DEC 01, 2022

WORKS FOR ME 1

Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferens cultures for photosynthetic efficiency and quantum yield of PSII

COMMENTS 1

DOI
dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1

Emily E. Chase[1], Gwen Stark[2], Alex Truchon[2], Steven W Wilhelm[2]

[1]University of Tennessee, Knoxville;
[2]The University of Tennessee, Knoxville

Emily E. Chase
The University of Tennessee, Knoxville

ABSTRACT

A protocol used to acquire photosynthetic efficiency (F_v/F_m) and quantum yield of photosystem II (Y(II)) of *Aureococcus anophagefferens* cultures. A maximal Fm (and minimal Fo) is acquired by dark adaption of the cultures beforehand.

DOI

dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1

PROTOCOL CITATION

Emily E. Chase, Gwen Stark, Alex Truchon, Steven W Wilhelm 2022. Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferens cultures for photosynthetic efficiency and quantum yield of PSII . **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1

KEYWORDS

Aureoccocus, photosynthetic efficiency, quantum yield, Fv/Fm, algae, brown tide, HAB, chlorophyll fluorescence

CREATED

Sep 19, 2022

LAST MODIFIED

Dec 01, 2022

PROTOCOL INTEGER ID

70236

GUIDELINES

The collection of quantum yield and photosynthetic efficiency is highly sensitive to modifications in sampling protocol. Careful attention must be given to the processing of individual samples in the same manner, and in the case of dark adapted samples, reducing sample exposure to light.

MATERIALS TEXT

PHYTO-PAM-II Compact Version and components
Laptop with a USB port, and PhytoWin_3 installed
Measurement cuvettes
At least 3 mL of culture material for each sample
70% Ethanol
KimWipes
Necessary materials and setup for both dark adapting cultures, and a no light or low light environment for sampling

BEFORE STARTING

Familiarise yourself with the PHYTO-PAM-II equipment, manufacturer's provided manual, and the basics of chlorophyll fluorescence parameters for full use of data collected and accuracy of results.

SAMPLE PREPARATION

1 Prepare 3 mL of each culture of *Aureococcus anophagefferens* to be measured in appropriately labelled, separate 15 mL Falcon tubes. Additionally, prepare at least one sample to carry out the auto-gain function if you do not know the appropriate gain (see **Step 3.1**).

2 For acquiring F_o and F_m, dark adapt all samples for 20–30 minutes. Both quantum yield (Y(II)) and photosynthetic efficiency (F_v/F_m) can be collected without dark adaption, but will be deduced when the photosystem II has not been completely cleared. Dark adaption is completed to obtain the maximal possible value of fluorescence (F_m) upon light application.

Note

Pay careful attention to the dark adaption set up so that individual samples can be easily retrieved with minimal exposure of light to other samples. It is important to keep dark adaption times and practices the same among all samples you would like to compare at present or from future experiments.

EQUIPMENT PREPARATION

3 Start the PhytoPAM-II Compact Version up by toggling the power, and plugging in the charger. Connect the equipment to a laptop or computer by USB. Select the PhytoWin_3 program from the computer, select the appropriate measuring head you are using (Compact Version = "Phyto Compact Unit"). See **Figure 1** for the

Citation: Emily E. Chase, Gwen Stark, Alex Truchon, Steven W Wilhelm Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferensÃÂ cultures for photosynthetic efficiency and quantum yield of PSIIÃÂ
https://dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1

software interface. In order to take measurements ensure that the program is in "MEASURE" mode. "VIEW" mode is exclusively for reviewing record files (*i.e.,* data collected). Ensure the "ML" (measuring light) light is selected (signalled by a green light), and that "AL" (actinic light) and "FR" (far red) are not selected. Wait for the light directly under the PAR input to be green before proceeding with any measurements (if the equipment is not ready the light will be red). If you have not deduced a gain to use during measurements then proceed to step 3.1, otherwise proceed to **Step 4**.

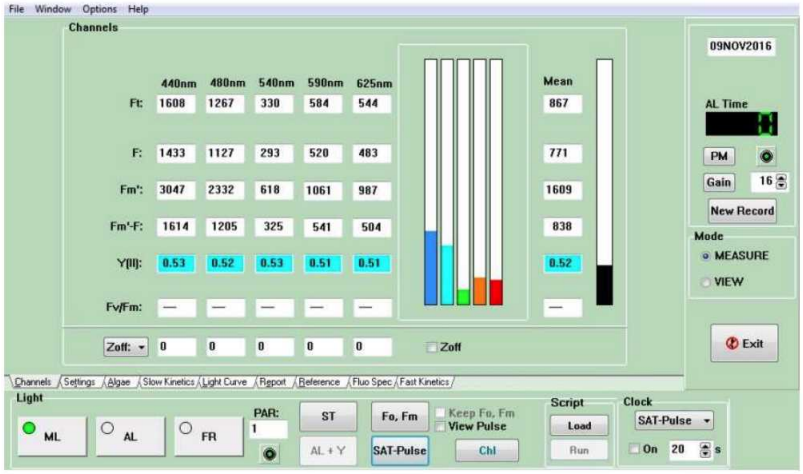

Figure 1. PhytoPAM-II Compact Version PhytoWin_3 program interface.

3.1 Transfer 3 mL of culture sample (dark adapted or not) to the measuring cuvette, wipe down the sides of the cuvette with a KimWipe, place the cuvette into the measuring head, replace the lid, and click "Gain" on the measurement green (right side). This will provide a gain automatically that will work well with your sample.

Note

Too high of a gain will overload the measurement, and too low will not register. The gain you select should be consistent throughout any experiments to ensure the results are comparable. Under a 12/12 light cycle at 19°C Aureococcus anophagefferens can normally be registered with a gain between 8–11. A very high density culture (10+ million cells per mL) will require a gain different than a culture with a "low" density (~1 million cells per mL).

MEASUREMENT ACQUISITION

4 Experimental measurements can now be collected. Set the gain to the appropriate level.

Note

Citation: Emily E. Chase, Gwen Stark, Alex Truchon, Steven W Wilhelm Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferensÃÂ cultures for photosynthetic efficiency and quantum yield of PSIIÃÂ
https://dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1

When running dark adapted measurements it is best to work in the dark or with very low light, and to remove samples from the dark only at the moment of sample collection. All measurements should be taken in the same way to allow for comparability between samples (e.g. mix each sample directly before measuring, transfer samples to the cuvette in the same way, etc.).

5 Add 3 mL of the culture to be measured into the cuvette, wipe down the sides with a KimWipe, place into the measuring head and cover with the cap. Wait for the indicator lamp to turn green (from red). Press the "F_o, F_m" button.

Note

Fingerprints and other debris on the sides of the cuvette may obscure the measuring light and therefore produce an inaccurate measurement.

6 Measurements have now been recorded in the "Channels" and "Algae" tab. Check relevant wavelengths and/or "Algae" settings for comparing samples/treatments. Once completed click "New Record" before insertion of the next sample. Ethanol (70%) should be used to clean the cuvette between different treatments.

Note

For Aureococcus anophagefferens we have recorded the "brown" algae results on the "Algae" tab, and the wavelengths with the highest F outputs (normally 440 nm). If "New Record" is clicked after the insertion of a fresh sample into the measuring head the Fo,Fm will be affected, thereby reducing the sample's comparability within the experiment.

7 Acquire data for all samples, record, and shut down the program, toggle the machine off, and unplug the charger and USB connection.

Note

It is recommended to store the equipment for prolonged periods (long term) with a full charge. If the equipment is sitting unused for months it is advisable to charge it to full capacity on occasion to prolong the life of the battery.

Citation: Emily E. Chase, Gwen Stark, Alex Truchon, Steven W Wilhelm Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferensÃÂ cultures for photosynthetic efficiency and quantum yield of PSIIÃÂ
https://dx.doi.org/10.17504/protocols.io.yxmvm2785g3p/v1