

Fluorescence imaging

for crop stress monitoring

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Plants are exposed to a various natural biotic and abiotic stressors. They can affect either directly or indirectly the photosynthetic performance of leaves and modify their optical and fluorescence properties. Early stress sign in plants, before visual damage symptoms are required. In the last few years, chlorophyll fluorescence techniques were widely applied as an efficient tool to describe and investigate the photosynthetic performance in plants. It is a non-invasive analysis that permits to assess photosynthetic performance in vivo.

Key words: Crop, Fluorescence, Monitoring, Stress

DIFFERENT techniques like thermal, reflectance and fluorescence imaging were used for detecting stress related changes in the pattern of light emission from plant leaves. These techniques can be applied on microscopic scales to airborne remote sensing. Several imaging techniques were used to detect the early signs of stress by monitoring changes in water status, photosynthetic efficiency, accumulation of secondary metabolites or structural modifications. At the leaf-scale, photosynthesis and CO₂ flux can be easily measured using gas-exchange systems (i.e. Infra-Red gas analysers, IRGAs). However, it is a rapid and reliable indicator of crop stress assessment but measurements are not appropriate. Eddy covariance towers was established over the last few decades which providing CO₂ flux data at high-temporal resolution But, they do not provide reliable measurement. This method is limited to only flat terrain with uniform vegetation. Alternatively, some remote sensing-based indices like Normalized Difference Vegetation Index (NDVI) and the Enhance Vegetation Index (EVI) were used to estimate plant stress over larger areas based on spectral reflectance. These indices

measure only the 'greenness' of crops and not photosynthesis itself. Therefore, they have little sensitivity to short-term leaf physiological changes. In a short duration green leaves remain green but there may be reduction in photosynthesis when they are under stressed. Only at longer time period, when prolonged stress causes premature senescence therefore, show as change in NDVI or EVI. Another techniques such as canopy temperature determined by infra-red thermometer can be used to estimate water-vapour loss by plants. Commonly, a more promising alternative is the use of chlorophyll fluorescence (ChF) which have a better agreement with photosynthesis than spectral reflectance and thermal indices.

Fluorescence excitation and emission fundamentals

Fluorescence is light re-emitted by a molecules during return from excited to non-excited states and used as an indicator of energy conversion. Generation of luminescence through excitation of a molecule is a phenomenon called photo-luminescence. On the basis of the electronic configuration of the excited state and the emission pathway, it can

be divided into two categories: (i) fluorescence and (ii) phosphorescence. Fluorescence can be explained by Jabolonski Energy Diagram (Fig. 1) in which green light shows excitation which takes 10⁻¹⁵ sec. After absorption of energy, molecules move to excited state (S₁, S₂) that depends on source energy. If blue light is excitation source, molecules comes to S₂ or higher state; and if red light is excitation source it comes to lower state (S₁). These excited molecules or electron can relax to S₁ state via vibrational relaxation or internal conversion. It takes 10⁻¹⁴ to 10⁻¹¹ sec. Molecules ultimately relax to S₀ state through photon emission that is called fluorescence. But sometimes due to inter-system crossing they changes its spin multiplicity. So the emission process that occurs without a change in spin multiplicity called fluorescence and a process that occurs with change in spin multiplicity, called phosphorescence.

Origin of fluorescence

On average, about 75% of visible solar radiation is absorbed by leaves, but a very small fraction is used for plant metabolic activity. About 10% radiation is reflected and 15%

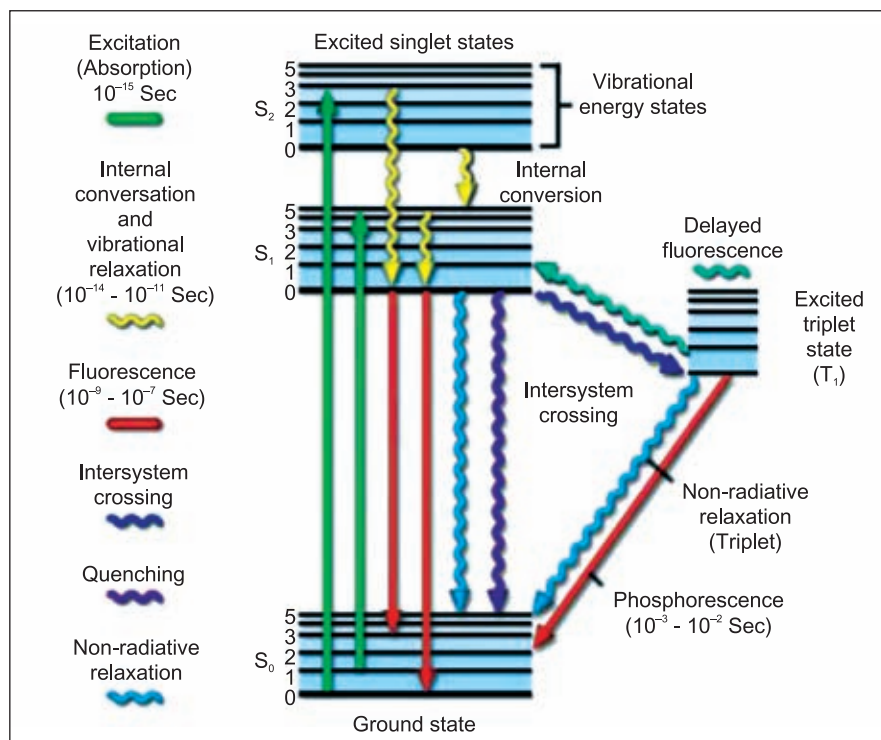


Fig. 1. Jablonski Energy Diagram

transmitted through leaves. Out of total 75 % absorbed light 2-3% used as biomass production, 96% heat dissipation and remaining 1% as fluorescence. When a chlorophyll (Chl) molecule absorbs the energy of a given wavelength, a part of it is dissipated by light emission at longer wavelengths within short period. This small amount of energy loss is known as the fluorescence emission. Plant stress modifies the proportions of absorbed light energy that are used for photosynthetic quantum conversion, chlorophyll fluorescence (ChlF), and heat emission (Figs 2 and 3). They also modifies the energy

flow of photons through the leaf, so altering light absorption, reflectance and transmittance properties and fluorescence.

Ultra-violet radiation induced fluorescence emission spectrum

Ultraviolet-radiation induced fluorescence emission spectrum of a green maize (*Zea mays*) leaf with fluorescence maxima in the blue (F 440 nm), green (F 520), red (F 690) and far-red (F 740) spectral regions (Fig 4). The four fluorescence bands, applied in the fluorescence imaging of leaves. The fluorescence images and the

corresponding fluorescence ratio images blue : red and blue : far-red are particularly sensitive to environmental change and stress.

Characteristics of the BGF and Red and far-red chlorophyll fluorescence

Blue-green fluorescence (BGF) 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. Generally Poaceae family showed this type of fluorescence. Veins and mid-vein of leaf exhibits BGF.

Red and far red type of fluorescence is generated by chlorophyll a in chloroplast of green leaf. It has two peaks, 690 nm and another 740 nm i.e. red and far-red β and respectively. Generally dicots exhibits this type of fluorescence. Vein free region exhibit R-FR fluorescence.

Fluorescence emission ratio

Fluorescence emission ratio showed about effect of environmental stress on chlorophyll. Generally four types of ratio used like- red (F 440/F 690), blue:far-red (F 440/F 740) and blue:green (F 440/F 520), and of the chlorophyll fluorescence ratio red : far-red (F 690/F 740) (Fig. 7). This ratio is sensitive to Chl content because of the process of re-absorption of the emitted fluorescence at 690 nm but it is

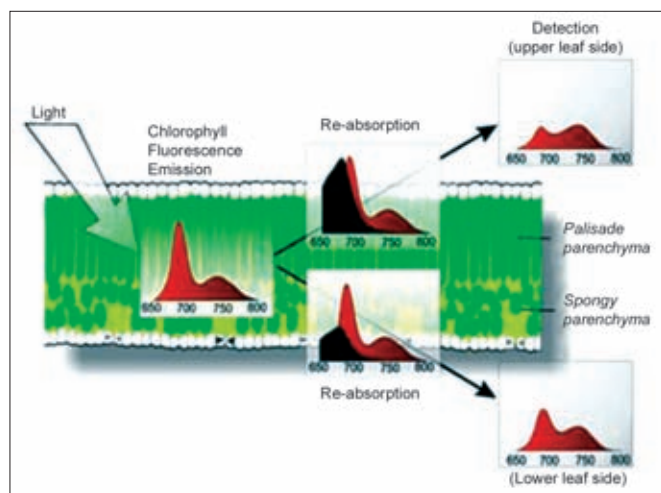


Fig. 2. Radiation is absorbed by leaves

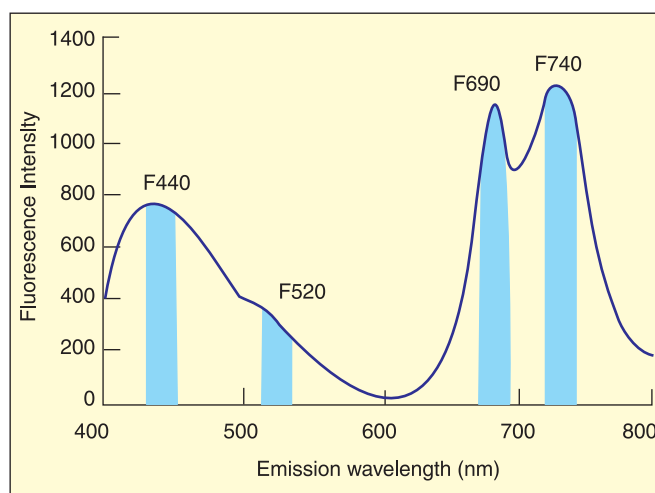


Fig. 3. Ultra-violet induced fluorescence emission

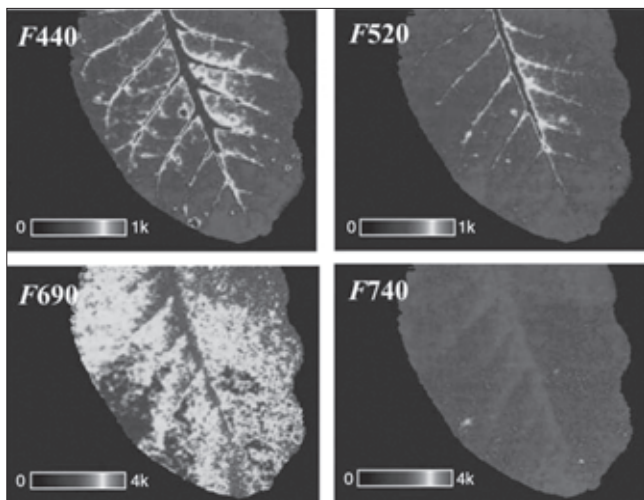


Fig. 4. Fluorescence in a leaf

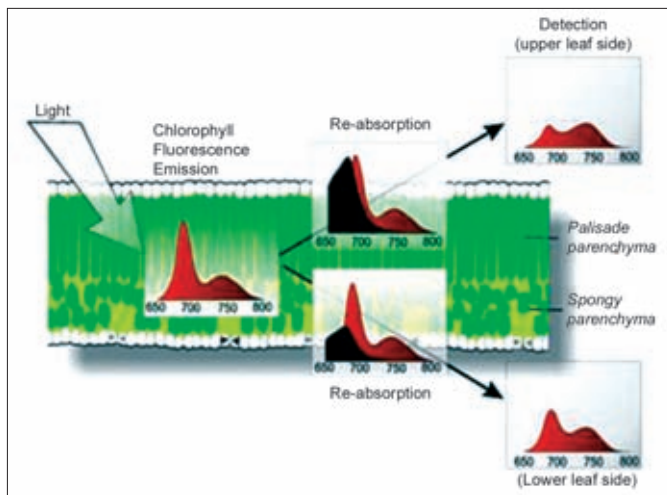


Fig. 5. Light absorption in leaf

sensitive to Chl changes only in lightly green leaves ($<25 \mu\text{g Chlcm}^{-2}$). A inverse ratio i.e. FRF/RF varies almost linearly with Chl content in the physiological range of crop leaves ($20\text{--}60 \mu\text{g Chlcm}^{-2}$).

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 cells, which forms 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 Fig. 7.

Fast chlorophyll fluorescence induction kinetics / Kautsky effect

When photosynthetic material is transferred from dark into the light,

an increase in fluorescence occurred over a time period of 1 sec. This reduction may be due to the reduction of electron acceptors in photosynthetic pathways particularly in Q_A . When PS II absorbs light then Q_A has accepted an electron, under this condition reaction centre said to be closed. So ultimately reduction in photosynthetic performance and corresponding increases in yield of fluorescence. After few minutes, fluorescence levels starts to fall again. It is called fluorescence quenching.

Pulse amplitude modulation (PAM) fluorometry

It is widely used chlorophyll fluorescence technique which is designed specifically to measure chlorophyll fluorescence yield at a high degree of sensitivity.

Fluorescence transient is generally

induced in dark-adapted leaves. The fluorescence emission rises rapidly from a minimal level and attains a maximum (F_m) shortly after the start of illumination. 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) is reached.

Fluorescence measurements during saturating light pulses are used to enumerate differences in non-photochemical fluorescence quenching. Different quenching parameters can be calculated by using formula as applicable in analysis.

Quenching analysis

It is of two types: (i) Photochemical quenching (qP) and (ii) Non-photochemical quenching (qN).

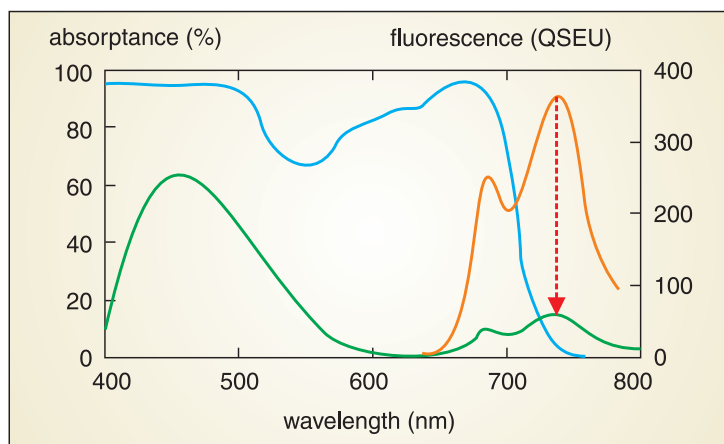


Fig. 6. Fluorescence emission ratio

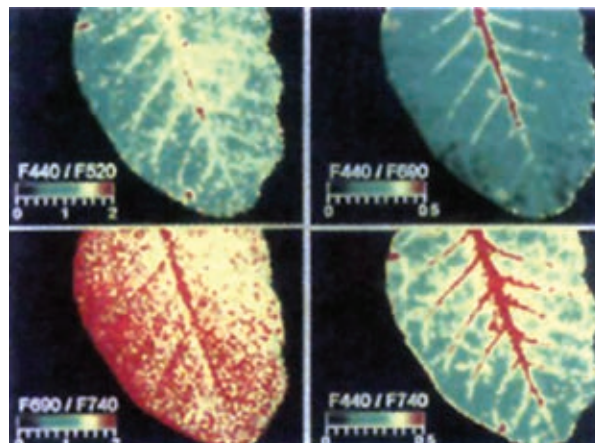


Fig. 7. As a consequence, the fluorescence ratios blue : red ($F_{440} : F_{690}$) and blue : far-red ($F_{440} : F_{740}$) strongly increase.

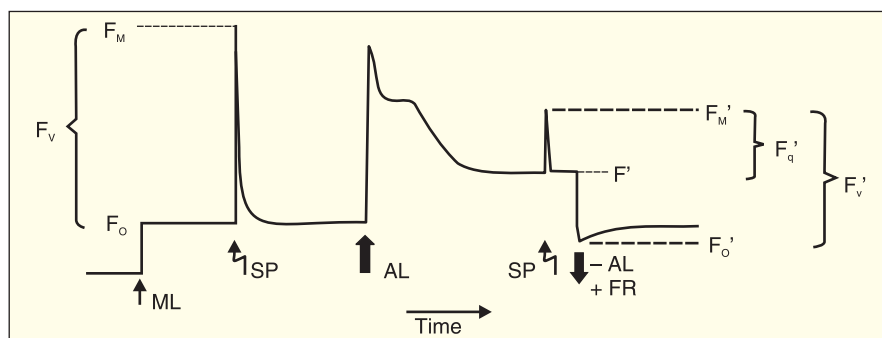


Fig. 8. Fluorescence transient study

Symbols	Parameter
F_0	Original fluorescence
F_v	Variable fluorescence
F_t	Fluorescence at steady-state
F_m	Maximal fluorescence in dark adapted state
F_v/F_m	Optimal quantum yield
qP	Photochemical quenching
qN	Non-photochemical quenching
Y	Effective quantum yield
ETR	Electron transport rate
$F' = F_s$	Steady state fluorescence
F'_0	Unrelaxed non-photochemical quenching
F'_m	Maximal fluorescence in light adapted state
F_q	$F'_m - F'$

Photochemical quenching (qP)

Quantum efficiency of PSII (x_{PSII}) = $(F'_m - F')/F'_m$

Maximum quantum efficiency of PSII $(F_v/F_m) = (F_m - F_0)/F_m$

Photochemical quenching (qP) = $(F'_m - F')/(F'_m - F'_0)$

Electron Transport Rate (ETR) = $J = x_{PSII} \times PFD_a \times 0.5$

where, J = Electron transport rate

PFD_a = Absorbed photon flux density ($\mu\text{mol photons m}^{-2}\text{s}^{-1}$)

0.5 = Factor for partitioning of energy between PS II and PS I

Yield of photochemical energy conversion (Y) = $(F'_m - F')/F'_m$

Non-photochemical quenching (qN)

Non-photochemical quenching is an indicative of the non-radiative energy or heat dissipation. It is sum total of photo-protective mechanisms (qE), state transition quenching (qT) and photo-inhibition (qI).

$$NPQ = qE + qT + qI$$

$$NPQ = (F_m - F'_m) - 1$$

Slowly and rapidly relaxing quenching can be calculated using the following formula :

$$NPQ_s = (F_m - F'_m) / F'_m$$

$$NPQ_f = (F_m/F'_m) - (F'_m/F'_m)$$

Applications of chlorophyll fluorescence imaging for agricultural crops

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

Limitations of chlorophyll fluorescence imaging

- The ratio of variable and maximum fluorescence (F_v/F_m) appears to be relatively insensitive to severe water limitation
- Non-flat tissue
- Less resolution

SUMMARY

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.

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Improved Carp polyculture in village ponds

Workshop on 'Improved Carp polyculture in village ponds' was organized by Anand Regional Research Centre of Central Institute of Freshwater Aquaculture, Bhubneshwar at Rajpipla, Narmada District, Gujarat as a part of Tribal Sub Plan Programme of CIFA, Bhubaneswar. Narmada has been identified as the tribal dominated Aspirational District by the Government of India.

Dr S.S. Mishra [Director (Acting), CIFA, Bhubaneswar] emphasized the improvement of livelihood conditions of tribal farmers through adoption of scientific aquaculture practices. He assured all technical help from CIFA for the benefits of fish farmers of Gujarat.

Shri R.V. Baria [Project Administrator, TSP Programme of Narmada District, Government of Gujarat] applauded the efforts of CIFA for socio-economic development of tribal population of the country. He said that it can be achieved through adoption of location specific scientific methods of aquaculture.

Dr B.C. Mohapatra [Chairman of TSP and Principal Scientist, ICAR-CIFA, Bhubaneswar] highlighted the demand for good quality fish seed in India which could be met through wider adoption of FRP carp hatchery technology and focused on dissemination of location specific aquaculture technologies for economic development of tribal farmers. He urged all members to join their hands to take up aquaculture in all types of water bodies in Gujarat.

One Leaflet Scientific Methods of Carp Polyculture in Village Ponds in Gujarati language was released in the workshop.