CHLOROPHYLL FLUORESCENCE ASSAY

PLANTING:

This protocol uses a specialized planting to allow On a Wednesday or Thursday, prepare one 96-plug flat with Redi-Earth Plug and Seedling Mix. Place flat code on tray and subflat code on bottom left position of each subflat of 12 (8 subflats). Add a small cheesecloth square (double thickness) to each pot. Add prepared soil mix using the soil bags in room 108A. Do not use greenhouse soil! Add a fine layer of sterilized vermiculite to the top of each pot. Bottom water the pots with Nutrient Solution so that pots are submerged in about ½ inch of solution. Cover with a clean clear plastic dome and tape the dome to one side of flat. Label the tape with the date and "Chlorophyll Fluorescence". Store in the cold room until planting on Friday.

On Friday, plant the designated box of seeds and controls. Using the laptop computer with the barcode scanner attached, open the Chlorophyll Fluorescence Assay database. Scan in the flat code, the subflat code and then the first seed stock code. To plant, add 6-8 seeds to a 2" square piece of glassine weighing paper which has been creased in the middle. Pour the seeds into the center of the pot, and then discard the weigh paper. Submit the pot to the database. Continue scanning and planting until the subflat is full, and then close the subflat by scanning in the subflat code. The order of planting for each subflat is top left to top right row by row, beginning at the top left subflat. The second subflat to be planted is the subflat adjacent to and to the right of the first subflat. Open the second subflat by scanning its subflat barcode into the database and continuing the planting process as described. When the last plug pot of the last subflat has been planted (bottom right plug pot of bottom right subflat which should be a high fluorescence mutant spike control), close the subflat and then close the flat by scanning the appropriate barcodes. Close out of the database. Store the flat in the cold room.

On Tuesday of the following week, remove the flat from the cold room and place it in a Growth Chamber (12/12 cycle) of Room 108A Biochemistry. This is **Week 0**. Friday, rotate the flat 180 degrees and check for germination. Crack the dome if all plants have germinated.

On Monday of **Week 1**, remove the dome, rotate the flat 180 degrees and bottom water as needed. On Tuesday, use a forceps to thin each pot to 3-4 plants. Rotate the flat on Wednesday and bottom water if needed. Rotate the flat and bottom feed with Nutrient Solution on Friday.

On Tuesday of **Week 2**, thin to one plant per pot.

On Monday of **Week 3**, add a fine layer of vermiculite to the soil to mask any algal growth, which would otherwise cause high background fluorescence. Assay the plants for chlorophyll fluorescence. Wednesday, perform Recovery Assay. Discard plants when the assay has been completed.

CHLOROPHYLL FLUORESCENCE ASSAY PROTOCOL

BEFORE HIGH LIGHT

Two boxes are at back of computer laptop. The top box is the battery charger the bottom is the controller for Maxi PAM. A green light indicates the battery is charged and must be so to run. If it is red, charge first. First thing on the Monday morning of an assay, plug in both boxes into the outlet on the left. Turn on the charger, NOT the controller so that battery gets charged. It takes about one hour. Once battery is charged, then turn on the controller.

The overhead light may be on. It is not sufficient to effect photosynthesis or the assay.

Software is ImagingWin. Open ImagingWin. Maxi: okay. Then turn on controller. There will be a warning message about a file not found, but ignore that. There will now be periodic pulses of $3\mu Ei \cdot m^{-2} \cdot sec^{-1}$ (purple) light. This is correct, but if a pulse of bright light flashes, quit the program and then open it again. If a subflat is under the light at that time, it must be dark-adapted (in the same room is fine) for 20-30 minutes before retesting. Be sure to remember this so the position is correct for later data entry. Set up the folders for storage of the data. The empty folder can be copied and renamed – use it as a template for saving future data.

Choose the Light Curve tab. The x axis represents light intensity. Choose edit, then open 2010CF folder, 2010CF(method).lcp. The program is set to run at 3µE for 60 seconds, following a flash of bright light and then at 533 µE for 3 minutes following a second blast of bright light.

Choose the Image tab. Manually position the subflat on the Maxi PAM platform (5 green tip boxes high for proper height). Choose the green Measure button at the bottom. Choose the visible light for easier viewing, and then choose the fluorescent radio button. Press New Record button. If it asks if you want to keep Fo/Fm, choose No.

Select the Light Curve tab, and press Start. The program will run automatically, just make sure no improper flashes of light occur at the wrong times.

Once the program has completed for that subflat (actinic light will be off and the 3uE pulsing light will go on indefinitely), the Save button at the bottom will no longer be grayed out. Choose Save, then select the proper folder (before HL, raw data). Saving format may change, but for now each subflat should be saved as Box XX-#(1-8 for subflat) subflat code#. Once that is done, remove the subflat from the platform and repeat with the seven remaining subflats for that flat. When computer asks if you want to save the prior record, choose no – it is already saved on file. Do not turn off computer or controller.

Water flat with about ½ inch of water. Wear sunglasses and check HL chamber with light meter to make sure it's 1500 to 1700$\mu Ei \cdot m^{-2} \cdot sec^{-1}$ on the shelf to be used. Place flat on

that shelf for 3 hours. Return flat to dark room to take the Fv/Fm readings. No NPQs at this time.

AFTER HIGH LIGHT (3 HOURS in 1,500-1,700uE)

Cover flat with black bottom tray of a second flat to stop any photochemical changes and remove flat from high light chamber. Return to darkroom. Make sure battery charging box has the green light illuminated. Open Imaging Win and turn on controller and select Maxi.

Position subflat on platform as before. In the Settings tab, choose the Default settings. Select the green Measure button, then New Record. When asked if want to save Fo/Fm, click No or Don't Save. Choose the Fv/Fm radio button at the bottom, and then rather than pressing Start, INSTEAD choose the Fo/Fm button at bottom center. Shield your eyes for the flash. Once the flash is completed, choose Save, and save in the After 3hr HL folder using the Box 31-(1-8subflat position) subflat barcode as the file name.

Repeat for remaining subflats.

Copy the BHL and AHL raw data onto a memory stick and then transfer that data onto a desktop computer having the ImagingWin software program. Data can be analyzed using cutoff values and subsequently entered into the Excel worksheet and 2010 database.

Return flat to its appropriate place in the growth chamber and allow to remain there for two days, not exceeding 48 hours.

On one of the Last Lab laptops containing the Imaging Win software, view images for each subflat, by opening the subflat file within the ImagingWin program. Choose the Fv/Fm button and on the Analysis page, dial in the high and low cutoff values (e.g., 0.420 and 0.000) to see if any mutants are found – would appear as red plants).

RECOVERY

Bring flat to the darkroom. Analyze for Fv/Fm as for After High Light Measurements, but there is no need to shield the flat from ambient light.

Copy the Recovery Analysis onto a memory stick, and enter into the Excel sheet and 2010 Database as above.

NOTES - DETAILED NOTES FOR TECHICIANS RUNNING THE ASSAY.
CHLOROPHYLL FLUORESCENCE (CF) MEASUREMENT

1. When in the darkroom B, make sure you touch a metal surface (the stool works well) before you touch the table with the computer/Maxi-PAM, as there is much static which can cause a shock and will crash the program.
2. Turn on the Maxi-PAM controller (bottom box). The charger (top box) should have already been turned on for at least one hour beforehand.
3. Turn on the computer. Select the Administrator icon. The password is “vitamine”.
4. Open the Imaging Win software. The Maxi-PAM should now be blinking a low purple light. (If it is bright, pulsing, or anything else, turn off the Maxi-PAM controller and close Imaging Win. Turn on the Controller again and then open Imaging Win. If the incorrect blinking happens again, shut the controller, program, and the computer down and try again.)
5. Once the Imaging Win software opens, you’ll get a message that says to make sure some setting is at7 msec. Ignore it, it will go away. You’ll be asked to select the Measuring Head. Select Maxi. It will then tell you some Corr file is not found, and just ignore this also by pressing Okay. (You may get an error message stating that Microsoft has encountered a problem, asking you to debug and/or send an error report to Microsoft. Choose Debug, but don’t send an error report. Then close the Imaging Win software, turn off the Maxi-PAM controller, and try again.)
6. Once everything is working, choose the Settings tab. At the bottom right of this screen, press the Default settings button.
7. Choose the Image tab. The low purple light should be blinking. Choose the Select Live Video (at right) radio button. Position the subflat on the green plastic stand so that you can see all twelve plants. Close the Live Video box by clicking the X in the top right corner, and the low purple blinking light returns. If the light changes to a constant or pulsing bright light, pull the subflat out immediately, turn off the controller and quit Imaging Win. Restart everything as above. Don’t test this subflat again for at least 10 minutes. Continue with the next subflat.
8. At the bottom of the screen, under Select Type of Image, choose the Fv/Fm radio button. Shield your eyes and then press the Fo,Fm button. One very bright purple pulse should occur. That is the entire measurement! (If the Maxi-PAM starts to pulse brightly and does not return to the low intensity blinking purple light, shut everything down and start over. Don’t test this subflat again for at least 10 minutes.)
9. Save this file (the save icon is toward the bottom left of the screen) in 2010 Project Mutants/Box ## (FCFXXXX)/Recovery/Raw Data. The filename format is: Box ##-1(or 2, 3, 4, 5, 6, 7, or 8) (FCF10XXX). Press Save.
10. Repeat steps 7-9 for the other seven subflats with this addition: for the subsequent subflats, once the subflat is positioned properly, choose the New Record button (near middle bottom of screen). You’ll be asked if you want to save the previous record. Choose No (this is important!). Then after you press the Fo,Fm button, you’ll be asked if you want to save the previous Fv/Fm value, and choose No again.
11. When all eight subflats have been measured, quit Imaging Win and turn off both the Maxi-PAM controller and camera charger (top box).

12. Insert the memory stick into the right side slot of the computer. Do not disturb the cable that's adjacent – it doesn't fit very tightly. Open Imaging Win again. You'll get a message stating, "No camera found. Running in Demo mode." Press Okay. Then choose Maxi head on the next window that pops up. In the Image tab window, choose the open file icon near the left bottom of the screen. Select the Recovery file folder you've just saved your measurements in and copy it onto the memory stick in the appropriate file folder within the Chlorophyll Fluorescence folder.
13. Close Imaging Win, remove memory stick, and shut down the computer. Make sure all the lights are off in the dark room and the ante rooms, and that the door leading to the darkrooms locks on your way out.
14. Place the tray insert and the subflats on the hallway table. Place a small piece of yellow tape on the top of the flat. Save the white tray, the black insert cover and the dome lid. The white tray and dome lid can go in our sink to be washed. Just save the black insert cover in the cardboard box for next time.

Kathleen Imre/edited by Rob Last
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