Photosynthesis measurement in Arabidopsis thalliana using the DUAL-PAM

Principle

DUAL-PAM-100 instruments can measure simultaneously a single-channel signal (e.g., chlorophyll fluorescence) and a two-channel signal (e.g. P700- dependent absorption changes at 820 nm relative to 870 nm). Also, a single wavelength and a dual wavelength absorption signal can be concurrently measured as in the case of the P515/535 setup for parallel determination of the electrochromic band shift and scattering changes.

This protocol follows the ones described in the "Instruction manual for DUAL-PAM-100" (http://www.walz.com/downloads/manuals/dual-pam-100/Dual-PAM_1e.pdf) that can be found on the Waltz Mess- und Regeltechnik website (http://www.walz.com/index.html).

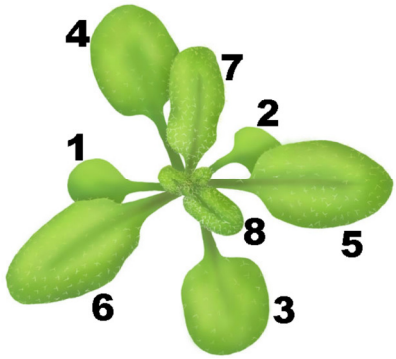
Equipment and Reagents

Machine/Product	Reference
Dual-PAM with software	Waltz Mess- und Regeltechnik
5 cm Petri dish	
Aluminum Foil	
Paper tissue wipe	
Tweezers	
Green Light	

Procedure

Leaf Selection

- For 3 week-old Arabidopsis plant, best results are obtained when using the 4th or 5th leaf.



- Start counting with the two cotyledon leaves, looking for the next leave outside the corner made by the previous two leaves.

Dark Adaptation

- Place a small piece of paper wipe in a 5 cm Petri dish and humidify the paper with demineralized water.
- Place the cut leaf on the wet paper wrap the Petri dish in aluminum foil to prevent exposition to light. Make sure you do not put the leaf upside down.
- Leave in the dark for at least 20 minutes.

Measurement

- Start up the DUAL-PAM and start up the software

- Under green light, remove the leaf from the Petri dish and place it on the measuring aperture using fine tweezers. Make sure you do not put the leaf upside down.
- In the tab [Slow Kinetics]: Manual → ind. Curv. (above START button)
- Start. After first fase: upper left corner Fv/Fm = write down in XLS sheet
- ± 5.5 minutes (red light off)
- When this is finished go to the tab [light curve]
- Press START
- New Fv/Fm → [NO]
- Measure for ± 4 minutes (→ up to 800 PAR)
- Finished = light out
- Go to the [REPORT] tab
- On top of the screen: press 🖫 (export)
- For a next report: NEW REPORT → YES

Calculation & Interpretation of the Results

- For the analysis of Y(II), ETR(II) and qP:
    - Fit the data to the continuous model of Platt without photoinhibition as used in Ralph and Gademan (2005) using a Marquardt-Levenberg curve fitting algorithm in statistical software package R, as was described before in (Biermans et al., DOI: http://dx.doi.org/10.1016/j.jenvrad.2013.03.011).
    - In the fitting data, an alpha parameter can be determined, accounting for the slope of the curve. The alpha values for different treatments can be compared.
    - Also the saturation points of the curve (i.e. last points of the curve) can be compared for the different treatments.
- For the analysis of Y(NO), Y(NPQ), qN and qL:
    - Compare the peak height and the saturation points of the curve for the different treatments.