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Chlorophyll Fluorescence Measurements in *Arabidopsis* Wild-type and Photosystem II Mutant Leaves

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[Abstract] Chlorophyll fluorescence measurement is a widely used technique to determine photosynthetic performance. Light energy absorbed by a chlorophyll molecule can be dissipated by driving photochemical energy conversion, as heat in non-photochemical quenching processes, or it is re-emitted as fluorescence. The loss of light energy as chlorophyll fluorescence is primarily derived from photosystem II. Photosystem II is a thylakoid-embedded multiprotein complex which provides the high redox potential needed to oxidize water. Within photosystem II photons of light are captured and used to energize electrons. Energized electrons are fed into the linear electron transport chain and photosystem II replenishes lost electrons with electrons from splitting of water. Chlorophyll fluorescence yield can be quantified using a modulated fluorometer device. In such a device, a modulated measuring light beam (switched on and off at a high frequency) and the parallel detection of fluorescence exclusively excited by the measuring light allows chlorophyll fluorescence measurements in the presence of photosynthetic (actinic) light. In addition, high intensity, but short duration light flashes (saturating pulses) are used to determine maximum fluorescence yields in dark and light adapted leaves. In this protocol the procedure to receive a typical fluorescence graph of *Arabidopsis* wild-type leaves is given. Furthermore, this procedure can be used to identify *Arabidopsis* mutant plants affecting photosystem II, on the basis of the respective fluorescence graphs and values.

Materials and Reagents

1. *Arabidopsis* wild-type plants (Ecotype: Col-0)
2. *Arabidopsis* mutant plant of interest (Ecotype: Col-0)
3. Stender Vermehrungssubstrat A210, 70 potting soil (Stender AG)
4. Planting pots (7 x 6 x 6 cm)
5. Plant tray and plastic wrap
6. Tweezers (not sterile)

Equipment

1. Cold room or refrigerator (4 °C)
2. Growth chamber (12 h light/12 h dark with 21 °C/18 °C and a PFD of ~ 100 µmol/m^2/s)

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3. Dual-PAM-100 for measuring chlorophyll fluorescence (Heinz Walz GmbH, model: Dual-PAM-100) connected to a PC and operated by the Dual-PAM software (see Note 1)
4. Dark room

Software

1. Dual-PAM software

Procedure

A. Growing *Arabidopsis* plants
   1. Seeds of *Arabidopsis* wild-type and mutants lines are transferred to planting pots (approximately 20 seeds per pot) filled with completely soaked soil. Place a plastic wrap to cover the plants to keep necessary humidity.
   2. Put the pots in a tray in 4 °C for 3 days to synchronize germination.
   3. Transfer tray into a growth chamber with the plastic wrap still covering.
   4. Normally seeds germinate within 4-5 days, remove the plastic wrap 2 days afterwards.
   5. Transfer individual plants into fresh pots (one plant per pot) filled with fresh, soaked soil after 7 further days using slightly curved tweezers.
   6. Cultivate *Arabidopsis* plants in the same condition as before for another 1 or 2 weeks. See Note 2.

B. Recording chlorophyll fluorescence
   1. Make sure that the Dual-PAM-100 device is properly connected to the PC and placed in a dark room.
   2. Place 3-4 week old *Arabidopsis* wild-type plants in a dark room for at least 30 min (this incubation is called dark adaptation). Perform subsequent steps in darkness. (Optional) Turn on green light for handling plants.

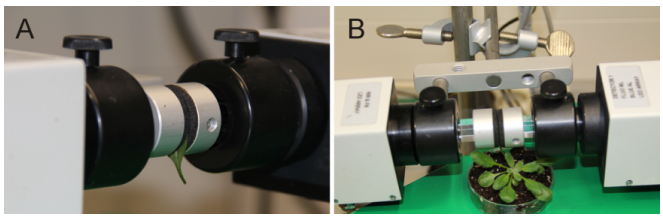

Figure 1. Set up sample leaves between the measuring head of the Dual-Pam-100 device. For the protocol given here (*e.g.* duration time 6 min) a detached leaf is fixed between the measuring head (A). For modified protocols it might be useful to keep the plant intact by using an attached leaf between the measuring head (B).

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3. Take a middle aged (*e.g.* the seventh leaf from the apex by counting down) wild-type rosette leaf and fix it between Dual-PAM-100 measuring heads (Figure 1A). (Optional) Try to fix the leaf, still attached to the remaining plant, between the measuring heads (Figure 1B).
4. Switch on the Dual-PAM software and define the settings. To measure chlorophyll fluorescence choose the <Fluo> and <SP-analysis> mode. A typical set up includes measuring light at 12 µmol/m^2/s, the saturated pulse (SP) at 10,000 µmol/m^2/s for 800 ms and the actinic light at 70 µmol/m^2/s. Save user settings, thus allowing quick start.
5. Open the <Slow Kinetics> window and apply measuring light for approximately 10 sec, fluorescence level is minimal (F_0).
6. Application of a saturated pulse will induce a maximal fluorescence level (F_m).
7. After another minute switch on actinic light. Adjust the light intensity of the actinic light to growth light (see above). Perform actinic light treatment for 5 min, the level of fluorescence at that point is called F_t (= F_0' in <Report> window).
8. A second saturated pulse is applied, which allows the measurement of the maximum fluorescence in the light (F_m').
9. Switch off actinic light, stop recording, export <Slow Kin. File> into a table calculation program (*e.g.* Excel) and recreate the graph (Figure 2A). Optional: Values can be averaged to recreate the graph. Repeat the measurement 3 times with different wild-type plants, but use leaves of corresponding age (*e.g.* the seventh leaf from the apex by counting down).
10. Repeat the measurement with a leaf of the dark-adapted mutant plant (Figure 2B).

C. Calculation of photochemical quenching parameters

1. Open the <Report> window. Values for F_0 and F_m are given. Calculate the maximum quantum yield according to the following formular (Maxwell and Johnson, 2000):
   $F_v/F_m = (F_m - F_0)/F_m$
2. Values for F_t (= F_0 in <Report> window) and F_m' are given in <Report>. Calculate the effective quantum yield of PSII photochemistry (Maxwell and Johnson, 2000):
   $\Phi_{II} = (F_m' - F_t)/ F_m'$

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Representative data

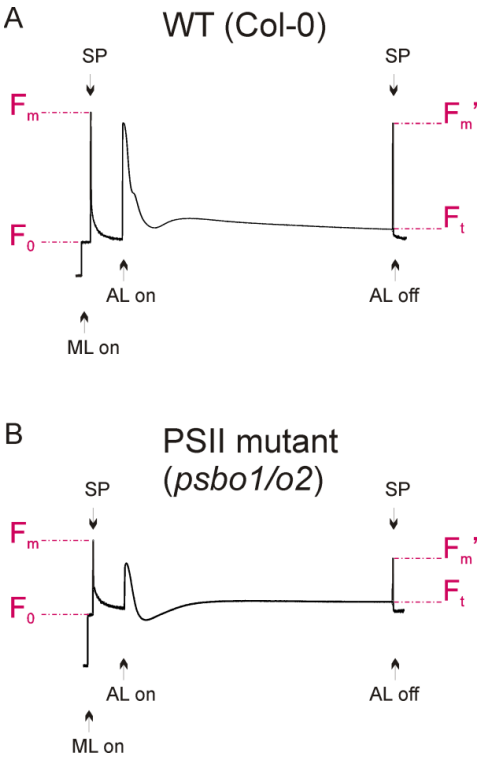

Figure 2. Fluorescence graphs of a wild-type (A) and a reference PSII mutant plant (B), see Note 3. Application of measuring light (ML) and saturated light pulses (SP) are indicated. Exposure to actinic light is shown and fluorescence parameters F_0, F_m, F_t and F_m' are denoted.

Notes

1. For first time users it is recommended to start with the Junior PAM device, because of its easy set up and use (Heinz Walz GmbH, http://www.walz.com/products/chl_p700/junior-pam/introduction.html).
2. It is important to grow healthy *Arabidopsis* plants, because any stress condition will eventually affect photosynthetic performance. Do not injure the root system upon transfer. Water plants approximately twice a week.
3. A typical wild-type fluorescence graph is shown in Figure 2A. The fluorescence graph of a reference photosystem II mutant (*psbo1/o2*) is shown in Figure 2B. This mutant was generated by crossing *psbo1-3* and *psbo2-2*, which carry a T-DNA insertion in exon 1 of *PsbO1* and in the 5' UTR of*PsbO2,* respectively (Figure 3A). Disruption of *PsbO1* and *PsbO2* entails a reduced PsbO protein level (Figure 3B). Particular mentionable is the drop of fluorescence below the F_0 value after switching on actinic light. This phenomenon concomitant with a reduced F_v/F_m value is typically observed in PSII mutants (Peng *et al.*, 2006; Armbruster *et al.*, 2010; Schneider *et al.*, 2014). Almost any PSII mutant with these characteristics is reduced in growth (Figure 3C).

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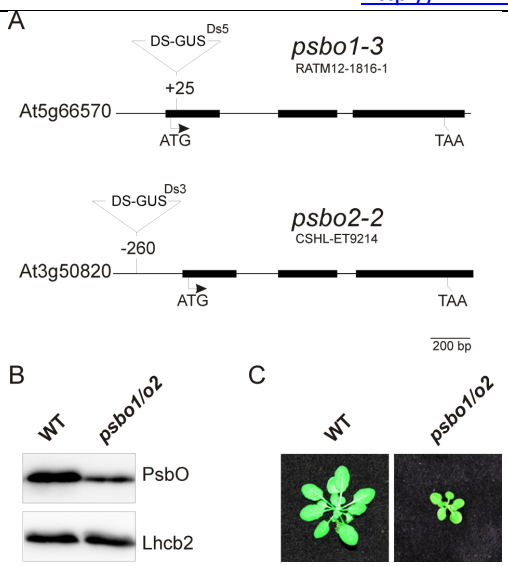

Figure 3. Generation of a reference PSII mutant plant (*psbo1/o2*) by crossing the homozygous T-DNA insertion line RATM12-1816-1 and homozygous T-DNA insertion line CSHL-ET9214 (A). The PsbO protein content is reduced in the *psbo1/o2* mutant line as deduced from immunoblotting using a PsbO- and anLhcb2-specific antibody (B). Wild-type and *psbo1/o2* mutant plants were grown for 4 weeks in a growth chamber (C).

Acknowledgments

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