

Review Article

<https://doi.org/10.20546/ijcmas.2017.604.068>**Fluorescence Imaging for Crop Stress Monitoring: A Review****Brijesh Yadav^{1*}, Chiranjeev Kumawat², Anil Kumar Verma²,
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Plants are exposed to a various biotic and abiotic stresses that can affect either directly or indirectly the photosynthetic performance of leaves. Several imaging techniques have been used to detect the early signs of stress by monitoring changes in water status, photosynthetic performance or structural modifications. Commonly, a more promising alternative technique is the use of chlorophyll fluorescence (ChF) which has a better agreement with photosynthesis and it has been used for the early detection of stress such as nutrient stress, heat stress, water stress, pathogen attack or herbicide toxicity. In plants, absorbed light can undergo three fates *viz.*, photosynthetic quantum conversion, chlorophyll fluorescence (ChF) and heat dissipation. Under stress condition absorbed light cannot completely used for carbon fixation and this excess energy is re-emitted back at longer wavelength, known as chlorophyll fluorescence. The fluorescence images and the corresponding fluorescence ratio blue: red and blue: far-red are particularly sensitive to environmental changes and stress. Different fluorescence parameters like maximum quantum efficiency of PSII (F_v/F_m), photochemical quenching (qP) and quantum efficiency of PSII (ϕ_{PSII}) are used for stress monitoring. This technique is limited up to the single leaves or seedling stage of crop and sometimes, this technique does not appear suitable for the early detection of water stress.

Keywords

Chlorophyll fluorescence, Biotic stress, Abiotic stress, FV/ Fm.

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Introduction

Plants are exposed to a various natural biotic and abiotic stresses. They can affect either directly or indirectly the photosynthetic performance of leaves and modify their optical and fluorescence properties. Stress can be detected prior to any visual symptoms could be observed in crop (Quilliam *et al.*, 2006; Soukupova *et al.*, 2003). Different techniques like thermal, reflectance and fluorescence imaging have been used for

detecting stress in crops. These techniques can be applied on microscopic scales (Oxborough and Baker, 1997; Osmond *et al.*, 1999; Küpper *et al.*, 2000; Rolfe and Scholes, 2002), to airborne remote sensing. These imaging techniques can detect the signs of stress by monitoring changes in water status, photosynthetic efficiency, and accumulation of secondary metabolites or structural modifications. But, these techniques do not

provide reliable measurements and they have little sensitivity to short-term leaf physiological changes. Commonly, a more promising alternative is the use of chlorophyll fluorescence (ChF), which has a better agreement with photosynthesis than spectral reflectance and thermal indices. In the last few years, chlorophyll fluorescence techniques have been widely used as an efficient tool to describe and investigate the photosynthetic performance in plants. Kautsky and Hirsch (1931) have been first time carried out an experiment with chlorophyll (Chl) fluorescence. It is a noninvasive analysis that permits to assess photosynthetic performance *in vivo*. Chlorophyll fluorescence analysis is widely used to evaluate the photosystem II (PSII) activity, which is an important target of biotic and abiotic stresses. By using these techniques for crop monitoring would allow us to alleviate stress at an early stage and thus substantially reducing yield losses.

Chlorophyll fluorescence

Solar radiation that absorbed by plant leaf can (i) drive photosynthesis (ii) used as heat dissipation and (iii) can produce fluorescence. These three fates have competition to each other and increase in the one process will leads to reduction in other two processes. In light reaction of photosynthesis, solar energy is absorbed by chlorophyll pigments and it transferred to reaction centers, which ultimately leads to storage of energy. In dark reaction, this energy is consumed for biochemical processes like carbon fixation and sugar production which known as photochemical quenching. During stress condition absorbed light cannot be completely used for carbon fixation and this excess energy is re-emitted back from the leaf at longer wavelength, known as chlorophyll fluorescence or be dissipated as heat (Krause and Weis, 1991; Maxwell and Johnson, 2000). Both processes *viz.*, chlorophyll

fluorescence and heat dissipation is called non-photochemical quenching (Fig. 1).

UV induced fluorescence emission spectrum

Ultraviolet-radiation-induced fluorescence emission spectrum of a green leaf with fluorescence maxima in the blue (F440 nm), green (F520), red (F690) and far-red (F740) spectral regions (Buschmann *et al.*, 2009), blue-green fluorescence is characterized by a high blue fluorescence band ranging from 440 nm and a lower shoulder in the green wavelength region near 520 nm. Blue-green fluorescence is primarily emitted by two cinnamic acids, p-coumaric acid and ferulic acid (Table 1). Generally poaceae family shows this type of fluorescence. Veins and mid veins of leaf exhibits blue green fluorescence, red and far red type of fluorescence is generated by chlorophyll-a in chloroplast of green leaf (Buschmann and Lichtenthaler, 1998). It has two peaks, 690 nm and another 740 nm *i.e.* red and far-red band respectively. Generally dicots and vein free region of leave exhibit red-far red fluorescence.

Fluorescence emission ratio

Ratio of fluorescence emission peaks explains the effect of environmental stress on chlorophyll content. Generally four types of ratio are used like- red (F440/F690), blue: far-red (F440/F740) and blue: green (F440/F520), and red: far-red (F690/F740). First two ratios are sensitive to changes in fluorescence emission while last one *i.e.* red: far-red ratio is sensitive to Chl content because of the process of re-absorption of the emitted fluorescence at 690 nm (Buschmann and Lichtenthaler, 1998; Chaerle and Van Der Straeten, 2000 and Buschmann *et al.*, 2008). But, this is sensitive to Chl changes only in lightly green leaves ($<25 \mu\text{gChl cm}^{-2}$). An inverse ratio *i.e.* FRF/RF, varies almost

linearly with Chl content in the physiological range of crop leaves ($20\text{--}60\mu\text{gChlcm}^{-2}$).

Under various stress conditions (high light exposure, enhanced UV radiation, drought and temperature stress) additional UV-absorbing or UV-scattering substances are formed in the epidermal cell, which acts as a barrier to the penetration of UV radiation into the mesophyll cells. The proportion of UV radiation that passes through the epidermis into the leaf mesophyll is strongly reduced and results in a dramatic reduction in chlorophyll fluorescence emission. As a consequence, the fluorescence ratios blue: red ($F_{440}: F_{690}$) and blue: far-red ($F_{440}: F_{740}$) strongly increases. The fluorescence images and the corresponding fluorescence ratio images blue: red and blue: far-red are particularly sensitive to environmental changes and stress. The fluorescence ratios are successfully used for detection of different stresses like nitrogen and zinc (on *Zea mays*) and heat and water stress (on *Rhododendron* sp.) (Lang *et al.*, 1996). Cadet and Samson (2011) also used fluorescence ratios to determine nitrogen, potassium and phosphorus status in sunflower. Burling *et al.*, (2011) have been used fluorescence ratios to discriminate nitrogen deficiency and disease infection in winter wheat.

Calculation of commonly used fluorescence parameters

To gain information about the photosynthesis in a plant through chlorophyll fluorescence, it is necessary to discriminate between the photochemical quenching and non photochemical quenching. The typical approach is to 'switch off' one of the two contributors, so that the fluorescence yield can be measured. Figure 2 shows a typical fluorescence pattern when a leaf is exposed to a combination of actinic light, darkness and saturation pulse. When leaf is exposed to light than chlorophyll fluorescence rise up for few

seconds due to reduction of electron carriers in the thylakoid membrane, the reaction centre in PSII *i.e.* P680, eject an electron to the electron acceptor QA via phenophytin. QA is unable to capture another electron from P680 until it passed to next carrier *i.e.* QB. This state of reaction centre is considered as closed state which leads to decline in quantum efficiency of PSII. After the initial rise of fluorescence, actinic light is applied and results in reduction of fluorescence signal for few minutes which is known as quenching (Krause and Weis, 1991) (Table 2).

When all PSII reaction centers are open *i.e.* dark adapted state, a modulating light of low intensity is switched on. In this state PSII shows a minimum value of fluorescence, F_0 . The fluorescence emission rises rapidly from a minimal level and attains a maximum (F_m) shortly after the start of illumination. In non-stressed plant or healthy plant, NPQ value is not obtained because material is completely dark adaptive. At this point, maximum fluorescence value is recorded, F_m .

The difference of F_0 and F_m is termed as variable fluorescence, F_v . The ratio of F_v and F_m is good indicator of stress in leaves (Krause and Weis, 1991). Subsequently, the signal declines because of quenching, which can be refereed as photochemical (use of light energy for CO_2 assimilation) and non-photochemical (photoprotective) reactions, until a steady-state value (F_s or F') is reached. Fluorescence measurements during saturating light pulses are used to enumerate differences in non-photochemical fluorescence quenching.

The application of saturation pulse under actinic illumination condition, close the reaction centre and produce a maximal fluorescence in light adaptive state *i.e.* F'_m . Another widely used parameter is the Photosystem II photochemistry (Φ_{PSII}) for detection of plant stress (Genty *et al.*, 1989).

Applications of chlorophyll fluorescence imaging for agricultural crops

This technique has been widely applied for various plant stresses like drought, chilling, freezing, salinity, nutrition, ozone pollution, wounding, high light, high metal accumulation and infection with fungi or virus.

Nutrient stress

Nitrogen is a part of chlorophyll and play a vital role in photosynthesis so, its deficiency easily can be assessed by this technique. Many studies found that chlorophyll fluorescence imaging is a good indicator for detection of nutrient stress. Application of this technique can help farmers to avoid overuse of fertilizers in precision farming (Chaerle *et al.*, 2007a). Thus, this technique seems to be useful in site specific nutrient management in sustainable manner.

Water deficit stress

Water availability is one of the limiting factor for plant productivity especially in arid and semi-arid regions (Flexas *et al.*, 2002). Consequently, a proper monitoring of water stress is necessary for the sustainable irrigation programmes (Penuelas and Filella, 1998). In plants water stress may leads to reduction in photosynthesis. In short term, it is due to either stomata closer or photo inhibition and in long term due to the inactivation of RuBis CO enzyme (Medrano *et al.*, 1997). Hence, Chlorophyll fluorescence can help in early detection of plant water stress and ultimately to irrigation scheduling.

Disease and pathogen

Many researcher found that chlorophyll fluorescence technique could be used for quantitative screening of disease and pathogen resistance (Barbagallo *et al.*, 2003; Chaerle *et al.*, 2005; Horie *et al.*, 2006).

Table.1 Characteristics of the blue-green and red and far-red chlorophyll fluorescence

Characteristics of the Blue-Green Fluorescence	
Fluorescing pigments	Cinnamic acids (p-coumaric acid and ferulic acid)
Location and origin	Cell walls and Vacuoles
Excitation	UV radiation
Maxima	Blue band F440, Green band F520
Exhibit plants	Poaceae family
Characteristics of the Red and Far-Red Chlorophyll Fluorescence	
Fluorescing pigments	Chlorophyll a
Location and origin	Chloroplasts of green leaf
Excitation	UV radiation
Maxima	Red band F690, Far-red band F740
Exhibit plants	Dicotyledons

Table.2 List of commonly used fluorescence parameters and their formulas

Parameters	Formula	Symbol description
1. Photochemical quenching (qP)		
Quantum efficiency of PSII (Φ_{PSII})	$(F'_m - F') / F'_m$	Steady state fluorescence ($F' = F_s$) Maximal fluorescence in light adapted state (F'_m)
Maximum quantum efficiency of PSII (F_v / F_m)	$(F_m - F_0) / F_m$	Original fluorescence (F_0), Variable fluorescence (F_v), Maximal fluorescence in dark adapted state (F_m)
Photochemical quenching (qP)	$(F'_m - F') / (F'_m - F'_0)$	Steady state fluorescence ($F' = F_s$), Maximal fluorescence in light adapted state (F'_m), Unrelaxed non-photochemical quenching (F'_0)
2. Non photochemical quenching (qN)		
NPQ	$(F_m - F'_m) - 1$	Maximal fluorescence in dark adapted state (F_m) Maximal fluorescence in light adapted state (F'_m)

Fig.1 Origin of chlorophyll fluorescence

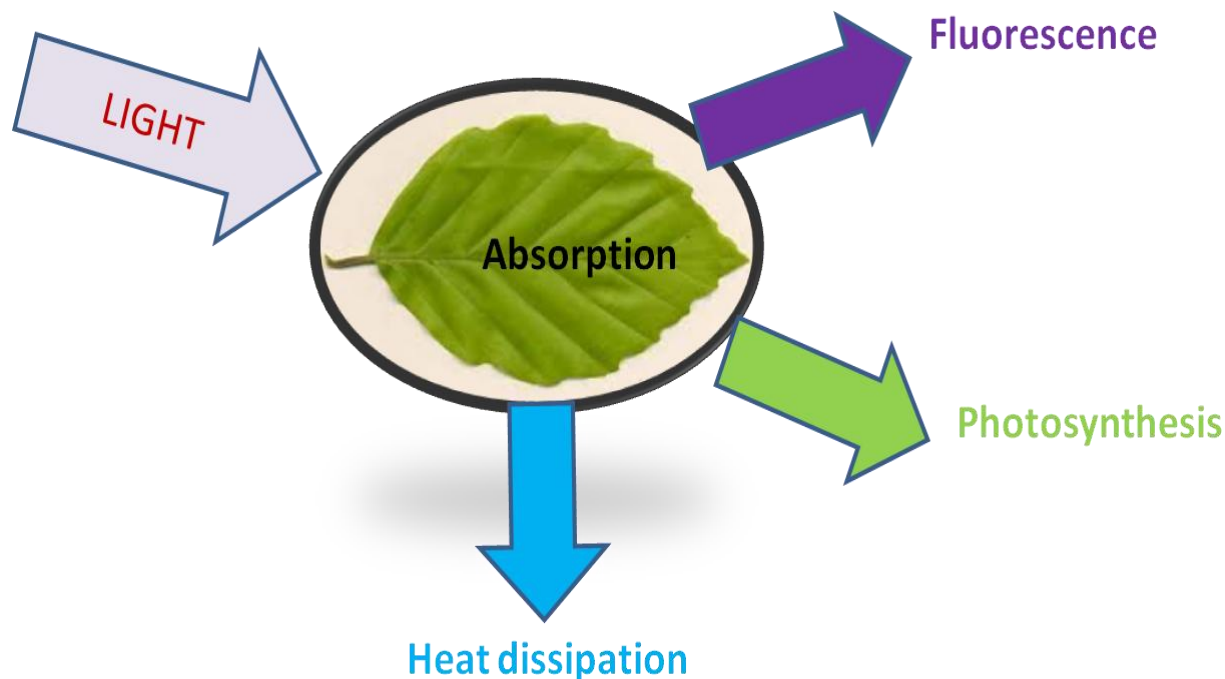
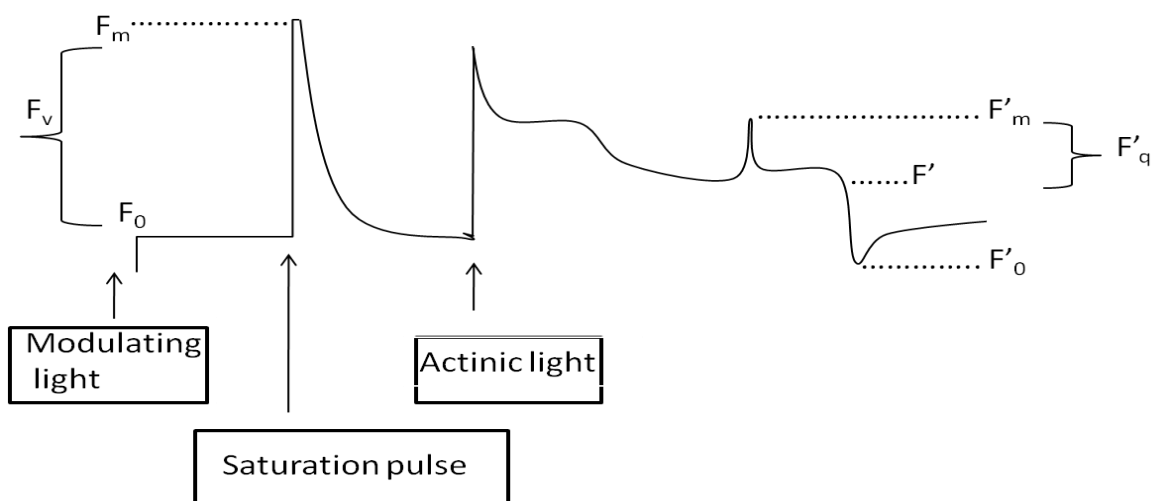


Fig.2 A typical fluorescence trace to measure photochemical and non-photochemical parameters



This technique has superior performance for damage evaluation of crop by different biotic stresses.

Elevated temperature stress

Excess high and cold temperature has adverse effect on plant growth and yield. Under elevated temperature, CO₂ assimilation is hampered which results F₀ increase and F_v/F_m decreases. Cold stress also reduces the values of F_v/F_m in the short term.

Excessive sun light intensity and UV exposition

Although solar radiation is the primary energy source for plant life which determines the crop growth and development but excess radiation is stressful for the plants. To protect the crop from the excessive sun light, farmers use different shade screens depending on the incoming light intensity. UV light is very harmful for crop production due to its interference with biomolecules viz., nucleic acid, lipids and proteins (Tevini, 2004). Thus, early detection of excess and UV light is important factor to achieve a good yield.

Herbicides toxicity

The application of herbicides alters the plant metabolism which directly or indirectly affects the photosynthesis. This leads to changes in chlorophyll fluorescence emission and it can be detected prior to any visual symptoms appear in crops (Barbagallo *et al.*, 2003).

Limitations of chlorophyll fluorescence imaging

Most Chlorophyll fluorescence techniques are limited up to the single leaves or seedling stage of crop. Sometimes, the ratio of variable and maximum fluorescence (F_v/F_m) appears to be relatively insensitive to severe water limitation so, this technique does not appear suitable for the early detection of water stress. Data analysis software is required for fluorescence image processing. Fluctuation in light intensity, during field measurement tends to produce results that entirely different from laboratory analysis. In addition, poor resolution and high power requirements may be limiting for its field applications.

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