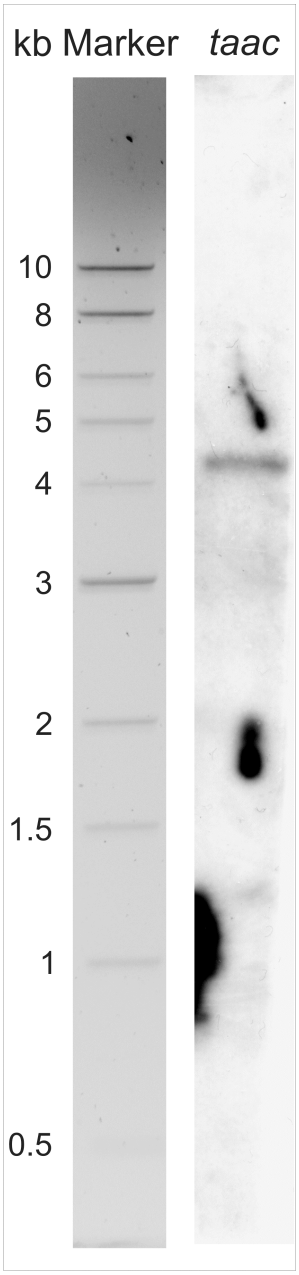
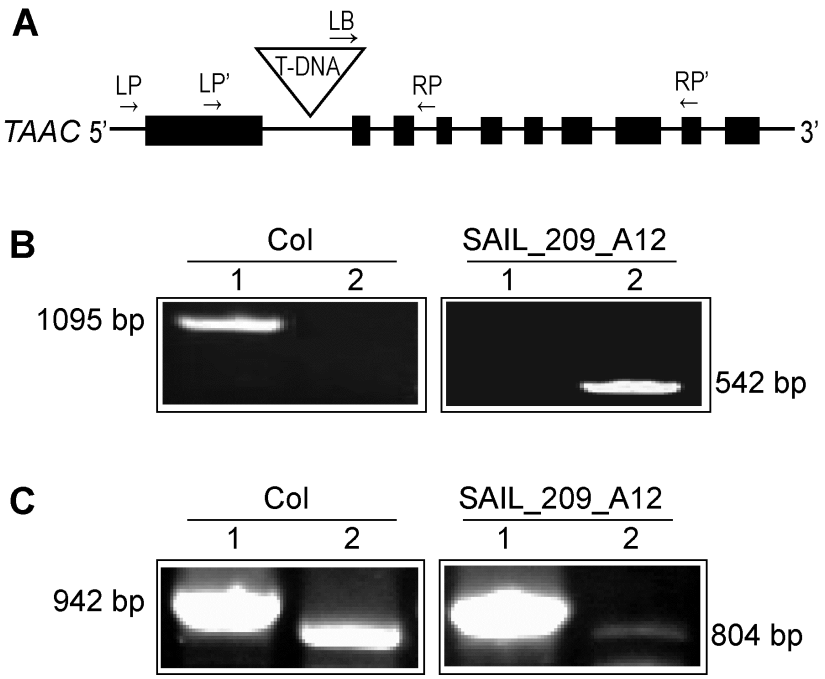
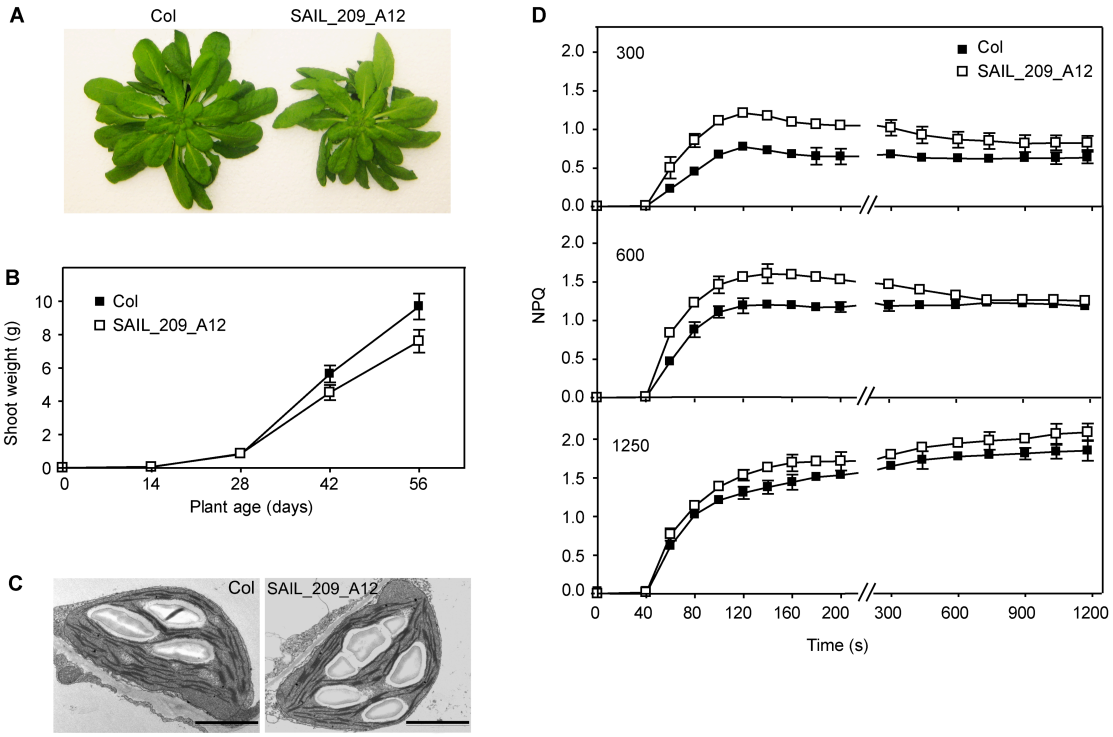
Supplemental Material



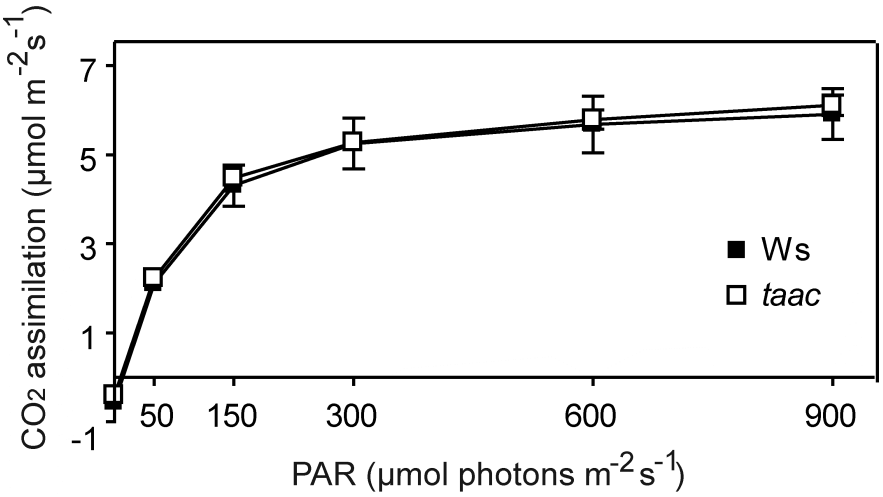
Supplemental Figure S1. Southern blot of the EcoR1-digested FLAG_443D03 *taac* mutant DNA probed with a radiolabeled T-DNA fragment indicating the presence of a single T-DNA insert with a predicted molecular weight of 4.5-kb in the mutant plants. The left panel shows markers for molecular weight on a ethidium bromide-stained gel. The dark spots are due to non-specific binding.



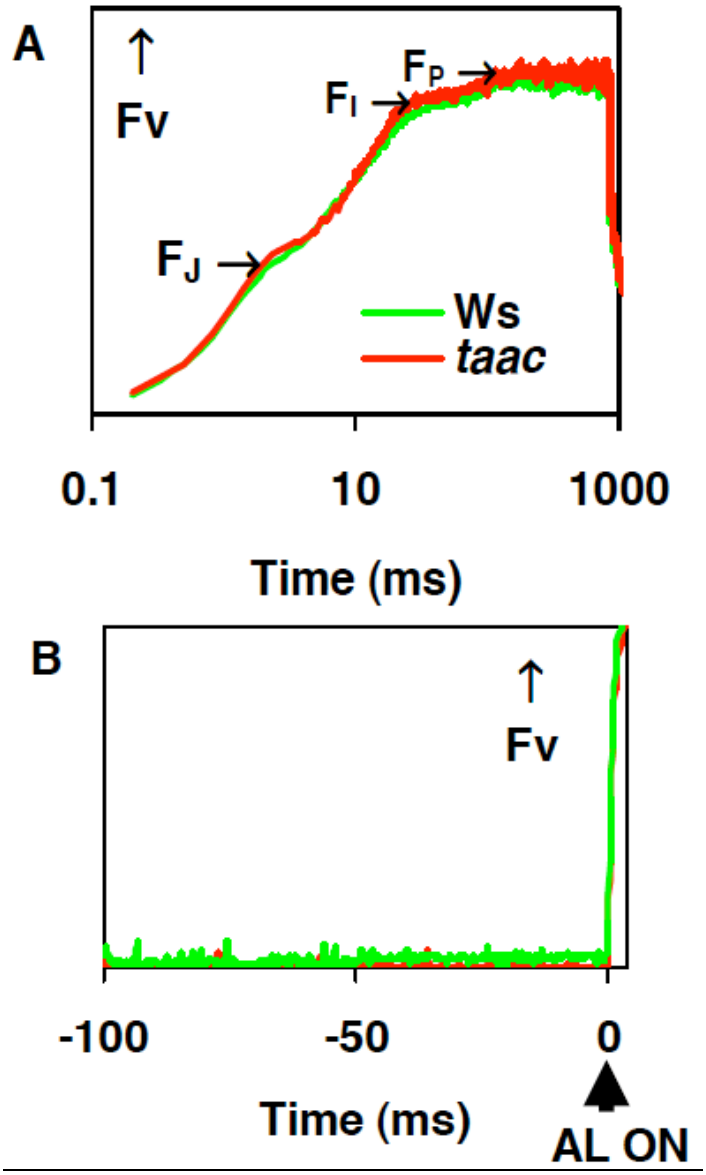
Supplemental Figure S2. Screening for homozygous Arabidopsis SAIL_209_A12 *taac* mutant. A, Localization of the T-DNA insertion in the *TAAC* gene (At5g01500), and of the primers used for PCR analysis (arrows LP, RP and LB) and for RT-PCR analysis (arrows LP' and RP'). The insertion site is indicated with a triangle, whereas black boxes represent exons separated by introns. B, Agarose gel stained with ethidium bromide of PCR products obtained from genomic DNA with the following sets of primers: LP and RP (*lane 1*), LB and RP (*lane 2*). As a control, wild type Columbia ecotype (Col) genomic DNA was analyzed with the same sets of primers. The size (bp) of the obtained PCR products is shown. C, Agarose gel stained with ethidium bromide of RT-PCR products obtained with 18S cDNA specific primers (*lane 1*) and *TAAC* cDNA specific primers LP' and RP' (*lane 2*).



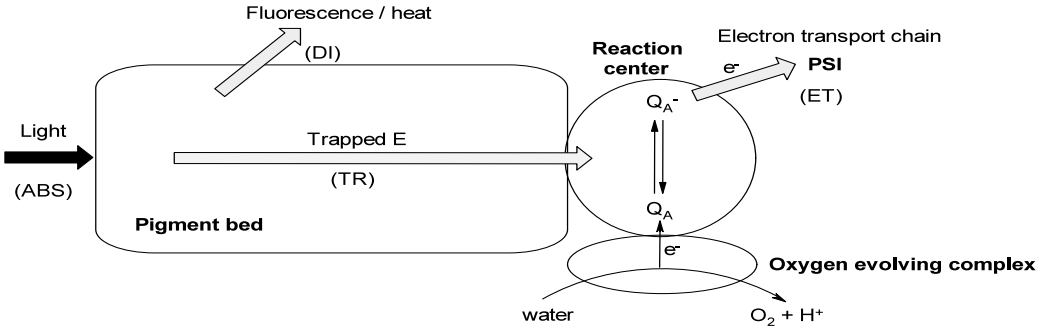
Supplemental Figure S3. Characterization of SAIL_209_A12 *taac* knockout mutant. A, The photographs of representative wild-type Col and mutant plants were taken at an age of 42 d of hydroponic growth at an irradiance of 120 µmol photons m^{-2} s^{-1}. B, Plot of shoot weight ±SD as a function of plant age (n=10). The difference in the weight is significant at 42 and 56 d of growth (Student's *t* test $P < 0.05$). C, Transmission electron microscopic images showing chloroplast ultrastructure were taken at an age of 28 d of growth. C, Kinetics for induction of non-photochemical quenching (*NPQ*). Chlorophyll fluorescence of leaves detached from 16-h dark-adapted plants was recorded during a 20-min exposure to light of the indicated intensities (300, 600 and 1250 µmol photons m^{-2} s^{-1}). Saturating pulses (1.0 s) of actinic light were applied to determine the maximum fluorescence yield F_m or F_m'. *NPQ* was calculated from fluorescence data as $(F_m-F_m')/F_m'$ and plotted ±SD as a function of illumination time (n=7-10). In some cases, the error bar was smaller than the symbol.



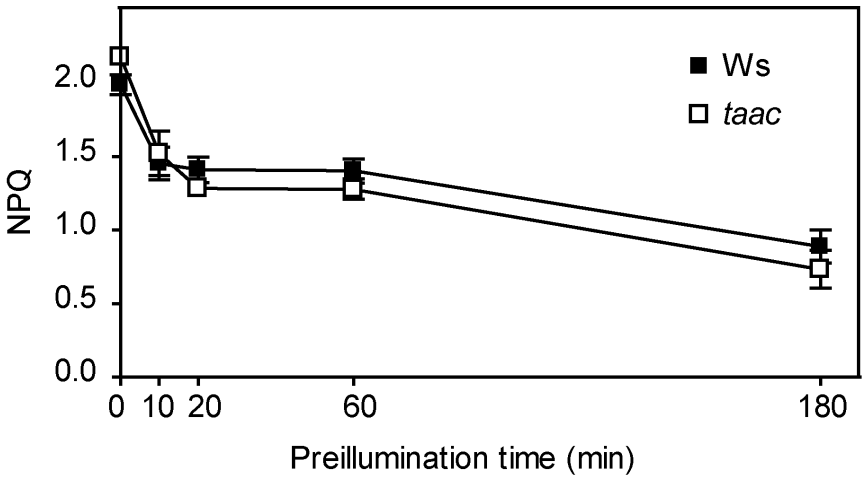
Supplemental Figure S4. CO_2 fixation in the *taac* mutant and respective ecotype (Ws). CO_2 assimilation rate was measured and plotted as a function of intensity of photosynthetically active radiation (PAR). The data are average of six independent measurements ±SD taken on plants at an age of 42 d old.



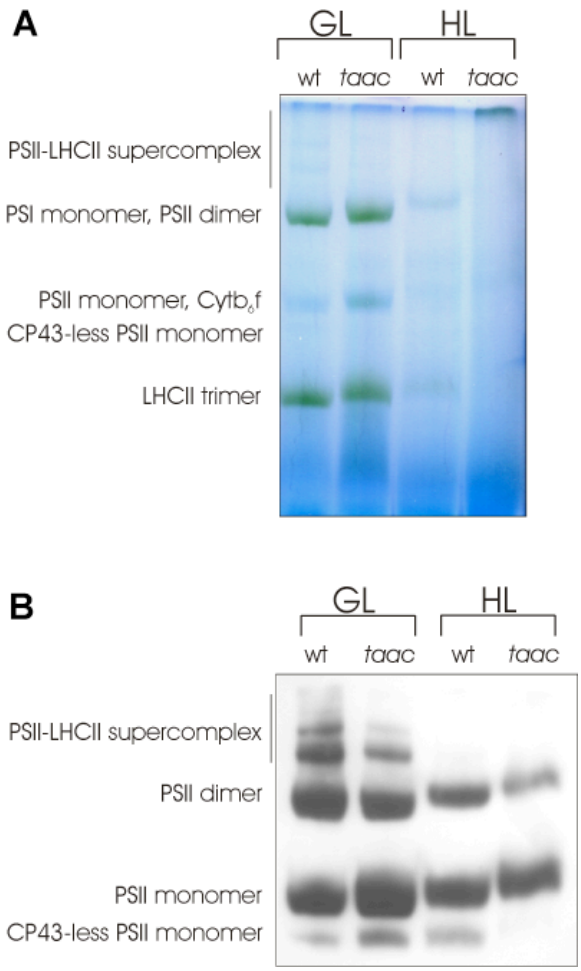
Supplemental Figure S5. Fast fluorescence kinetics of wild-type Arabidopsis Wassilewskija ecotype (Ws) plants and the *taac* mutant grown at an irradiance of 120 µmol photons m^{-2} s^{-1}. A, Typical Chl *a* fluorescence transient O-J-I-P curve recorded and plotted on a logarithmic time scale. The fluorescence yield at 2 ms (F_J), at 30 ms (F_I) and the maximum fluorescence yield (F_P) are indicated by arrows. B, Recording of the variable fluorescence yield (F_v) as the mean of the fluorescent signal over the 200 ms before applying the saturating actinic light (AL).



Supplemental Figure S6. A highly simplified model presenting the energy fluxes *per* reaction center (RC) in photosystem II. ABS: flux of photons absorbed by the antenna pigment bed; TR: flux of excitations channelled to the RCII and trapped, ET: electron flux; DI: flux of energy dissipated as fluorescence and/or heat.



Supplemental Figure S7. Steady-state levels of non-photochemical quenching in high-light stressed leaves. Leaves detached from wild-type Wassilewskija ecotype (Ws) and *taac* plants were preilluminated at 950 µmol photons m^{-2} s^{-1} for the indicated periods of time, allowed to dark incubate for 5 min before recording NPQ induction curves for 5 min at 1250 µmol photons m^{-2} $s^{-1.}$ The steady state levels ±SD are plotted as a function of preillumination time (n=3).



Supplemental Figure S8. Analysis by Blue Native gel electrophoresis (BN-PAGE) of protein complexes from wild-type Wassilewskija ecotype (wt) and *taac* plants grown for 21 d at an irradiance of 120 µmol photons m^{-2} s^{-1} (GL) and then for 14 d under GL or high-light (HL= 950 µmol photons m^{-2} s^{-1}) conditions. Thylakoids were isolated and solubilized followed by BN-PAGE (3.75 µg Chl/lane*)*. A. Representative unstained BN-gel. B, Western blot with anti-D1 antibody of gel as in A.

Supplemental Table S1. *Photosynthetic characteristics of wild type Arabidopsis of Columbia ecotype (Col) and SAIL_209_A12 taac plants grown at an irradiance of 120 µmol photons m^{-2} s^{-1}.*

The parameters were measured on 42 d old plants, and expressed as means ±SD. *, Significantly different from the wild type (Student's *t* test P = 0.001).

Strain	Leaf Chlorophyll content			Oxygen evolution[a]		
	µg mg^{-1} (n=12-18)	*µg cm^{-2}* (n=12-18)	*a*/*b* (n=12-18)	*µmol O_2 mg Chl^{-1} h^{-1}* pPBQ (n=4-6)	pPBQ + NH_4Cl (n=2-4)	MV (n=2-4)
Col	3.10±0.34	47.01±7.53	4.06±0.23	132.5±8.8	143.8±6.5	-117.6±11.9
SAIL_209_A12	2.93±0.34	41.98±11.38	4.12±0.15	135.8±10.7	163.6±9.1*	-119.4±4.2

[a]Light-saturated rate of oxygen evolution measured in thylakoid membranes in the presence of 0.5 mM phenyl-*p*-benzoquinone (pPBQ) without or with the addition of 10 mM NH_4Cl (uncoupler) or in the presence of 100 µM methyl viologen (MV) and 5 mM sodium azide.

SUPPLEMENTAL MATERIALS AND METHODS S1

Southern blotting

Southern blotting of 2 μg of EcoR1-digested FLAG_443D03 mutant line (*taac*) genomic DNA was performed according to Sambrook et al. (1989). A 698-bp probe was PCR-amplified from the mutant's genomic DNA using T-DNA specific primers 5'- TATCCGCTCACAATTCCACA-3' and 5'- CTCCACCATGTTGACGAAGA-3'.

PCR and RT-PCR analyses

Homozygous T-DNA insertion mutant for the Arabidopsis *TAAC* gene (At5g01500; SAIL_209_A12, Columbia ecotype) was identified from Salk SIGnAL T-DNA express Arabidopsis Gene mapping tool (http://www.signal.salk.edu/cgi-bin/tdnaexpress) by PCR analysis. Genomic DNA isolated from wild type Columbia ecotype (Col) and mutant leaves was analyzed by two sets of PCR reactions using gene specific forward 5'-GAAATTCTCAGGCGAGATTCC-3' and reverse 5'-CCAAAAGGATGCAAGAAATTG-3' primers and the T-DNA left border 5'-GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC-3' primer. The PCR cycle conditions (35 cycles) used were denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 35 sec. Homozygoty was confirmed in the next generation.

Characterization of the *TAAC* mRNA expression in the SAIL_209_A12 mutant was performed by non-quantitative RT-PCR as described in Lundin et al. (2007). Forward and reverse primers for the 18S cDNA, used as control, were 5'-AAACCCCGACTTATGGAAGG-3' and 5'-AGTTTCAGCCTTGCGACCATA-3'. Primers specific for *TAAC* cDNA were 5'-TTTCGGTGGTGATTCCGAAG-3' (forward) and 5'-ACAACTCCTTCACGCGCAAT-3' (reverse). The PCR cycle conditions (30 cycles) used were denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec.

CO_2 fixation

Gas exchange measurements were carried out using an open-gas portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebrasca). CO_2 assimilation rate was measured by using leaf chamber equipped with blue-red light diodes (LED). Leaf chamber was adjusted to control relative humidity of 55 %, temperature to 23°C and CO_2 level to 400 μmol^{-1}. The intensity of photosynthetic active radiation (PAR) was varied between 0 and 900 µmol photons $m^{-2}s^{-1}$.

Chlorophyll fluorescence

Chl *a* fluorescence O-J-I-P transient was recorded using dual PAM-100, and analyzed according to the JIP-test (Strasser et al., 1996). The following parameters were determined from the recorded data: the maximal fluorescence yield (F_P), which is equal to F_m since the excitation intensity was high enough to close all the PSII reaction centers (RC); the minimal fluorescence yield (F_0), which is the mean of the fluorescence signal over the 200 ms preceding the actinic illumination; the fluorescence yield at 300 µs ($F_{300\mu s}$) required for the calculation of the initial slope (M_0) of the relative variable fluorescence kinetics; the fluorescence yield at 2 ms (F_J) and at 30 ms (F_I). Using these fluorescence parameters, the energy fluxes *per* reaction center (RC) were calculated according to the equations (Strasser et al., 1996) presented below.

The index 0 refers to the time zero, *i.e.* at the onset of the fluorescence induction.

Flux for absorption: $ABS/RC = (M_0/V_J)/[1 - (F_0/F_m)]$
Flux for trapping: $TR_0/RC = M_0/V_J$
Flux for dissipation: $DI_0/RC = (ABS/RC) – (TR_0/RC)$
Flux for electron transport: $ET_0/RC = (M_0/V_J)/(1 - V_J)$

$V_J = (F_J – F_0)/(F_m - F_0)$
$M_0 = 4\ (F_{300\mu s} – F_0)/(F_m - F_0)$

Pigment analysis

For determination of the xanthophyll cycle activity, the leaves illuminated with PAR of various intensities were detached from the plants, and immediately frozen in liquid nitrogen. The samples were grinded in methanol, and the extracts clarified by centrifugation at 25 000 g for 10 min. Each sample was dried under nitrogen gas and stored in darkness at -20°C or immediately used for HPLC analysis. For pigment extraction, all manipulations were performed at 22°C under a green safe light as recommended by Schoefs (2002).

Pigment analyses were run on a Beckman Gold HPLC device (Beckman Coulter, USA) consisting of a solvent-delivery system (module 126) and a photodiode-array detector (module 168). The time and wavelength resolution were 1.1 ± 0.1 s and 3 nm, respectively. The pigment extract was injected with a Rheodyne (Cotati, CA, USA) Model 7725 sample valve equipped with a 20-µl loop. Up to 20 µl of methanolic extracts were injected. Separations were carried out with a Zorbax Original reversed-phase column (Agilent Technologies, Interchim, France, particle size of the packing 4.65 µm; 25 cm × 4.6 mm I.D.) with the method described by Darko et al. (2000). The pigments were identified on the basis of their absorption spectra and their retention times described in the literature (Darko et al., 2000). The pigment concentrations were corrected to the wavelengths of the detection (430 and 445 nm). After each measurement, the column was re-equilibrated for 20 min with the solvent mixture used initially. All HPLC analyses were carried out at 22ºC and the flow-rate was 1 ml min^{-1}. All the solvents were of HPLC grade and purchased from Sigma-Aldrich. Because the detector could only record absorbance variations between 190 and 600 nm, it was not possible to separately quantify pheophytin *a* and β-carotene during the same run as for the other pigments (Darko et al., 2000).

Literature cited

Darko E, Schoefs B, Lemoine Y (2000) Improved liquid chromatograpic method for the analysis of photosynthetic pigments of higher plants. J Chromatogr **876:** 111-116

Lundin B, Hansson M, Schoefs B, Vener AV, Spetea C (2007) The Arabidopsis PsbO2 protein regulates dephosphorylation and turnover of the photosystem II reaction centre D1 protein. Plant J **49:** 528-539

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Strasser RJ, Eggenberg P, Strasser BJ (1996) How to work without stress but with fluorescence. Bull Soc R Sci Liège **65:** 330-349.