Antisense reductions in the PsbO protein of photosystem II leads to decreased quantum yield but similar maximal photosynthetic rates

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Supplementary Material

Supplementary methods

Conversion of a P700 transmittance signal to absorbance units

According to the Beer-Lambert law absorbance of light by a substance in an aqueous state is related to the concentration of the substance, *c*, the extinction coefficient for the substance, *ε*, and the path length, *l*:

$$A = \varepsilon l c$$

In the context of $P700^+$ absorbing 810 nm light (relative to the 870 nm), it can be assumed that *ε* is a constant. Given the low absorption of the signal by other species in the leaf there is a lot of scatter and *l* is long. It is therefore assumed that while *l* is not constant, changes between similar leaves will be small as a percentage of the overall path length. As such, the greatest influence on *A* is *c*, and it in the case of $P700^+$ it can be said that *A* at 810 nm is closely proportional to the $P700^+$ content.

Transmittance can be measured directly by I/I_0, where *I* is the intensity of transmitted light and I_0 the intensity of the incident light, and is related to *A* logarithmically according to the Beer-Lambert law by:

$$A = -\log_{10}\frac{I}{I_0} = \log_{10}\frac{I_0}{I}$$

Consider two absorbance signals, A_0 and A_t. A_0 is in the dark when the P700 pool is completely reduced and the transmitted intensity is given by I_1. A_t is at any given time after A_0 where transmittance is I_t/I_0

$$A_0 = \log_{10}\frac{I_0}{I_1}$$

$$A_t = \log_{10}\frac{I_0}{I_t}$$

The difference between the two, $\Delta A = A_t - A_0$, can be formulated as:

$$\Delta A = \log_{10}\frac{I_0}{I_t} - \log_{10}\frac{I_0}{I_1} = \log_{10}\left(\frac{I_0}{I_t}\cdot\frac{I_1}{I_0}\right) = \log_{10}\frac{I_1}{I_t} = -\log_{10}\frac{I_t}{I_1}$$

The raw I_1 signal is off-set to zero (baseline) in the dark. The running signal is given as a deviation from the baseline, $\Delta I = I_t - I_1$ in arbitrary units. The P700 emitter detector unit (ED-

P700DW, Walz. Effeltrich, Germany) simulates a 0.1% change in the transmittance signal by changing the absolute intensity of the 810 nm measuring light and keeping the 870 nm reference beam constant. This allows calibration of the ΔI signal to account for variations due to beam intensity, signal amplification, sample geometry, *etc*. This 0.1% signal change is used to convert ΔI to a (calibrated) change in transmittance, ΔT_c:

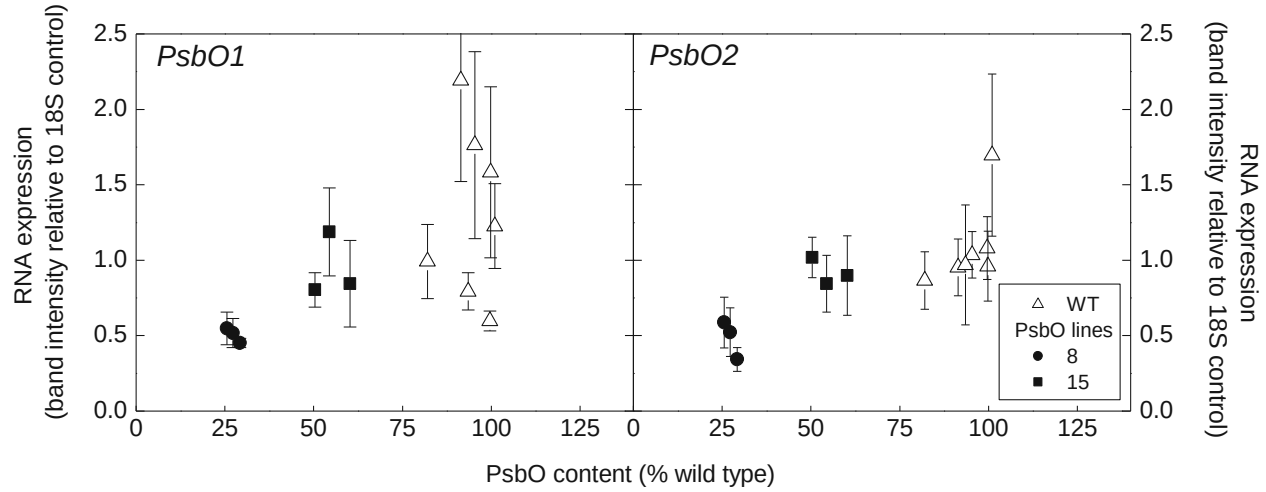
$$\Delta T_c = \frac{\Delta I}{I_1} = \frac{I_t - I_1}{I_1} = \frac{I_t}{I_1} - 1$$

$$\rightarrow \frac{I_t}{I_1} = 1 + \Delta T_C$$

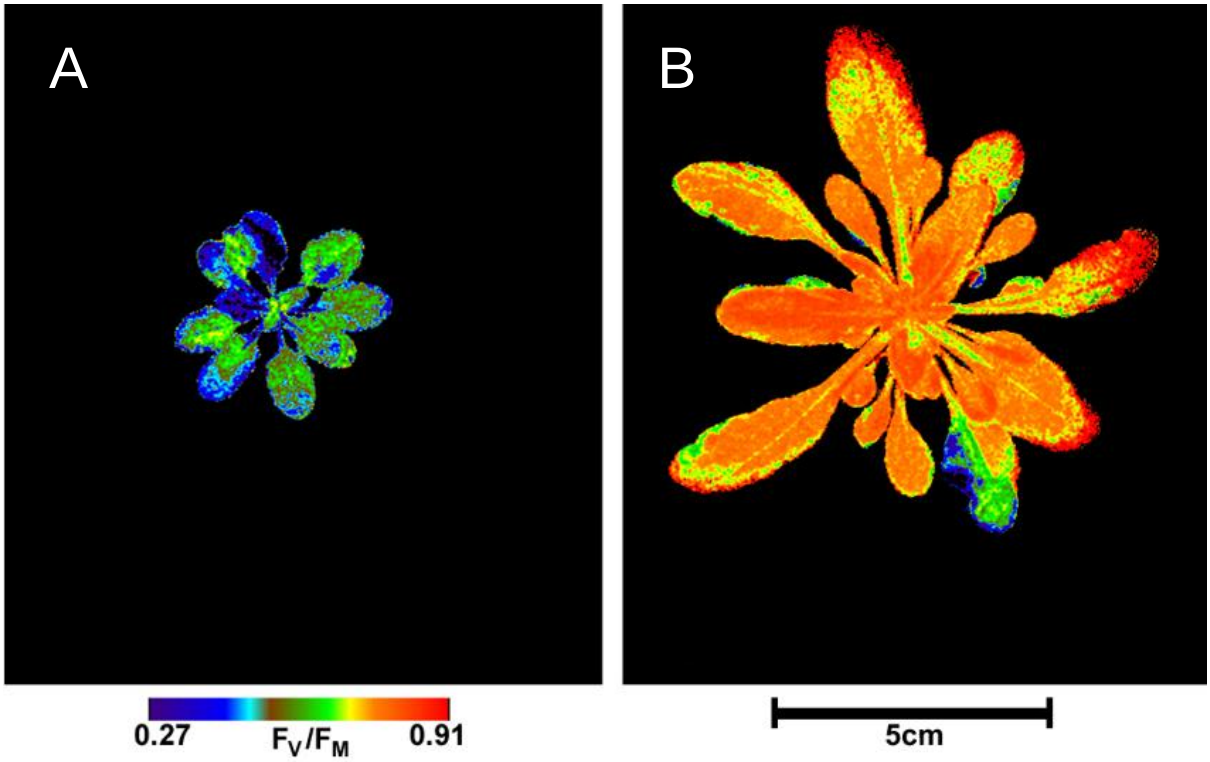
From this, the measured signal ΔT_c can be substituted into the equation for ΔA to estimate the $P700^+$ content from absorbance change at 810 nm according to the Beer-Lambert law as:

$$\Delta A = -\log_{10}(1 + \Delta T_c)$$

Supplementary data



Supplementary Fig. S1. Semi-quantitative PCR on *PsbO1* and *PsbO2* expression Semi-quantitative PCR on several key photosynthetic genes representing the major protein complexes involved in photosynthesis. Each point is the average of at least three PCR replicates of a single plant sample, ± the standard error of these PCR replicates. Quantification was relative band intensity of PCR product run on a 4% agarose gel with 18S ribosomal RNA (24 PCR cycles) as the control (AT2G01010, Albrecht *et al.*, 2006). Spearman rank order correlation test showed a significant positive relationship between PsbO protein content and expression of PsbO1 (p=0.021) and PsbO2 (p=0.001) genes. RNA was extracted from leaf discs sampled and snap frozen at midday, in TRIzol reagent (Invitrogen) according to the manufacturer's instructions, then treated with DNase (RQ1, Promega). RNA concentration in samples was quantified spectrophotometrically (ND-1000, NanoDrop, Delaware, USA) and diluted to equal concentration in RNase free water. First strand cDNA was synthesised from 1.25 µg RNA using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) using the supplied Oligo (dT)20 primers. The psbO1 (AT5G66570) was amplified using the forward primer TCATGTTATTATATTTTCCGTTGAT, and the reverse primer, CGGTACTGTTTTTACGATGAAGG. For psbO2 (AT3G50820) the forward primer was TGAAGTGATCGGAGTGTTCG and the reverse primer AAACTTGATTTCTCAAATTATT. The PCR products were run on a 4% agarose gel and visualised with SYBR Safe (Invitrogen) using a digital fluorescence imager (Versadoc MP 4000, Bio-Rad). Band intensity was quantified in Quantity One (v 4.6.3, Bio-Rad) relative to the 18S ribosomal RNA control band. To account for technical error, PCR was repeated at least three times independently on each cDNA sample.



Supplementary Fig. S2. Chlorophyll fluorescence images of an antisense plant with low PsbO content (A) and a WT plant (B), demonstrating the consistency of F_V/F_M changes within and between leaves. Both plants are shown to scale and were imaged 22 days after transfer to soil, corresponding to the final day of growth measurements (Table 1). Images were obtained from the same chlorophyll fluorescence imaging instrument as used for growth analysis.