was approx. 20% higher than that of the next highest genotype (Tsu-0; 79%, Supplementary table S1). Both genotypes also displayed high levels of the STN8 kinase. Four genotypes (C24, Per-1, Ws-4 and Tsu × Ws-4) displayed moderate

levels of phosphorylation but high levels of STN8 kinase*,* Five accessions displayed low D1 protein phosphorylation (Bur-0, Pa-2, Mt-0, Bor-4, Can-0, and Ely) although had high STN8 levels. Sha, L*er*-1, and ELB had very high STN8 levels (>120% of Col-0) but low D1 phosphorylation. Thus, there was no correlation between the STN8 protein level and the level of D1 phosphorylation, though *stn8,* which had the lowest level of phospho-D1, lacked any STN8 (figure 2*b*). The Tsu × Ws-4 hybrid and the ELB backcross were moderate or lower than their parents in the D1 phosphorylation levels, despite having high(er) levels of the STN8 kinase. These data indicate that there must be another limiting/regulating factor for D1 protein phosphorylation under GL conditions than the substrate or the kinase levels.

D1 phosphorylation in HL ranged between 2-117% of Col-0 (figure 2*b*). As in GL, there was little variation in D1 protein level (87-114%), whereas STN8 abundance showed greater variation (32-121%) among accessions (Supplementary table S2). Under HL conditions, there was a much tighter grouping of genotypes than in GL with the exception of *stn8*, Ely and ELB showing low D1 phosphorylation (compare figure 2*a* to figure 2*b*). There was a general upward trend in D1 phosphorylation, and Col-0, whilst still high, was no longer an outlier. Six accessions displayed high phosphorylation levels and also high STN8 levels (Col-0, Bur-0, Mt-0, Per-1, Bor-4 and Tsu × Ws-4) (Supplementary table S2 and figure 2*b*). Other three displayed moderate phosphorylation levels and also moderate STN8 levels (Sha, C24 and Ws-4). The *stn8* mutant displayed residual D1 phosphorylation. The remaining genotypes displayed either high levels of phospho-D1 despite moderate STN8 kinase levels (Pa-2, Tsu-0 and L*er*-1) or moderate levels of phospho-D1 and low kinase levels (Can-0). Ely and ELB were found low phosphorylation accessions despite high STN8 protein levels, suggesting that kinase abundance was not limiting. No significant correlation was obtained between phospho-D1 level and STN8 abundance if all accessions were included. Nevertheless, a weak but significant correlation was obtained if Ely and ELB were excluded from the analysis (figure 2*b*).

When comparing GL to HL conditions, the phosphorylation of the D1 protein increased by 70%, whereas the STN8 level remained stable in Col-0 (figure S1 *a, b*). The other accessions also displayed increased levels of phospho-D1, but the level of the STN8 protein either decreased (ELB, Pa-2, L*er*-1, Can-0, Tsu-0, Ws-4 and Tsu × Ws-4), remained quite stable (C24, Bur-0, Per-1, Bor-4 and Ely) or even increased (Mt-0) upon transfer from GL to HL. All genotypes showed reduced transcription of *STN8* in HL with the exception of ELB, C24, Bur-0, Pa-2, Sha and Mt-0, which either showed an increase or no difference in transcription (figure S1*c*).

(b) ***LHCII protein phosphorylation in growth and high light conditions***

Phosphorylation of LHCII proteins in GL varied between 7-102 % of Col-0 (figure 3*a*). With the exception of Ely and ELB, the level of Lhcb2 showed limited variation, whereas STN7 protein levels varied between 70-213% of Col-0 (Supplementary table S1 and figure 3*a*). There were seven high phosphorylation genotypes (Col-0, *stn8*, Sha, Mt-0, Per-1, Tsu-0, Tsu × Ws-4), seven moderate (C24, Bur-0, Pa-2, Bor-4, Can-0, Ws-4 and L*er*-1), and two low accessions (Ely and ELB). All high phosphorylation accessions displayed high levels of STN7 kinase, including Col-0 and the *stn8* mutant. Accessions displaying moderate levels of LHCII phosphorylation accumulated STN7 at either high or very high (>120%) levels, indicating that other factors limited the kinase activity. Tsu × Ws-4 displayed similar high phospho-LHCII as Tsu-0 although it had much higher levels of STN7 (213 versus 70%). One striking observation was that both genotypes with the atrazine-resistant cytoplasm, Ely and ELB, showed Lhcb2 levels of 143 and 126%, respectively, relative to Col-0, and yet showed extremely low levels of LHCII phosphorylation. The reduced phosphorylation levels in these

two genotypes cannot be explained by the abundance of the kinase, which was 71 and 163% relative to Col-0, and indicate that the STN7 kinase was not properly activated.

Phosphorylation of LHCII proteins in HL varied between 5-107% relative to Col-0, whereas Lhcb2 and STN7 protein levels varied between 89-123% and 58-179% relative to Col-0, respectively. Col-0, Sha, C24, Bur-0, Pa-2, Mt-0, Tsu-0, Tsu × Ws-4 and L*er*-1 were high phosphorylation accessions, Per-1, Bor-4 and Ws-4 were moderate, and Can-0, Ely and ELB were low phosphorylation accessions (Supplementary table S2). As in GL conditions, the level of kinase did not appear to correlate with the level of LHCII phosphorylation (figure 3*b*). Genotypes with high phosphorylation, including Col-0 and the *stn8* kinase, displayed high to very high levels of STN7 kinase. Also the moderate and the low phosphorylation accessions displayed high STN7 levels, indicating that factors related to activation rather than the amount of kinase may limit the phosphorylation reaction.

When comparing GL to HL, LHCII phosphorylation decreased by 30% in Col-0, whereas STN7 abundance remained stable (figure S2*a*, *b*). With few exceptions (Ler-1, C24 and Bur-0), phospho-LHCII also decreased in the other accessions, whereas STN7 abundance varied between the two light regimes. The *STN7* transcript level in GL was comparable among genotypes (figure S2*c*). Upon transfer to HL, the *STN7* transcript abundance decreased in all genotypes except ELB and Mt-0. The extent of reduction in transcript abundance varied considerably, with Col-0, C24, Per-1 and Ely showing a much more pronounced reduction than the other accessions. Interestingly, the *stn8* mutant did not show the same response as Col-0 wild type, retaining relatively higher expression of STN7 in HL.

4. DISCUSSION

(a) *Variation in PSII protein phosphorylation and factors involved*

The reversible and differential phosphorylation of PSII proteins is dependent on the interplay between the STN7 and STN8 kinases. This process has been intensively studied in the standard lab accession Col-0 and *stn* mutants in the Col-0 background. A recent report compared phosphorylation levels in Col-0 with those in L*er*-0 and Ws-4, found that Ws-4 displayed 50% lower phospho-D1 and attributed this difference to 50% less STN8 kinase than in the other two accessions [23]. Here we report on the occurrence of variation in D1 and LHCII protein phosphorylation ranging between approx. 5-120% in *Arabidopsis* accessions of diverse geographic origins, when expressed relative to Col-0. The large differences observed in GL in this set of accessions were not caused by variation in the amount of substrate or STN7 and STN8 protein levels. In HL, the levels of D1 phosphorylation correlated with the STN8 kinase levels, indicating that kinase abundance can be a limiting factor for phosphorylation under these conditions.

Among the genotypes we analyzed, some resembled the standard lab accession Col-0 in phosphorylation levels and were classified as high accessions. However, the other accessions displayed moderate or even low phosphorylation levels. Why would *Arabidopsis* accessions have variable phosphorylation of PSII proteins? Is this an adaptive mechanism facilitating survival and reproduction across the range of environmental conditions where *Arabidopsis* naturally occurs? In support of this hypothesis, we found a significant correlation between longitude and both D1 and LHCII phosphorylation in GL conditions (figure 4 *a, b*). This suggests that there may be some form of selective pressure that correlates with longitude. In order to test this, climate data were obtained from the WorldClim database [37] (http://www.worldclim.org/). Bioclimatic variables 4 (temperature seasonality) and 7 (temperature annual range) correlated significantly with phospho-LHCII in GL conditions (figure 4 *c, d*). Whilst there was some correlation between these climatic variables and

phospho-D1, it was not found to be significant. This correlation between temperature variability and PSII protein phosphorylation in GL conditions is interesting and may be the outcome of a photoprotective mechanism similar to that observed in evergreen trees which must maintain functioning leaves in very cold conditions [38]. Interestingly there was no correlation between protein phosphorylation and longitude in HL conditions, which could be due to a stronger, more geographically uniform, selective pressure in the HL response. This makes sense considering the damage an inappropriate response to HL can cause. To better understand the relationship between phosphorylation and the natural habitat, many more accessions will need to be investigated from a wide range of environments.

In line with published data [17, 23], we show that HL-treated plants contained more phospho-D1 whereas GL-treated plants displayed a higher extent of LHCII phosphorylation. The amount of kinase involved could be one mechanism to regulate enzyme activity, as indicated by the significant correlation between phospho-D1 levels and STN8 abundance in HL (figure 2*b*). In the case of *Arabidopsis* STN7 or its *Chlamydomonas* homologue Stt7, it has been suggested that their amounts are regulated by the redox status of the electron transport chain, by phosphorylation and by transcript abundance [24, 25]. In our panel we had two genotypes, Ely and ELB, that displayed reduced LHCII phosphorylation under both GL and HL conditions but high STN7 protein levels (figure 3). These genotypes are atrazine resistant due to a deficient binding of quinones in the Q_B pocket, and as a result have reduced PSII efficiency [30]. Therefore, they are likely to have a more oxidized PQ pool especially at limiting irradiances. Although this requires experimentation, we use as support of our assumption the fact that at low, light-limiting irradiances Chl *b* deficient barley mutants displayed a more oxidized PQ pool than the wild type due to diminished PSII activity relative to PSI activity [39]. However, the unaffected abundance of STN7 protein relative to Col-0 in our study indicates that the redox state did not alter STN7 expression level. Therefore, the observed reduced level of LHCII phosphorylation is most likely due to reduced kinase activity.

(b) ***Regulation of D1 protein phosphorylation***

The factors regulating the amount of STN8 kinase in the membrane have not yet been investigated. D1 protein phosphorylation requires the presence of the STN8 kinase since phospho-D1 is hardly detected in the *stn8* mutant, and what remains is most likely due to a partial replacement by STN7 or other yet unknown kinases [8]. This potential redundancy between STN7 and STN8 is illustrated by the much higher *STN7* transcript and also protein levels in the *stn8* mutant in HL when compared to Col-0. The mechanism by which this difference is mediated is not clear and may be either direct or indirect. This may be due to the absence of functional STN8 protein stimulating additional *STN7* transcription and translation under HL conditions. However, the *stn8* mutant displayed unaltered LHCII phosphorylation levels, suggesting that the STN8 kinase does not play any role in this process. STN8 levels in Col-0 did not change upon transfer from GL to HL conditions in line with [26].

All studied accessions displayed reduced D1 phosphorylation relative to Col-0 in GL despite high levels of the D1 substrate and STN8 kinase. One cause could be a poor activation of the STN8 kinase in GL, which could be related to the redox state of the PQ pool, as in the case of STN7. In support of this possibility is the low phosphorylation in Ely and ELB. As previously discussed with reference to STN7, due to low PSII efficiency, the PQ pool may be more oxidized which results in reduced activation of the kinase. ELB contains L*er*-1 nuclear DNA but the organellar DNA of Ely, and as such allows us to compare the phosphorylation level of PSII proteins in the same nuclear background. At both HL and GL ELB displayed half the phosphorylation level of D1 as that found in L*er*-1 (figure 2), thus resembling Ely in the deficient activation of STN8. Since ELB is effectively identical to L*er*-1 as far as nuclear

genome is concerned, the difference reflects the strong effect of the cytoplasm on the level of D1 phosphorylation. In all accessions except Ely and ELB, a weak but significant correlation was found in HL between phospho-D1 level and STN8 abundance. This indicates that under these conditions, the abundance of STN8 may be either limiting or plays a regulatory role in D1 phosphorylation.

(c) ***Regulation of LHCII protein phosphorylation***

The phosphorylation of LHCII proteins enables the excitation and redox balance between PSII and PSI under low irradiance. This process requires the STN7 kinase, which is activated by a reduced state of the PQ pool under these light conditions [5, 22]. Upon exposure to high light, the kinase is inactivated by a thioredoxin-mediated reduction of disulfide bonds [22]. In our study, many accessions displayed moderate LHCII phosphorylation in GL, whereas the genotypes with atrazine-resistant forms of D1, Ely and ELB, displayed only residual phosphorylation levels, whilst at the same time showing much higher levels of Lhcb2 protein than Col-0 (Supplementary table S1). There may be a common cause with D1 phosphorylation, namely the inability to fully reduce the PQ pool, and thus to activate the kinase. ELB resembles Ely in low levels of LHCII protein phosphorylation, thus in the deficient activation of STN7. The reduction in PSII efficiency in these genotypes [30] may result in both increased antennae size and reduced phosphorylation in order to increase PSII light-absorption relative to that of PSI under light-limiting conditions.

In our experimental conditions STN7 levels did not change upon shift from GL to HL in Col-0 despite decrease in LHCII phosphorylation levels (Supplementary figure S2), indicating that STN7 is regulated at activity rather than protein level. This observation is in contrast with a recent report about down-regulation of STN7 at both protein and transcript levels [26]. The reason for this discrepancy could be the distinct light regimes used in this study or other yet unknown factors. However, the protein levels did change in other accessions (Supplementary figure S2). Based on our results, the *STN7* transcript abundance decreased but did not correlate with the abundance of the STN7 protein (Supplementary figure S2). This is in contrast to a recent study which showed that the accumulation of the STN7 protein was controlled at the level of transcript abundance [25]. However, that study was performed on Col-0 wild type and mutants in the Col-0 background, and based on our results it appears that Col-0 does not show a typical level of transcription for *STN7*.

The picture that emerges from these primary studies is that of highly diverse levels of PSII protein phosphorylation in nature, in which kinase activation may play a central role at least under GL conditions. It is likely that under HL conditions PSII core protein phosphorylation is in addition regulated by STN8 protein abundance in the thylakoid membrane. Longitude and temperature variability may be involved at least under GL conditions in variation of PSII protein phosphorylation. In conclusion, the significant variation found in both traits highlights our lack of understanding of the role these processes play in plant performance in nature. Using knockout mutants it has been shown that a complete absence of the STN7 kinase, and to a lesser extent the STN8 kinase, results in reduced fitness. This fitness cost is much more pronounced in the double mutant [3], once again illustrating that there is some degree of functional redundancy. A further conclusion from this work is that Col-0 appears to be an outlier accession. It operates at the phenotypic extreme for this trait and as such is most likely not representative of thylakoid protein phosphorylation in *Arabidopsis*. Based on an analysis of both *STN7* and *STN8* sequences in the many re-sequenced *Arabidopsis* accessions as found in the '1001 Genomes' website (http://www.1001genomes.org/) it appears unlikely that the diversity of phosphorylation phenotypes observed is due to sequence variation in the kinase genes themselves. It is much more likely that the observed variation is due to variation elsewhere in the process, be that

upstream signaling or downstream dephosphorylation rates. In order to identify the genes responsible for such variation, genetic mapping studies using either Recombinant Inbred Line (RIL) populations or genome wide association mapping panels could be undertaken [40]. However currently the main limiting factor to such a study is not the availability of suitable genetic material but rather the ability to screen the necessary number of individuals (more than two hundred genotypes would be required), in sufficient replicates to allow for detection of the genetic loci involved, and even more to identify the causal sequence variation [41]. Identification of such genes is likely to provide us with additional insights into the regulation of the photosynthetic process and the selective pressures acting upon this trait. Such knowledge will not only be of use to fundamental research but is likely to provide new avenues to crop improvement whereby the photoprotective processes can be optimized for different agricultural or climatic conditions [29, 42].

Acknowledgements
We thank Prof. Maarten Koornneef (Max-Planck-Institute for Plant Breeding, Cologne, Germany/Wageningen University) for initiating collaboration between the two laboratories. We thank Drs. Ross Alexander and Bas Dekkers (Wageningen University) for advice on qPCR analysis and Dr. Joost van Heerwaarden (Wageningen University) for help with climate data. This work was supported by funding from the Swedish Research Council and the Olle Enkvists Foundation (to C.S.), the Netherlands organisation of Scientific Research section Earth and Life Sciences (NWO-ALW), the Technological Top Institute Green Genetics, and the BioSolar Cells research programme (to P.J.F., J.H. and M.G.M.A.).

REFERENCES

1. Rochaix J.D. 2013 Redox regulation of thylakoid protein kinases and photosynthetic gene expression. *Antioxid Redox Signal* **18**, 2184-2201. (doi:10.1089/ars.2012.5110).
2. Külheim C., Ågren J., Jansson S. 2002 Rapid regulation of light harvesting and plant fitness in the field. *Science* **297**, 91-93. (doi:10.1126/science.1072359).
3. Frenkel M., Bellafiore S., Rochaix J.-D., Jansson S. 2007 Hierarchy amongst photosynthetic acclimation responses for plant fitness. *Physiol Plant* **129**, 455-459. (doi:10.1111/j.1399-3054.2006.00831.x).
4. Vener A.V., van Kan P.J., Rich P.R., Ohad I., Andersson B. 1997 Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. *Proc Natl Acad Sci USA* **94**, 1585-1590.
5. Bellafiore S., Barneche F., Peltier G., Rochaix J.D. 2005 State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* **433**, 892-895. (doi:10.1038/nature03286).
6. Aro E.M., Suorsa M., Rokka A., Allahverdiyeva Y., Paakkarinen V., Saleem A., Battchikova N., Rintamaki E. 2005 Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. *J Exp Bot* **56**, 347-356. (doi:10.1093/jxb/eri041).
7. Tikkanen M., Nurmi M., Kangasjarvi S., Aro E.M. 2008 Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. *Biochim Biophys Acta* **1777**, 1432-1437. (doi:10.1016/j.bbabio.2008.08.004).
8. Vainonen J.P., Hansson M., Vener A.V. 2005 STN8 protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of photosystem II core proteins. *J Biol Chem* **280**, 33679-33686. (doi:10.1074/jbc.M505729200).
9. Bonardi V., Pesaresi P., Becker T., Schleiff E., Wagner R., Pfannschmidt T., Jahns P., Leister D. 2005 Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature* **437**, 1179-1182. (doi:10.1038/nature04016).
10. Rochaix J.D. 2007 Role of thylakoid protein kinases in photosynthetic acclimation. *FEBS Lett* **581**, 2768-2775. (doi:10.1016/j.febslet.2007.04.038).
11. Pesaresi P., Pribil M., Wunder T., Leister D. 2011 Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. *Biochim Biophys Acta* **1807**, 887-896. (doi:10.1016/j.bbabio.2010.08.002).
12. Vainonen J.P., Sakuragi Y., Stael S., Tikkanen M., Allahverdiyeva Y., Paakkarinen V., Aro E., Suorsa M., Scheller H.V., Vener A.V., et al. 2008 Light regulation of CaS, a novel phosphoprotein in the thylakoid membrane of *Arabidopsis thaliana*. *FEBS J* **275**, 1767-1777. (doi:10.1111/j.1742-4658.2008.06335.x).
13. Reiland S., Finazzi G., Endler A., Willig A., Baerenfaller K., Grossmann J., Gerrits B., Rutishauser D., Gruissem W., Rochaix J.D., et al. 2011 Comparative phosphoproteome profiling reveals a function of the STN8 kinase in fine-tuning of cyclic electron flow (CEF). *Proc Natl Acad Sci U S A* **108**, 12955-12960. (doi:10.1073/pnas.1104734108).
14. DalCorso G., Pesaresi P., Masiero S., Aseeva E., Schunemann D., Finazzi G., Joliot P., Barbato R., Leister D. 2008 A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. *Cell* **132**, 273-285. (doi:10.1016/j.cell.2007.12.028).
15. Fristedt R., Willig A., Granath P., Crevecoeur M., Rochaix J.D., Vener A.V. 2009 Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in Arabidopsis. *Plant Cell* **21**, 3950-3964. (doi:10.1105/tpc.109.069435).

16. Tikkanen M., Grieco M., Kangasjarvi S., Aro E.M. 2010 Thylakoid protein phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. *Plant Physiol* **152**, 723-735. (doi:10.1104/Pp.109.150250).
17. Rintamaki E., Salonen M., Suoranta U.M., Carlberg I., Andersson B., Aro E.M. 1997 Phosphorylation of light-harvesting complex II and photosystem II core proteins shows different irradiance-dependent regulation in vivo. Application of phosphothreonine antibodies to analysis of thylakoid phosphoproteins. *J Biol Chem* **272**, 30476-30482.
18. Tikkanen M., Piippo M., Suorsa M., Sirpio S., Mulo P., Vainonen J., Vener A.V., Allahverdiyeva Y., Aro E.M. 2006 State transitions revisited - a buffering system for dynamic low light acclimation of Arabidopsis. *Plant Mol Biol* **62**, 779-793. (doi:10.1007/s11103-006-9044-8).
19. Nath K., Poudyal R.S., Eom J.S., Park Y.S., Zulfugarov I.S., Mishra S.R., Tovuu A., Ryoo N., Yoon H.S., Nam H.G., et al. 2013 Loss-of-function of OsSTN8 suppresses the photosystem II core protein phosphorylation and interferes with the photosystem II repair mechanism in rice (*Oryza sativa*). *Plant J* **76**, 675-686. (doi:10.1111/tpj.12331).
20. Zer H., Vink M., Keren N., Dilly-Hartwig H.G., Paulsen H., Herrmann R.G., Andersson B., Ohad I. 1999 Regulation of thylakoid protein phosphorylation at the substrate level: reversible light-induced conformational changes expose the phosphorylation site of the light-harvesting complex II. *Proc Natl Acad Sci U S A* **96**, 8277-8282.
21. Vink M., Zer H., Herrmann R.G., Andersson B., Ohad I. 2000 Regulation of Photosystem II core protein phosphorylation at the substrate level: Light induces exposure of the CP43 chlorophyll *a* protein complex to thylakoid protein kinase(s). *Photosynth Res* **64**, 209-219. (doi:10.1023/A:1006455926748).
22. Rintamaki E., Martinsuo P., Pursiheimo S., Aro E.M. 2000 Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. *Proc Natl Acad Sci USA* **97**, 11644-11649. (doi:10.1073/pnas.180054297).
23. Yin L., Fristedt R., Herdean A., Solymosi K., Bertrand M., Andersson M.X., Mamedov F., Vener A.V., Schoefs B., Spetea C. 2012 Photosystem II function and dynamics in three widely used *Arabidopsis thaliana* accessions. *PLoS One* **7**, e46206. (doi:10.1371/journal.pone.0046206).
24. Willig A., Shapiguzov A., Goldschmidt-Clermont M., Rochaix J.D. 2011 The phosphorylation status of the chloroplast protein kinase STN7 of Arabidopsis affects its turnover. *Plant Physiol* **157**, 2102-2107. (doi:10.1104/pp.111.187328).
25. Wunder T., Liu Q., Aseeva E., Bonardi V., Leister D., Pribil M. 2013 Control of STN7 transcript abundance and transient STN7 dimerisation are involved in the regulation of STN7 activity. *Planta* **237**, 541-558. (doi:10.1007/s00425-012-1775-y).
26. Wunder T., Xu W., Liu Q., Wanner G., Leister D., Pribil M. 2013 The major thylakoid protein kinases STN7 and STN8 revisited: effects of altered STN8 levels and regulatory specificities of the STN kinases. *Front Plant Sci* **4**, 417. (doi:10.3389/fpls.2013.00417).
27. Lemeille S., Turkina M.V., Vener A.V., Rochaix J.D. 2010 Stt7-dependent phosphorylation during state transitions in the green alga *Chlamydomonas reinhardtii. Mol Cell Proteomics* **9**, 1281-1295. (doi:10.1074/mcp.M000020-MCP201).
28. Grouneva I., Gollan P., Kangasjärvi S., Suorsa M., Tikkanen M., Aro E.-M. 2013 Phylogenetic viewpoints on regulation of light harvesting and electron transport in eukaryotic photosynthetic organisms. **237**, 399-412. (doi:10.1007/s00425-012-1744-5).
29. Flood P.J., Harbinson J., Aarts M.G. 2011 Natural genetic variation in plant photosynthesis. *Trends Plant Sci* **16**, 327-335. (doi:10.1016/j.tplants.2011.02.005).
30. El-Lithy M.E., Rodrigues G.C., van Rensen J.J., Snel J.F., Dassen H.J., Koornneef M., Jansen M.A., Aarts M.G., Vreugdenhil D. 2005 Altered photosynthetic performance of a

natural Arabidopsis accession is associated with atrazine resistance. *J Exp Bot* **56**, 1625-1634. (doi:10.1093/jxb/eri157).
31. Noren H., Svensson P., Andersson B. 1999 Auxiliary photosynthetic functions of Arabidopsis thaliana--studies in vitro and in vivo. *Biosci Rep* **19**, 499-509.
32. Porra R.J., Thompson W.A., Kriedemann P.E. 1989 Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-*a* and chlorophyll-*b* extracted with 4 different solvents - verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy. *Biochim Biophys Acta* **975**, 384-394. (doi:10.1016/S0005-2728(89)80347-0).
33. Fristedt R., Carlberg I., Zygadlo A., Piippo M., Nurmi M., Aro E.M., Scheller H.V., Vener A.V. 2009 Intrinsically unstructured phosphoprotein TSP9 regulates light harvesting in Arabidopsis thaliana. *Biochemistry* **48**, 499-509. (doi:10.1021/bi8016334).
34. Onate-Sanchez L., Vicente-Carbajosa J. 2008 DNA-free RNA isolation protocols for Arabidopsis thaliana, including seeds and siliques. *BMC Research Notes* **1**, 93.
35. Czechowski T., Stitt M., Altmann T., Udvardi M.K., Scheible W.-R. 2005 Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiol* **139**, 5-17. (doi:10.1104/pp.105.063743).
36. Dekkers B.J.W., Willems L., Bassel G.W., van Bolderen-Veldkamp R.P., Ligterink W., Hilhorst H.W.M., Bentsink L. 2012 Identification of reference genes for RT–qPCR expression analysis in Arabidopsis and tomato seeds. *Plant Cell Physiol* **53**, 28-37. (doi:10.1093/pcp/pcr113).
37. Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. 2005 Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* **25**, 1965-1978. (doi:10.1002/joc.1276).
38. Verhoeven A., Osmolak A., Morales P., Crow J. 2009 Seasonal changes in abundance and phosphorylation status of photosynthetic proteins in eastern white pine and balsam fir. *Tree Physiol* **29**, 361-374. (doi:10.1093/treephys/tpn031).
39. Andrews J.R., Fryer M.J., Baker N.R. 1995 Consequences of LHC II deficiency for photosynthetic regulation in chlorina mutants of barley. *Photosynth Res* **44**, 81-91. (doi:10.1007/BF00018299).
40. Bergelson J., Roux F. 2010 Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nat Rev Genet* **11**, 867-879.
41. Koornneef M., Alonso-Blanco C., Vreugdenhil D. 2004 Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu Rev Plant Biol* **55**, 141-172. (doi:10.1146/annurev.arplant.55.031903.141605).
42. Murchie E.H., Niyogi K.K. 2011 Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiol* **155**, 86-92. (doi:10.1104/pp.110.168831).

Tables

Table 1. The names, stock numbers, abbreviations, geographic origin and habitat of all genotypes used in this study (source: TAIR, www.arabidopsis.org). N/A, not applicable.

Stock Number	Accession name	Abbreviated name	Country	Latitude (°)	Longitude (°)
CS76113	Columbia-0	Col-0	N/A	N/A	N/A
SALK060869	*stn8-1* (in Col-0)	*stn8*	N/A	N/A	N/A
CS76227	Shakdara	Sha	Tadjikistan	38.35	68.48
CS76106	C24	C24	Portugal	41.25	-8.45
CS76105	Burren-0	Bur-0	Ireland	52.9	-9
CS28595	Palermo	Pa-2	Italy	38.07	13.22
CS76192	Martuba	Mt-0	Libya	32.34	22.46
CS76210	Perm	Per-1	Russia	58.00	56.31
CS76100	Borky	Bor-4	Czech	49.40	16.23
CS76109	Canary Island	Can-0	Spain	29.21	-13.48
CS28780	Tsushima	Tsu-0	Japan	34.43	136.31
N/A	Tsu-0 × Ws-4	Tsu × Ws-4	N/A	N/A	N/A
CS5390	Wassilewskija-4	Ws-4	Belarus	52.3	30
CS76164	Landsberg *erecta*	L*er*-1	Poland	52.71	15.23
N/A	((Ely × L*er*) × L*er*)BC6	ELB	N/A	N/A	N/A
CS28631	PHW-31 (Ely)	Ely	England	52.39	0.26

Figures

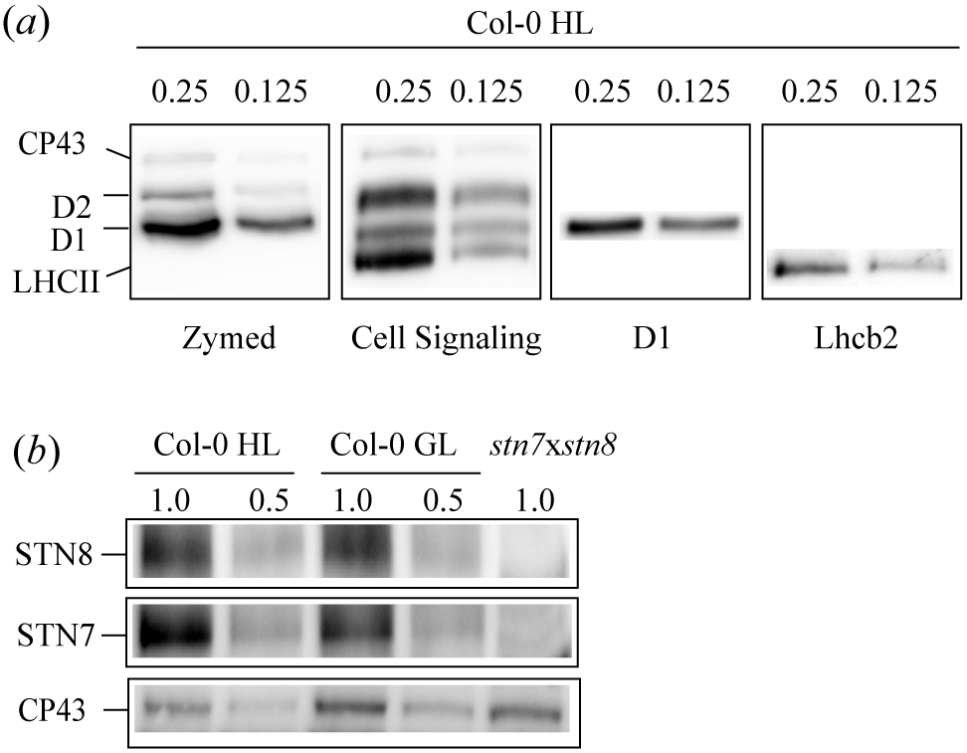

Figure 1. Representative western blots of thylakoid proteins isolated from Col-0. The plants were illuminated for 3 h with growth light (GL, 150 μmol m^{-2} s^{-1}) and subsequently transferred for 3 h to high light (HL, 600 μmol m^{-2} s^{-1}). Thylakoid membranes were isolated in the presence of NaF and the proteins were separated by gel electrophoresis. (*a*) The phosphorylated PSII proteins were immunodetected with anti-phospho-Thr antibodies from Zymed and Cell Signaling. Control blots with anti-D1 and Lhcb2 antibodies are also shown. (*b*) The levels of STN8 and STN7 protein kinases are shown in parallel with control CP43 blots. Thylakoids isolated from the *stn7*×*stn8* double mutant were used as a control for specificity of the anti-STN8 and STN7 antibodies. Two chlorophyll loadings (μg/lane) are shown to demonstrate the linearity of the immunodetected signal from each antibody used.

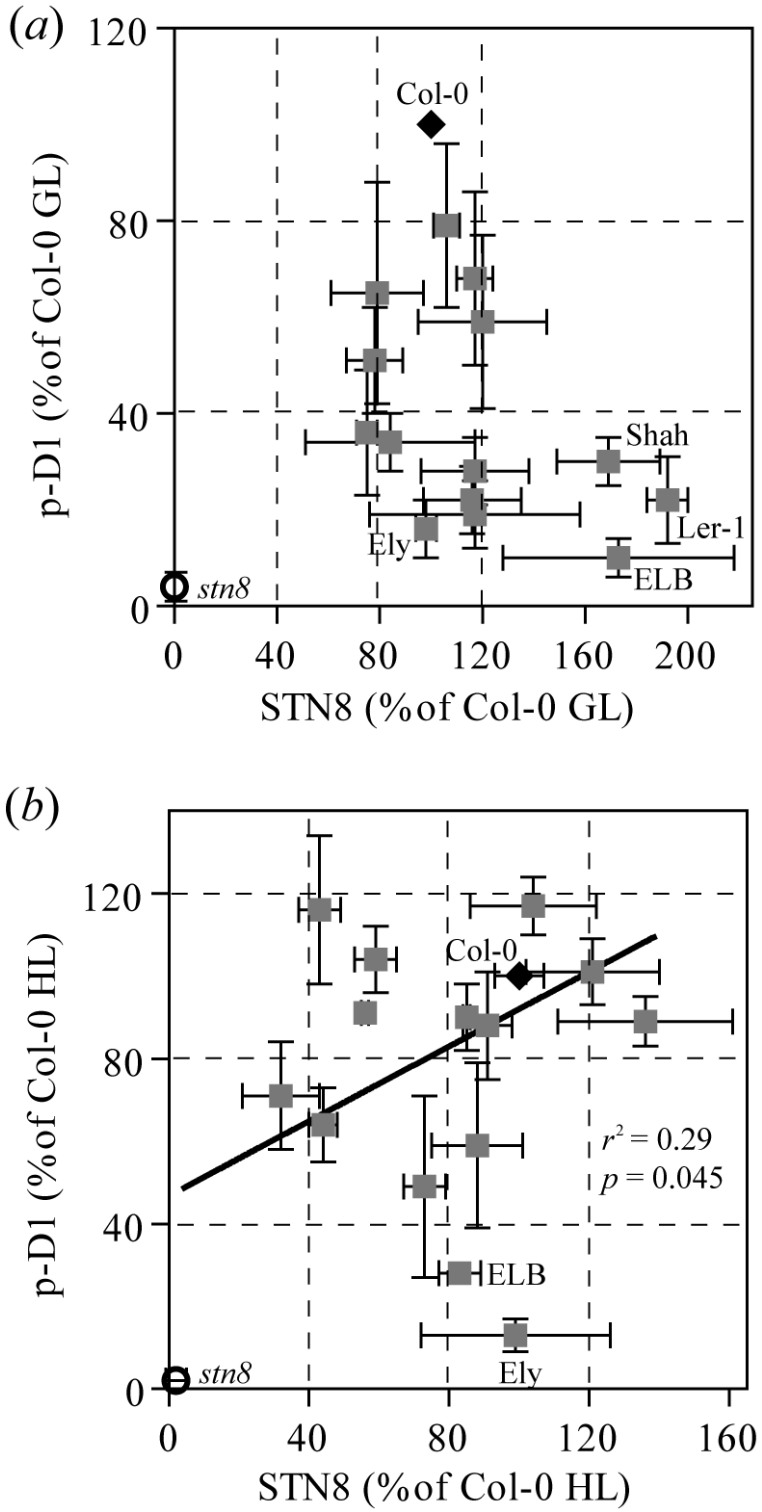

Figure 2. Scatterplots comparing the levels of phospho-D1 (p-D1) relative to STN8 protein level under growth light (GL) (*a*) and high light conditions (HL) (*b*). The plotted data are expressed relative to Col-0 and are means of 2-3 technical replicates ±SD. ◆ represents Col-0, ○ represents the *stn8* mutant, and ■ all other genotypes. Some extreme genotypes are also labelled in panel (a) with their names. No significant correlation was found in (*a*). A weak but significant correlation was found in (*b*) if Ely and ELB were excluded from the regression analysis. Broken lines delimit high, moderate and low D1 phosphorylation and STN8 protein levels.

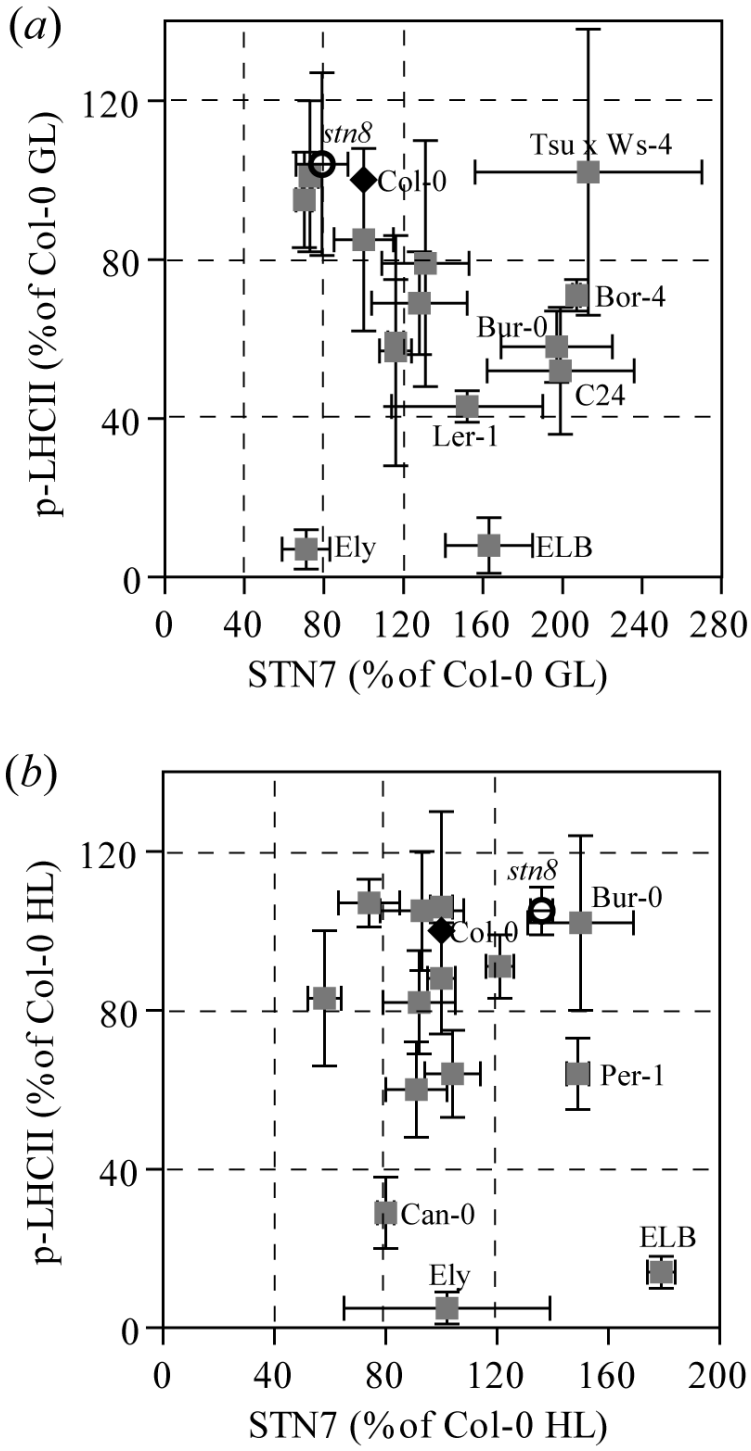

Figure 3. Scatterplots comparing the levels of phospho-LHCII (p-LHCII) relative to STN7 protein level under growth light (GL) (*a*) and high light conditions (HL) (*b*). The plotted data are expressed relative to Col-0 and are means of 2-3 technical replicates ±SD. ◆ represents Col-0, ○ represents the *stn8* mutant, and ■ all other genotypes. Some extreme genotypes are also labelled with their names. No significant correlation was found between LHCII phosphorylation and STN7 kinase abundance. Broken lines delimit high, moderate and low LHCII phosphorylation and STN7 protein levels.

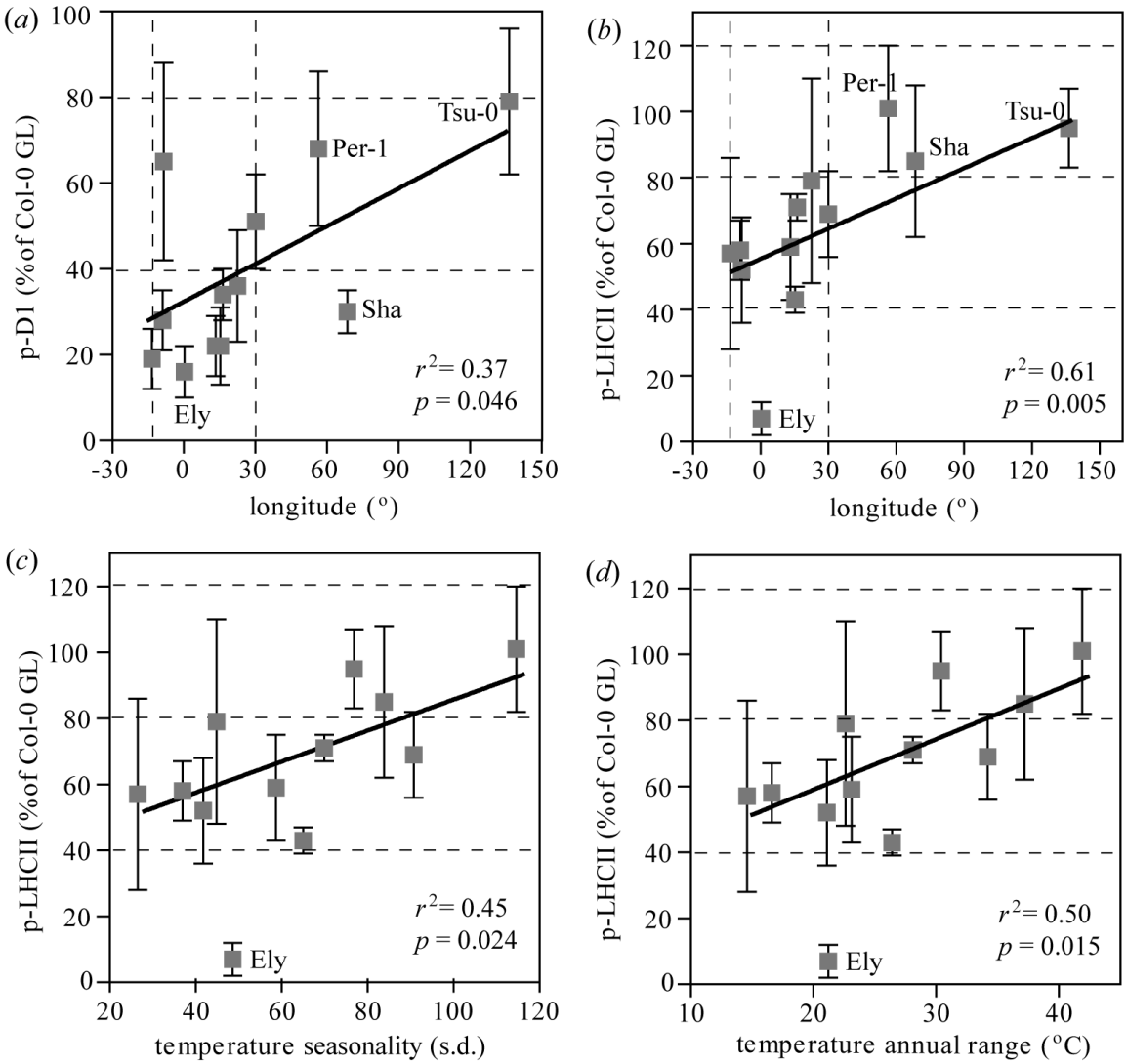

Figure 4. Scatterplots comparing the levels of phospho-D1 (p-D1) (*a*) and phospho-LHCII (p-LHCII) (*b-d*) in growth light (GL) relative to geographic (*a, b*) and climatic factors (*c, d*). A significant correlation with longitude was found for both p-D1 (*a*) and p-LHCII levels (*b*). Vertical broken lines delimit longitude for European accessions (-14° to 30°). Per-1, Sha and Tsu-0 are labelled because they are non-European accessions. A significant correlation was found between the levels of p-LHCII and the temperature seasonality (*c*) and the temperature annual range (*d*). Correlation of p-D1 with the parameters in (*c*) and (*d*) was low but a trend was visible however not significant (r^2 = 0.23, p = 0.128 and r^2 = 0.24, p = 0.123, respectively). Temperature data were obtained from the WorldClim database (http://www.worldclim.org/). The phosphorylation data in all panels are expressed relative to Col-0 and are means of 2-3 technical replicates ±SD. Ely is labelled because it was excluded from regression analysis in all panels. Horizontal broken lines delimit high, moderate and low phosphorylation levels in all panels.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rstb.2013.0499 or via http://rstb.royalsocietypublishing.org.

Table S1. Protein abundance and phosphorylation, gene expression, and chlorophyll a/b ratio in growth light (100 μmol m^{-2} s^{-1}).

Table S2. Protein abundance and phosphorylation, gene expression, chlorophyll *a*/*b* ratio in high light (600 μmol m^{-2} s^{-1}).

Table S3. AGI codes, gene names, primer sequences and efficiencies for all genes tested.

Figure S1. Bar charts of D1 protein phosphorylation (*a*), of the STN8 protein (*b*) and of the *STN8* transcript levels (*c*) in growth (GL) and high light (HL) expressed relative to Col-0 levels in GL conditions. Transcript levels are expressed on a log scale.

Figure S2. Bar charts of LHCII protein phosphorylation (*a*), of the STN7 protein (*b*) and of the *STN7* transcript level (*c*) in growth (GL) and high light (HL) expressed relative to Col-0 levels in GL. Transcript levels are expressed on a log scale.