

Desk-Top Stress Guide

The following pages represents a compilation of research done using chlorophyll fluorescence for stress detection and measurement. It is organised by stress type with important introductory notes listed first.

The best tests for different types of stress are listed on the following pages. Test are listed in order with the best tests listed first. This guide is intended as a starting point for research. Results may sometimes vary by plant species. Results were complied by OptiSciences Inc. from world wide published research independent of fluorometer brand name.

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Fv/Fm and Yield

Fv/Fm and Yield are both very robust tests that have been shown to correlate well with carbon fixation under most conditions. However, they do have some limitations in stress measurement. Some types of plant stress do not affect PSII especially in early phases. *To compensate for these limitations inventive researchers have found ways to solve some of these issues by developing unique working assays. These improvements are listed under specific stress categories.*

Limitations of the two most commonly measured parameters:

Fv/Fm – Dark adapted test - a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. 0.83 is the approximate optimal value for most plant species with lowered values indicating plant stress.
(Maxwell K., Johnson G. N. 2000) (Kitajima and Butler, 1975). Fv/Fm has a photochemical component and a non-photochemical component (Baker 2004).

- 1. Not sensitive to early or moderate water stress. (Bukhov & Carpentier 2004) (Zivcak 2008)
- 2. Fv/Fm is not sensitive to nitrogen stress until very low levels are reached. (Baker 2004)
- 3. Fv/Fm is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004)
- 4. Not sensitive to DCMU herbicide stress. (Nedbal & Whitmarsh 2004)
- 5. Not sensitive to some other herbicide stress types. (Nedbal & Whitmarsh 2004)
- 6. Not sensitive to nickel stress. (Joshi & Mohanty2004)
- 7. Not sensitive to zinc stress. (Joshi & Mohanty2004)
- 8. Not sensitive to NaCl stress in rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)

Yield or △F/Fm' - Light adapted test – a measurement ratio that represents achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions. (Genty 1989), (Maxwell K., Johnson G. N. 2000), (Rascher 2000) It is affected by closure of reaction centers and heat dissipation caused by non-photochemical quenching. (Schreiber 2004)

- 1. Fs a component of Yield is sensitive to moderate water stress at saturation light levels. (Flexas 1999),(Flexas 2000), (Flexas 2002)
- 2. Yield with heat applied is sensitive to very early water stress. (Burke 2007)
- 2. Yield is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004)
- 3. Not sensitive to early or moderate CO2 stress. (Siffel & Braunova 1999)
- 4. Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)

Notes for stress measuring:

1. It is Common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997)

2. **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 of 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 photochemical plastoquinine 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).

Rapid acting photo-protective non-photochemical mechanisms activated by exposure to variable light intensities (designated in the parameters qE and Y(NPQ) are the xanthophyll cycle and thylakoid lumen \triangle ph. 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 2-4 minutes.

Non-photochemical 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 5% of total quenching. State transition quenching relaxes in less than twenty minutes in terrestrial plants. (Lichtenthaler H. Burkart S 1999)

It has been shown that the effects of non-photochemical 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)

Notes for stress measuring:

Dark Adapting –Continued

When making longer non-photochemical quenching and quenching relaxation 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 could 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 source for dark adaptation in corals, and (Consalvey 2004) is a good source for Algae. For information regarding dark adaption and 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 reoxidize 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).

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 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.

3. For reliable Yield measurements photosynthesis must be at steady state, an equilibrium condition reached after a few minutes of exposure to existing light radiation conditions. Maxwell and Johnson (2000) tested 22 different species of British plant and found that steady state occurred in fifteen to twenty minutes in the plants measured. Measurements taken under variable lighting conditions may not provide reliable Yield results (Rascher 2000). No dark adaptation is required.

Water Stress:

Best Tests

 Yield with heat treatment
 This is a light adapted test can also be used for very early water stress. Leaf disc samples are collected with a leaf punch and heated to 39 degrees centigrade. Yield measurements are made before heating and after heating. Sensitive to water stress within 24 hours after cessation of irrigation. (Burke 2007) Par Clip recommended.

- Fs/Fo & Fs
 -Fast light adapted steady state fluorescence test. <u>The best modulated test for</u> <u>moderate water stress.</u> Tested in C3, C4, and CAM plants. Fs/Fo is normalized ratio using Fs that allows comparison between samples. Fo is a predawn value of Fo. Actinic light is used at saturating levels between 800 to 1250 umls. (Flexas 2002), (Flexas 2000), (Flexas 1999)
- ETR/A Fast light adapted steady state fluorescence test. In C4 Plants The ratio of ETR to water assimilation, ETR/A, is known to be consistent. This is not true in C3 plants. ETR requires a PAR Clip. (J Cavender-Bares & Fakhri A. Bazzaz 2004) (Cerovic 1996)
- Fast dark adapted test for water stress within three days after cessation of irrigation on wheat using OKJIP protocol. This is a normalized OJIP parameter for comparing data between samples. Sensitive to changes of Relative Water Content in the range of 80% and below. The test correlates well with CO2 gas exchange data during water stress measurements. (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008) (Thach 2007)
- K Step Fast dark-adapted test for water stress using OJIP protocol (Strasser 2004).

Stepped Actinic Test – Slow test that helps identify water as the cause of stress. This is a longer light adapted test. Fs has been found to decrease as light intensity increases. (Flexas 2000)

Other Tests

- Yield Fast light adapted test can also be used for water stress in steady state not as sensitive to water stress as its component Fs/Fo and Fs. (Flexas 1999)
- NPQ Slow test, increases with moderate to late water stress. This is a dark adapter test. (Cavender-Bares J. & Fakhri A. Bazzaz 2004)
- Fv/Fm Leaf treated with high light irradiation and polyethylene glycol to induce water stress. 20 mm leaf plugs are collected and treated with polyethylene glycol PEG at 6000 mol weight at various concentrations to induce water stress and exposed to 1500 to 1800 umls for two hours before dark adaption. (Nair D. B., Alam B., Jacob J. 2005)

Non Sensitive Water Stress Tests:

 Fv/Fm - Fast dark-adapted test is <u>not</u> sensitive to early or moderate water stress. (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008) In some species Fv/Fm is more sensitive to water stress than in other species. (Deng X. Hu Z., Wang H., Wen X., Kuang T. 2003)

Light Stress:

Best Tests

Quenching and Quenching Relaxation Test	– Best test to study photo-protection mechanisms
	the \triangle ph of the thylakoid lumen, and the
	xanthophyll cycle. NPQ, qN, qP, qL, qE, qт, qı,
	Y(NPQ), Y(NO). This is a longer dark adapted
	test (Muller, Niyogi 2001) (Kramer 2004)
	(Hendrickson 2004) For standardized definitions see
	(van Kooten O., & Snel J.F. 1990)

- Stepped Actinic Test The effects of light level increases and decreases can be studied easily. This is a longer dark adapted or light adapted test. (Muller, Niyogi 2001) (Kramer 2004), (Hendrickson 2004).
- Yield Fast light adapted test can also be used for light stress in steady state sensitive to light stress. (Cavender-Bares & Bazzaz 2004)
- PI Fast dark-adapted test sensitive to light stress using OKJIP protocol (Thach 2007).
- **Fv/Fm** Fast dark-adapted test can be used to detect light stress. (Adams & Demming-Adams 2004) PI is more sensitive to light stress that Fv/Fm (Thach 2007)

Other Tests

- Light Curves Long (usually dark-adapted test) where light levels are increased after steady state photosynthesis has been reached. These are curves that show the results of light level on yield and Electron transport rate. Yield is accurate. (Rascher U. 2000)
- Rapid light Curves –A longer dark adapted test that takes less than five minutes. Steady state photosynthesis is not reached. Data from several measurements at different times of day are required for reliable results. This is a test that is used for aquatic plants and under canopy plants where light is constantly variable and other methods of testing will not work. Parameters ETRMAX and PPFD_{sat} are possible. (Rascher U. 2000)

Heat Stress:

Best Tests

Quenching and Quenching Relaxation Test – Best test to study moderate heat stress	
in Spin	ach plants. NPQ (Tang Y., Wen X.,
Lu Q.,	Yang Z., Cheng Z., & Lu C. 2007). Other
quench	ing parameters include qN, qP, qL,
qe, qt, c	qı, Y(NPQ), Y(NO). (Schreiber U. 2004)
For def	initions of quenching parameters see (Muller
P., Xiao-Ping L., Niyogi K. (2001) & (Kramer D. M	
Johnson G., Kiirats O., Edwards G. 2004)	
For star	ndardized definitions see
(van Ke	ooten O., & Snel J.F. 1990)

Other Tests

- **Fv/Fm** Fast dark adapted test could be used to detect severe heat stress in cotton above 45 degrees centigrade. (Crafts-Brander and Law 2000)
- **Fv/Fm** Fast dark adapted test can be used for heat stress. (Schreiber U. 2004) (Baker and Rosenqvist 2004)
- Yield Fast light adapted sensitive test can also be used for heat stress in steady state. Dascaliuc A., Ralea t., Cuza P., (2007) (Schreiber U. 2004)
- K Step Fast dark adapted test sensitive to heat stress using OJIP protocol sensitive (Strasser 2004)
- **PI** Fast dark adapted test sensitive to heat stress using OJIP protocol. This is a normalized parameter for comparing different samples.(Strasser 2004) results reported at 44 degrees centigrade and above.

Nutrient Stress:

Best Tests

- Yield Fast light adapted test that can also be used for <u>nutrient</u> stress in steady state. (Cavender-Bares and Bazzaz 2004) (Baker and Rosenquist 2004)
- K Step Fast dark adapted test that is sensitive to <u>nutrient</u> deficiency. Sensitive to nitrogen deficiency. (Strasser 2004).
- **PI** Fast dark adapted test sensitive to <u>nutrient</u> stress using OJIP protocol. (Strasser 2004) (Thach 2007).

<u>Nitrogen</u>

- **FRFex360/FRFex440** Fast test that can be done in light adapted or dark adapted work. Very sensitive to <u>nitrogen</u> deficiency. It allows the investigator to <u>separate nitrogen</u> <u>deficiency from sulfur deficiency</u> (Sampson and Treblay 2000).
- Yield *at high light levels* Fast light adapted test that can also be used for <u>nitrogen</u> stress in steady state for C3 plants. Various nitrogen levels can be distinguished better using high light levels (Cheng 2001)
- **CCI or SI** These are reflectivity indices not fluorescent parameters that measure greenness of a leaf and leaf thickness. They are used in chlorophyll content meters for fertilizer and nitrogen management programs. (Peterson 2006)
- K Step Fast dark adapted test that is sensitive <u>nitrogen</u> deficiency in soybean & maize (Strasser 2004)
- **qP** Slow modulated test that shows some nitrogen deficiency, but not sulfur deficiency. (Baker and Rosenqvist 2004)
- Yield Fast light adapted test that can also be used for <u>nitrogen</u> stress in steady state. (Cavender-Bares and Bazzaz 2004) (Baker and Rosenqvist 2004)

<u>Boron</u>

Yield and ETR – Fast Light adapted test sensitive to Boron deficiency in sunflowers (Kastori R., Plesnicar M., Pankovic D., Sakac Z., 1995)

<u>Calcium</u>

Fv/Fm – Was found to detect Ca stress in tomato plants (Shmidts-Eiberger, Haefs, Noga)and apple trees (Shmidts-Eiberger, Haefs, Noga 2002).

<u>Chlorine</u>

- Lack references

<u>Cobalt</u>

Yield - Cobalt. (Joshi & Mohanty2004)(Tripathy 1983)

<u>Copper</u>

- Yield <u>Copper.</u> Sensitive test (Joshi & Mohanty2004) (Lanaras 1993)
- Fo/F_{5min} A slow dark adapted test that is sensitive to <u>copper</u> deficit. (Adams, Norvell, Philpot & Peverly 2000), (Kriedemann 1985)

Iron

K Step – Fast dark adapted test that is sensitive <u>iron</u> deficiency in soybean & maize (Jiang, Gao, & Zou 2006)

<u>Magnesium</u>

PI – PI has been shown to be sensitive to Mg deficiency (Hermans C, Johnson GN, Strasser RJ, Verbruggen N, 2004)

<u>Manganese</u>

Fo/Fv - A fast dark adapted test very sensitive to <u>Manganese</u> deficiency. (Adams, Norvell, Philpot & Peverly 2000), (Kriedemann 1985) (Hannam 1985)

<u>Molybdenum</u>

- Lack references

<u>Nickel</u>

ETR - <u>Nickel</u>. Fv/Fm is not a good indicator of Nickel stress. (Joshi & Mohanty2004), (Tripathy 1981)

<u>Phosphorus</u>

- Fv/Fm Has been shown to be sensitive to <u>phosphorus</u> stress (Stark, Niemyska, Bogdan & Tawlbeh 2000)
- PI PI is sensitive to <u>phosphorus stress</u> in Sorghum (Ripley, Redfernand, Dames 2004)

<u>Potassium</u>

Yield, NPO, and qP - were effective in detecting <u>K</u> deficiency in rice plants. Experiments with K deficiency in reference to photoprotection mechanisms. (Weng, Zhen, Xu, Sun 2008)

<u>Sulfur</u>

- **CCI or SI** These are reflectivity indices not fluorescent parameters that measure greenness of a leaf and leaf thickness. They are used in chlorophyll content meters for fertilizer and nitrogen management programs. Readings for sulfur stress and nitrogen stress are indistinguishable. (Yara fertilizer management guide on line),(Peterson 2006). Fluorescence is not a good indicator of sulfur stress. (Baker and Rosenqvist 2004)
- **Fv/Fm** was found to detect <u>severe sulfur</u> stress in Chlamydomonas (Antal T., Volgusheva A., Kukarskikh G., Krendelva T., Tusov V., Rubin A. 2005)

<u>Zinc</u>

Fs in Yield - <u>Zinc</u> - Fv/Fm is <u>not</u> a good indicator of zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980) (Krupa 1993)

Non-sensitive Nutrient tests:

- Fv/Fm Fast dark adapted test that is only sensitive to nitrogen content at very low levels, and Sulfur at starvation levels. (Baker and Rosenqvist 2004)
 It is also not a good test for Zinc (Joshi & Mohanty2004).
 It is also not a good test for nickel. (Joshi & Mohanty2004)
- Yield Fast light adapted test is sensitive to Sulfur deficiency at starvation levels . (Baker and Rosenqvist 2004)
- **qP** Slow modulated test is sensitive to Sulfur deficiency at starvation levels . (Baker and Rosenqvist 2004)

Cold Stress: All tests below are important in Cold stress studies.

ETR/ CO2 Assimilation- The ratio of ETR in PSII to CO2 assimilation changes in cold stress indicating other electron sinks in cold stress. (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998)

- Yield Fast light adapted sensitive test can also be used for moderate cold stress in steady state. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).
- Fv/Fm Fast dark adapted test can be used for moderate cold stress. (Oquit and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994,1995), (Ball 1995).
- Stepped Actinic Test Light response curves and the effects of light level increases and decreases with cold stress can be studied easily. This is a longer light adapted test. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994, 1995), (Ball 1995).
- ETR This is a short or long light adapted test related to yield and PAR or light level. A PAR clip is required. (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994, 1995), (Ball 1995).

Quenching and Quenching Relaxation Test – Test to study relaxation kinetics after exposure to light and chilling temperatures. Studies of the △ph of the thylakoid lumen, xanthophyll cycle, and photo-inhibition with NPQ, qN, qP, qL, qE, qT, qI, Y(NPQ), Y(NO). This is a longer dark adapted test. (Cavender-Bares J., Bazzaz F., 2004)

Over-Wintering Stress

- Yield Fast light adapted sensitive test can also be used for moderate cold stress in steady state. (Adams & Demming- Adams 2004) (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994,1995), (Ball 1995).
- Fv/Fm Fast dark adapted test can be used for moderate cold stress. (Adams & Demming- Adams 2004), (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994,1995), (Ball 1995).

Quenching and Quenching Relaxation Test – Test to study relaxation kinetics after exposure to light and over-wintering plants. Studies of qI mechanisms become possible as well as the △ph of the thylakoid lumen, xanthophyll cycle, and photo-inhibition with NPQ, qN, qP, qL, qE, qT, qI, Y(NPQ), Y(NO). This is a longer dark adapted test. (Adams & Demming-Adams 2004) (Cavender-Bares J., Bazzaz F.,2004)

Stepped Actinic Test – The effects of light level increases and decreases with cold stress can be studied easily. This is a longer light adapted test. (Adams & Demming- Adams 2004), (Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams 1994, 1995), (Ball 1995).

CO₂ Stress:

Best Tests

- Fv/Fm Fast dark adapted test is sensitive to early CO₂ stress. (Siffel & Braunova 1999)
- **PI** Fast dark adapted test sensitive to CO₂ stress using OJIP protocol. (Strasser 2004)
- **qP** A longer slow light or dark adapted test that has been used in compound stress situations related to water and light stress with CO₂ stress (Bukov & Carpentier 2004), (Cornic 1989), (Brestic 1995)

Non sensitive CO₂ Stress tests

- Fast light adapted test that is not sensitive to CO₂ stress initially and has been show to actually increase early on. It will decline after a period of time. While it is not valuable to detect CO₂ stress, it may be valuable to identify it in conjunction with Fv/Fm, and NPQ. (Siffel & Braunova 1999)
- **NPQ** This is a longer dark adapted measurement. It has been shown there is no quenching in the total absence of CO₂. (Siffel & Braunova 1999).

Air Pollution Stress

- **Fv/Fm** Fast dark adapted test is sensitive to ozone stress. (Mikkelsen 1994) (Calatayud, Pomares, and Barreno 2006)
- Yield Fast light adapted test can also be used for ozone stress in steady state.
 (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)
- **qP** Slow test. Ozone stress showed a lower qP (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)
- NPQ Slow test, ozone stress showed an increase in NPQ stress. This is a dark adapter test. (Calatayud, Pomares, and Barreno 2006) (Carrasco-Rodriguez J. and del Valle-Tascon S., 2001)

Herbicide Stress:

Different herbicides work in various ways. Some parameters are successful with certain types of herbicide stress and not for others.

For example: Fv/Fm is not sensitive to DCMU stress but VJ is sensitive to DCMU stress.

Herbicides are listed in alphabetical order and the test used to identify stress is listed on the left.

- VJ-OJIP Atrizine, a PSII inhibitor, by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003) (Percival 2005)
- Yield & NPQ -Basta (AgrEbo) is composed of 18.5 % Glufosinate-ammonium <Ammonium -DL-homoalanine-4-YL-(methyl)phosphinate> Yield and NPQ are sensitive tests for Basta herbicide stress. (Takayama K., Konishi A., and Omasa K.. 2003)
- VJ- *Bentazone*, a PSII inhibitor, VJ (or FvJ) is the fluorescence rise from O to J in the OJIP test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)
- VJ- OJIP DCMU has little effect on Fv/Fm (Nedbal & Whitmarsh 2004). However by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003) (Percival 2005)
- **NPQ** *DCMU*. A longer dark adapted test will provide stress information on DCMU. (Nedbal & Whitmarsh 2004)
- NPQ DDT. A sensitive test for DDT that is also dependent on zeaxanthin quantity in leaves. If there is little or no zeaxanthin production, NPQ can detect DDT stress. If zeaxanthin has been produced, NPQ is not affected by DDT. (Bilger & Bjorkman 1994)
- VJ-OJIP *Diuron* by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise In J provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003) (Percival 2005)
- VJ *Fluorochloridone* a PDS inhibitor, VJ (or FvJ) is the fluorescence rise from O to J in the OJIP Test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)
- VJ- *Glycosate* an EPSPs inhibitor, VJ (or FvJ) is the fluorescence rise from O to J in the OJIP test, provides a sensitive test for stress. (Christiansen, Teicher and Streibig 2003)

- VJ-OJIP TU-1178 by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in I provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003)
- VJ-OJIP TU-1282 by observing the transition from Fo to Fm in the OJIP test, a rise in Fo and a rise in I provide a sensitive test for stress. (Hiraki, van Rensen, Vredenberg, & Wakabayashi 2003)

Herbicide effects on Arabidopsis at standard dose:

Ĩ	2,4D in the phenoxy group, is a synthetic auxin herbicide. These parameters were sensitive to 2,4D use after 48 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)
Fv/Fm, 1-(Fo/Fp), 1-(Fi/Fp)–	<i>Asulam.</i> These parameters were sensitive to Asulam use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)
Fv/Fm, 1-(Fo/Fp), 1-(Fi/Fp)–	<i>Bifenox</i> . These parameters were sensitive to Bifenox use after 48 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)
	<i>Diclofop-methyl</i> . These parameters were sensitive to Diclofop-methyl use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)
Fv/Fm, 1-(Fo/Fp), 1-(Fi/Fp)–	<i>Glycosate</i> . These parameters were sensitive to Glycosate use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)
Fv/Fm, 1-(Fo/Fp), 1-(Fi/Fp)–	<i>Imazapyr</i> . These parameters were sensitive to Imazapyr use after 6 hours. Baker uses Fi instead of J as his designation but they are the same. (Baker and Rosenqvist 2004)

Fo = O, Fp = P, Fi = J in the OJIP protocol

Pesticide Stress:

Copper based Algicides and Fungicides – are main sources of Cu stress in plants, see Copper stress under Chemical Stress.

Mercury based Organo-mercury fungicides – A main source of Mg stress in plants, see Mercury stress under Chemical Stress.

- PI, FvFm Lindane. Sensitive test on cyanobacteria Anabaena (Bueno, Fillat, Strasser, Rodriguez, Marina, Smienk. Moreno, Barja 2004)
- Yield Trimax stress on Cotton Germ M., (Gonias E. D. Oosterhuis D.M., Bibi A.C. & Brown R.S. 2003)

Chemical Stress:

Listed by chemical.

While some types of chemical stress can be measured by various parameters including Fv/Fm, some require specific parameters for measurement.

Fv/Fo - Aluminum (Joshi & Mohanty2004), (Pereira 2000)(Baker and Rosenqvist 2004)

- VJ *Aluminum* Fi is = J in OJIP VJ = Fi-Fo/ Fm-Fo (Joshi & Mohanty2004), (Moustakas 1993, 1995, 1997)
- **Fv/Fm** -*Aluminum* (Joshi & Mohanty2004), (Moustakas 1996) (Baker and Rosenqvist 2004)
- **qP**, & **qN** *Aluminum* (Joshi & Mohanty2004), (Moustakas 1996)
- **qN** *Cadmium*. qN is more sensitive to Cadmium concentration than Fv/Fm. (Joshi & Mohanty 2004) (Krupa 1993) Skorzynska and Baszynski 1997)
- Fv/Fm -*Cadmium*. Not as sensitive as qN (Baker and Rosenqvist 2004)
- Yield *Cobalt*. (Joshi & Mohanty2004)(Tripathy 1983)
- Yield *Copper*. Sensitive test (Joshi & Mohanty2004) (Lanaras 1993)
- **Fv/Fm** -*Copper* (Baker and Rosenquist 2004)
- **Rfd** *Copper*. Sensitive test (Joshi & Mohanty2004))
- Fv/Fm -Lead (Joshi & Mohanty2004), (Parys 1998) (Romanowska 1998)
- J & I in OJIP -Mercury (Joshi & Mohanty2004), (Haldimann P., and Tsimilli-Michael M.2002)

Fv/Fm -Mercury (Baker and Rosenqvist 2004), (Joshi & Mohanty 2004)

- **qN** -Mercury (Joshi & Mohanty2004), (Lee 1995), (Xylander 1998)
- **ETR** *Nickel*. Fv/Fm is not a good indicator of Nickel stress. (Joshi & Mohanty2004), (Tripathy 1981)
- **qN** –*NaCl.* **qN** is a very sensitive indicator of salt stress in <u>*Rice*</u>. <u>Fv/Fm and yield were not sensitive</u> to salt stress in Rice (Moradi & Ismail 2007)

qN, qP, Fv/Fm, Yield, & ETR - *NaCl.* All parameters were sensitive to salt stress in Cereal *Sorghum* (Moradi & Ismail 2007) (Netondo 2004)

- **Fv/Fm** *NaCl*. Fv/Fm was sensitive to salt stress in the red mangrove, *Rhizophora mangle* L. (Biber 2006)
- **Fv/Fm** *NaCl*. Fv/Fm was sensitive to salt stress in chickpea seedlings (Eyidogan 2007)
- Yield NaCl. Yield was sensitive to salt stress in chickpea seedlings (Eyidogan 2007)

Fs in Yield - *Zinc* - Fv/Fm is <u>not</u> a good indicator of zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980)

Ph Stress

Fv/Fm – Fv/Fm was found to detect severe acid rain stress at a ph of 1.8 or below. (Velikova, Yordanov 1996)

Biotic Stress: The fluorescence parameter best suited to the type of infection is dependent on the type of Infection (Nedbal & Whitmarsh 2004) Therefore it is important to have versatile capability

The tests listed in this category are not listed in order of sensitivity or effectiveness. While many references below involve fluorescence imaging, spot measurement can also be used for study.

Due to early site specific infections, multiple point measurements on the same leaf in different areas are recommended. (Claus Buschmann 2008)

- NPQ This is a longer dark adapted measurement for crown rust on oat leaves (Sholes & Rolfe 1996)
- NPQ This is a longer dark adapted measurement for tobacco mosaic virus on tobacco (Osmond 1998), (Lohaus 2000)
- Fv/Fm Fast dark adapted test can be used for <u>Bean rust</u> (Peterson & Aylor 1995)
- **Yield -** Fast light adapted test used for <u>cedar fungus</u> (Ning 1995)
- **Fm-Fs/Fm** This is a longer dark adapted test that requires several minutes to reach steady state photosynthesis. <u>tobacco mosaic virus on tobacco</u> (Osmond 1990)
- **Fv/Fm** Fast dark adapted test can be used for biotic stress <u>chick pea leaves fungus</u> (Esfield 1995) (Weiss 1998)
- **Fv/Fm** Fast dark adapted test can be used for biotic stress <u>lemons infected by Penicllium digitatum</u> (Nebal 2000)
- **Fo/Fv** Fast dark adapted test can be used for biotic stress <u>Brassica Blackspot by destruxins</u> (Buchwaldt & Green 1992)
- **NPQ** This is a longer dark adapted measurement recommended for virus infection in higher plants and algae. (Balachadran & Hurry 1997)
- **Fv/Fm** Fast dark adapted test can be used for biotic stress recommended for virus infection in higher plants and algae. (Balachadran & Hurry 1997)
- Fv/Fo Fast dark adapted test can be used for biotic stress Maize rust resistance. (Duraes 2001)

Fv/Fm - Fast dark adapted test can be used for biotic stress. Maize rust resistance. (Duraes 2001)

Herbivory – (Animal Stress):

- Yield Fast light adapted sensitive test for Arthropod damage showing greater damage than the size of the hole indicates stress.
 (Aldea, Hamilton, Resti, Zangerl, Berenbaum, Frank and Deluca 2006), (Zangerl 2002)
- **Fv/Fm** Fast dark adapted test can be used to test for damage caused by insect larval foot hooks. (Hall, MacGregor, Nijsse, and Bown 2004)

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