Plant Leaf Dark Adaptation - How long is long enough?

Dark adaptation is a technique used in some chlorophyll fluorescence measurements to fix a non-stressed reference point relative to various measurements (Maxwell and Johnson 2000). Deciding where to put that reference is based on an understanding plant mechanisms that can affect measurements, and what one wants to measure.

Dark adaption times of ten minutes, twenty minutes, thirty minutes, forty minutes and sixty minutes are common for terrestrial plants, and some researchers use pre-dawn values.

To obtain reliable modulated Fv/Fm or OJIP test values, decisions need to be made for control and test measurements. The plant mechanisms listed below will lower Fm, and possibly raise Fo, changing OJIP and Fv/Fm measurements downward like other types of plant stress. One must decide which mechanisms are of concern for specific types of plant stress measurement and dark adapt accordingly.

Fv/Fm is affected by both photochemical and non-photochemical factors. If a leaf is dark adapted and measured, then subjected to high light levels, then dark adapted and re-measured, the first measurement will be higher then the second measurement. The decline in Fv/Fm measurement may be due to a decrease in reaction centers capable of photochemistry or un-reversed non-photochemical quenching. (Baker N.R., Oxborough K. 2004)

Papageorgiou reports that results may vary greatly depending on how long dark adaptation is done. A few minutes of dark adaptation is enough to re-oxidize the plastoquinone pool and the CaMn4OxCly cluster, while longer periods deplete respiratory substrates through respiration in cyanobacteria and chlororespiration in higher plants and algae. Longer times will also deplete ATP pools, and trans-membrane ion concentration gradients. Dark adaptation also shifts higher plants and algae toward state 1 conditions and cyanobacteria to state 2 conditions. (Papageorgiou G.C. Tismmilli-Michael M. Stamatakis K. 2007)

Full activation of Rubisco takes between three and four minutes in vascular plants as well as photoplankton. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton. The longer deactivation is thought to offer an advantage for species subjected to erratic bright light for maximum utilization of light (MacIntyre 1997).

Rapid acting photo-protective mechanisms activated by exposure to variable light intensities (designated in the parameters qE and Y(NPQ) are the xanthophyll cycle and thylakoid lumen Δph. They relax in a few minutes during dark adaptation. (Muller, Niyogi 2001),(Kramer D. M., Johnson G., Kiirats O., Edwards G. (2004). According to Lichtenthaler (1999) this time is 4-6 minutes.

State I – State 2 transition quenching (called qT) is most significant at lower light levels in terrestrial plants and can represent more than 60% of quenching at low light levels. At high light levels it represents about 6% of total quenching. State transition quenching relaxes in ten to twenty minutes in terrestrial plants. (Lichtenthaler H. Burkart S 1999)

It has been shown that the effects of acute photo-inhibition caused by exposure to high light intensities can be reversed with 20 to 30 minutes of dark adaption (Theile, Krause & Winter 1998), where as reversal of chronic photo-inhibition caused by several hours of exposure starts to

relax at about 40 minutes and may take 30 to 60 hours to fully relax under dark adaptation (Lichtenthaler H. & Babani F. (2004) (Theile, Krause & Winter 1998)

When making longer quenching and quenching relaxation parameter measurements related to photo-inhibition and photodamage mechanisms that are common in chronic high light stress, high heat stress, cold stress and over wintering stress, one should understand that it can take days for full relaxation or repair of the non-photochemical quenching parameters, qI and Y(NO), to pre-stress conditions. To get an accurate control value for Fm and Fo under chronic photo-inhibition conditions (components of non-photochemical quenching parameters) it is common to dark-adapt for a full night, or 24 hours. (Maxwell and Johnson 2000) In some cases longer times may be appropriate.

In Aquatic Plants Gorbunov (2001) is a good source for corals, and Consalvey (2004) is a good source for Algae. For information regarding dark adaption for rapid light curves Rascher 2000 is a good source.

The use of far-red pre-illumination that is available on some fluorometers is designed to rapidly re-oxidize PSII by activating PSI. While this can be valuable in fieldwork (Maxwell and Johnson 2000), it does not affect the relaxation of non-photo-chemical quenching mechanisms Consalvey (2004).

Dark adaptation can be accomplished by using dark adaptation leaf clips or cuvettes. Some researchers use hundreds of inexpensive clips to make measurements on larger population quickly. Shrouds or darkened rooms may also be used.

In review, it is important to take a few things into account. Reliable dark adaptation times can vary by species, plant photo-history, the fluorescence parameter of interest, and the type of stress that needs to be measured. When dealing with a new species, or an unknown photo-history it is probably best to test for maximum and stable Fv/Fm at different dark-adapted times for best results. When testing for optimal dark adapting times it is important to use samples that have been exposed to the maximum light conditions that will occur during the experiment for reasons discussed above.

For a complete free "Stress Guide" that deals with research, references, and recommendations on all kinds of plant stress contact Opti-Sciences by phone or E-mail.

Dark Adaptation Tests:

OS30p - Dark-adapted tests available: Fv/Fm with Fo and Fm and basic OJIP.

OS1p - Dark-adapted tests available: Fv/Fm, NPQ, Y(NPQ), Y(NO), Fod (or Fo'). Adjustments for far-red pre-illumination and post illumination are included.

OS5p - Dark-adapted tests available: Fv/Fm, OJIP, NPQ, qN, qE, qT, qI, qP, qL, Y(NPQ), Y(NO), Fod (or Fo'). Adjustments for far red pre-illumination and post illumination are included.

Light Adapted tests are also available on the OS1p, OS5p. Yield or (ΔF/Fm'), ETR, qL, Y(II) and light curves via the stepped actinic test.

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