SCREENING FOR ACCELERATED RELAXATION FROM PHOTOPROTECTION IN *GLYCINE MAX* (L.)

Laidlaw Scholars
Undergraduate Research and Leadership Programme

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BACKGROUND

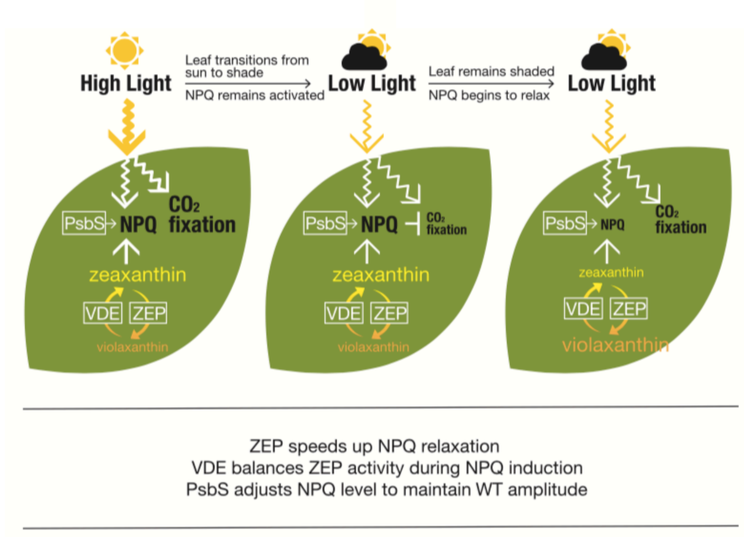

Figure 1. Interaction between photoprotection and CO_2 fixation during sun-shade transitions. Source: Kromdijk et al. (2016). DOI: 10.1126/science.aai8878

- The slow rate of recovery from photoprotection has been estimated to result in 7.5-30% loss of CO_2 fixation (Long et al., 1994; Zhu et al., 2004).
- The recovery of this lost biomass means higher plant productivity and, thus, improved yield. This is an exciting window of opportunity into sustainably meeting the need for a two-fold increase in food production by 2050 in the face of growing population size.
- Accelerating the xanthophyll cycle and increasing PsbS results in more rapid relaxation from NPQ and a corresponding 15% increase in dry plant matter productivity, providing proof of concept for the abovementioned (Kromdijk et al., 2016).

METHODS

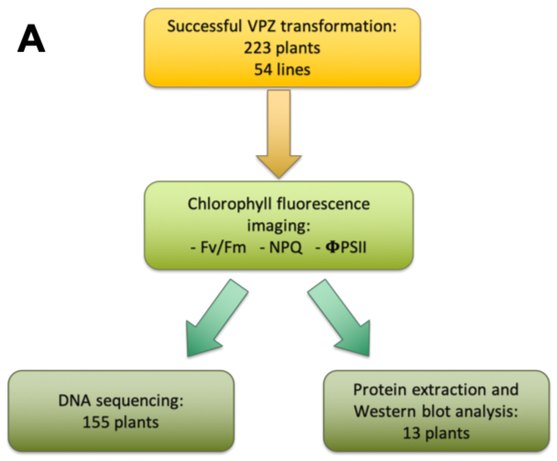

*Figure 2. **A)** Flowchart showing the steps in the screening process – 155 out of 223 starting plants were chosen for DNA sequence analysis based on their NPQ relaxation rate relative to wild type plants. 13 plants with results closest to the tobacco model were chosen for protein extraction and Western blot analysis; **B)** Sample image from chlorophyll fluorescence imager– data on maximum potential quantum efficiency of PSII (Fv/Fm), non-photochemical quenching (NPQ), and photochemical yield of PSII (ΦPSII) were obtained for six technical repeats per plant by subjecting plant material to alternating high and low light conditions; **C)** Experimental setup in the greenhouse.*

RESULTS

NPQ

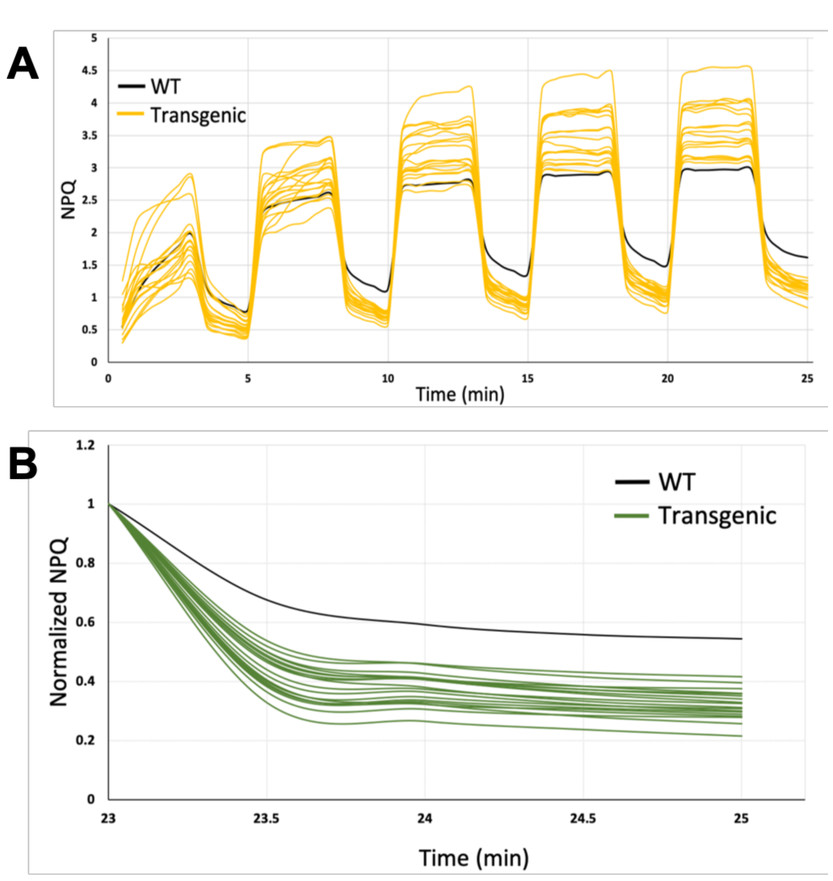

*Figure 4. **A)** NPQ in fluctuating light conditions – the selected transgenic plants consistently perform better than the WT (black), as shown by the lower NPQ values at low light conditions ($p<0.05$ second cycle onwards); **B)** Normalized NPQ values at final dark relaxation stage – the rate of dark relaxation of NPQ (τ_1) was calculated by fitting a double exponential model to the non-normalized values. Both τ_1 and NPQ are significantly lower in transgenic plants compared to WT (in black; $p<0.05$); **C)** A table showing τ_1 values for the best performing transgenic plants and the corresponding p-value for the comparison with WT*

C

Plant number	τ_1	p-value
WT	0.3466	
20	0.0703	0.002316
21	0.0747	0.002702
26	0.0757	0.0028
37	0.1125	0.009604
70	0.0635	0.001809
77	0.1127	0.009655
78	0.0662	0.001993
104	0.0932	0.005115
115	0.166	0.044997
158	0.0847	0.003832
164	0.1162	0.010776
179	0.0852	0.008058
180	0.0796	0.006822
181	0.0983	0.006076
184	0.0412	0.000781
203	0.0955	0.00553

Fv/Fm

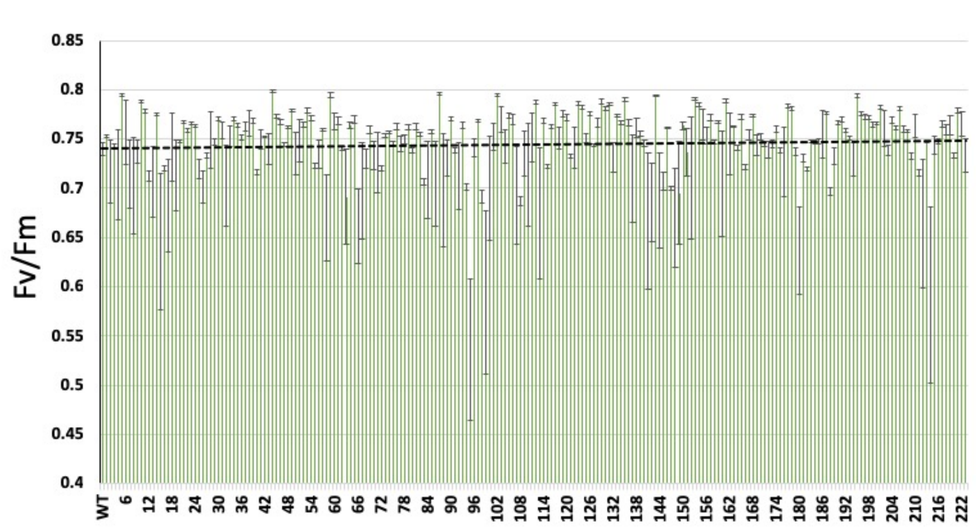

Figure 3. Fv/Fm values for the 223 starting plants – averaged across the six technical repeats for each plant (error bars show the SEM of technical repeats; the dashed line is a linear regression between Fv/Fm values and plant ID). The majority of Fv/Fm values lie within the theoretical range of 0.7-0.8, indicating that the photosynthetic capacity of the transgenic plants is unaffected.

Western blot analysis

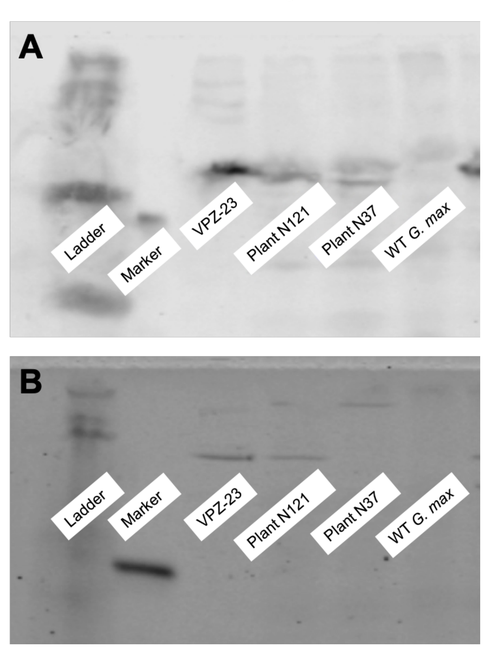

*Figure 5. Preliminary immunoblots for **A)** VDE and **B)** ZEP – VPZ* N. tabacum *was used as a positive control*

SUMMARY

- Initial screening of transgenic *G. max* grown in greenhouse conditions showed that accelerating the xanthophyll cycle and increasing PsbS results in a more rapid relaxation from NPQ when transitioning from high to low light condition.
- Maximum quantum efficiency of PSII of the transgenic plants shows that they have the same photosynthetic capacity as the wild type control.
- Preliminary results from Western blot analysis show that the proteins of interest are present in the plant tissue in detectable amounts. Further analysis is required to quantify the relative amounts of the proteins of interest in the best performing plants.
- More thorough analyses of the photosynthetic rates in fluctuating light conditions, as well as further chlorophyll fluorescence and dry plant matter measurements are required to be performed in the future with fully segregated plants with more than one biological repeats to confirm the findings of this study and to further narrow down to the best performing lines.

ACKNOWLEDGEMENTS

Thanks to Steven Burgess, Amanda Cavanagh and Chris Harvey for helping with the protein extraction and Western blot analysis, Yu Wang for helping with the double exponential model, and to all the fellows who helped in the greenhouse and in the lab.

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