Journal of Innovative Agriculture
Review Article

Chlorophyll fluorescence imaging technique: horticultural perspective

Lal Rinmawii[1], Sukjai Dhar[2], Songthat William Haokip[3*]

[1]Department of Fruit Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra-444001, India.
[2]Department of Fruit Science, SKUAST, Srinagar, Jammu & Kashmir-190017, India.
[3]Department of Fruit Science, College of Horticulture and Forestry, CAU(I), Pasighat, Arunachal Pradesh-791102, India.

[*]**Correspondence**
Songthat William Haokip
williamhort05@gmail.com

Volume: 11, Issue: 2, Pages: 11-16
DOI: https://doi.org/10.37446/jinagri/ra/11.2.2024.11-16
Received: 16 January 2024 / Accepted: 27 May 2024 / Published: 30 June 2024

Chlorophyll fluorescence is a quick, non-lethal, and low-cost approach for evaluating Photosystem II activity in plants. It is denoted by Fv/Fm. The reason why it measured only PSII activity and not PSI is the fluorescence formed in PSI is constant and not variable like PSII. As PSII activity is responsive to abiotic as well as biotic factors, this approach is useful for studying photosynthetic systems and also providing a reliable benchmark of the way plants give a response to environmental variation. Though, Chlorophyll Florescence Imaging Technique is a valuable and propitious method for horticulture, it has significant shortcomings that must be addressed. Imaging of fluorescence signs, for instance, might be hampered by inflated tissues due to variances in light assimilation (e.g., curly leaves or spherical fruits), by highly reflective exteriors such as waxes or hairs, and by dust adulteration on the surface. Two leaves with different chlorophyll fluorescence release, for example, could not be compared if the light engrossed was not the same. Kautsky and Hirsch were the first to conduct experiments with the fluorescence of chlorophyll (Chl) by exposing a plant to blue light and saw it through a red filter to witness chlorophyll (Chl) fluorescence with naked eyes. This method has advanced quickly since then.

Keywords: *abiotic, biotic, chlorophyll, florescence, environmental and photosyst*

Introduction

Chlorophyll fluorescence analysis has become a popular technique in plant research owing to the development of conventional non-imaging fluorometers (chlorophyll fluorescence meters). Overtime, imaging fluorometers were developed, Kautsky & Hirsch (1931) exposed a plant to blue light and saw it through a red filter to witness chlorophyll (Chl) fluorescence with naked eyes. The plant initially emitted a dull red glow, yet as they observed, the stationary image of plant was substituted with a lively image as the red glow quickly ascended to a perkier red and then gradually dropped. This potentially represents the inaugural instance of capturing chlorophyll fluorescence from leaves in motion, uncovering the close association amongst the dynamics of chlorophyll fluorescence and intricate processes occurring within the photosynthetic light responses and the photosynthetic carbon reduction cycle. At present, chlorophyll fluorescence can serve as a tool to monitor various aspects, including the proficiency of Photosystem II (PSII), the oxidation-reduction phase of the primary quinone acceptor (QA) of PSII, the status of the plastoquinone pool's redox state, changeovers between the different S-states of the oxygen-evolving complex, the rate of continuous linear electron transport, the cyclic electron transport of Photosystem I (PSI), and both biotic and abiotic stresses, along with other facets of photosynthetic processes (Heredia & De Las Rivas, 2003). Even if exertions to scan Chl fluorescence has been continuing for years, recent developments in the know-how of light emission, imaging detectors, and speedy data controlling has enabled the construction of effective, user-friendly, and affordable modern instrumentation. Chl fluorescence imaging differs from consolidative techniques like gas exchange or non-imaging Chl fluorometry where it can resolve photosynthetic performance all over a leaf's surface. The discovery of lateral heterogeneities of fluorescence characteristics, which reflect physiological heterogeneities, is the most important new information that Chl fluorescence

imaging offers. The ability to evaluate multiple samples simultaneously under the same circumstances is the primary distinction between the imaging fluorometer and a conventional fluorometer. CFI provides a swift indication of the fluorescence release pattern of cells, leaf or plants and delivers quick, logical, graphic data on plant stress Calatayud (Calatayud et al., 2006). It is common knowledge that even physiologically vigorous foliage has "patchy" stomatous opening. Moreover, stress-induced constraints are not equally spread across the entire leaf surface. On time discovery of such stress-induced injury may be feasible through the use of fluorescence imaging. This technology allows for the investigation of the spatial-temporal heterogeneousness of Chl fluorescence signs across entire leaf surface, caused by a variety of interior plant features (Nedbal & Whitmarsh, 2004), and that can't be espied by non-imaging Chl fluorescence point measurements (Ellenson & Amundson, 1982; Omasa & Takayama, 2003; Takayama, et al., 2003; Wang et al., 2018). Incorrect color pictures or arithmetical data derived through photochemical reaction kinetics are two ways to display fluorescence fluctuation. To portray the heterogeneity, pixel by pixel, in photographs, false color palettes are frequently employed, wherever dissimilar colors encrypt for dissimilar arithmetical values. It has mostly been used in horticulture studies to diagnose different types of stress or in pre and post-harvest scenarios. In order to identify genotypes having better resistance to biological and insensate distress early on, genotype screening using chlorophyll fluorescence imaging is suitable (Guidi et al., 2019). This technology has the valuable ability to notice stresses prior to the arise of visible symptoms.

Imaging Technology

This technology relies on four primary procedures:

1. Image capture: Illumination, data capture, making digital as well as information transmission to the mainframe.
2. Image dissection: Using assortment techniques to identify important areas for investigation.
3. Analysis: Computation of fluorescence properties and oscillation for every picture segment and
4. Data visualization: Visualizing the data

Light origins, imaging sensor, control unit, power supply as well as a computer make up the fundamental imaging hardware. The light sources and detectors are the parts which govern goodness of the picture and system cost. An image fluorometer's utility is significantly influenced by the software that controls the instruments, conducts the analysis, and acts as the user-instrument interface. The software system should offer data management procedures which enable the users to quickly envisage pictures and segment images into chosen areas using premeditated fluorescence parameters. The whole operation call for pixel-by-pixel arithmetic calculations of parameters over specific locations. Additionally, the software needs to make it possible for users to create advance investigational procedures that regulate light sources and image capture procedures (Nedbal & Whitmarsh, 2004; Weber et al., 2017).

The spectral energy received by chlorophyll in leaves has three possible outcomes:

1. It has the potential to be utilized in powering photosynthesis.
2. It could be debauched in the form of heat.
3. It has the ability to re-emit as chlorophyll fluorescence.

A. Sources of light

Constant light sources

Formerly, imaging apparatuses utilized a sole, high-intensity nonstop light source which help in assisting direct photosynthesis and stimulate the fluorescence emission captivated by the imaging detector (Ning et al., 1995). The advantage of this design is a fairly strong fluorescence signal, that boosts the signal-to-noise ratio, yet it also produces fluorescence transients that are too quick for a CCD camera to capture. About 25 pictures are captured per second by a standard CCD camera. The issue is that PSII turns over repeatedly in high light throughout the lengthy integration period, raising the fluorescence level and making it difficult to estimate Fo. The actinic light's intensity can be reduced to obtain accurate values of Fo, however the resulting luminescence fleeting falls short of the Fm maximum fluorescence level. With a constant light source that can be regulated to produce minimal luminescence to govern Fo and dousing luminescence to govern Fm can help solve this issue.

Modulated light sources

The development of controlled measuring light sources, that significantly increased the energetic range of Chl fluorescence measurements over a huge array of synthetic light strengths, was a significant advancement in non-imaging

fluorometry (Schreiber et al., 1986). The first experiment using controlled light excitation to photograph chlorophyll fluorescence from a leaf made use of xenon lamp flashes (Fenton & Crofts, 1990). LEDs are extra adaptable moderated light source available today. To offload power of flash-lamps, LEDs' flash period may be tuned lower to the sub-microsecond scale, and brightness points could be adjusted from low light to immediate luminescence that can be up to two scale brighter than sunlight (Nedbal et al., 1999). Unlike any other imaging method, we are aware of; the brief measurement flashes from LEDs enable a precise calculation of Fo images.

B. Detectors

Although conventional photography is capable of capturing fluorescence images of plants, the analysis is only possible with static images (Yanase & Andoh, 1992; Jensen & Siebke, 1997). The development of CCD cameras, which offer dynamic images of Chl fluorescence that can be digitally captured then uploaded to a mainframe, has changed this. With the help of a two-dimensional range of photosensitive spots connected to the pixels that make up the final image, CCD cameras operate by capturing light.

C. Data handling

For each image taken during a measurement, luminescence imageries are saved in the mainframe in data pitches that correspond to all of the photosensitive CCD elements. After being stored, the data is divided into useful image segments using software tools. According to a fluorescence insignia, such as DCMU-inhibited or entirely dynamic sections, for instance, data may be segmented. Both manual and automated segmentation are possible. After the data has been segmented, each pixel inside a chosen section is combined to get the average value at that certain period. The distinctive fluorescence purview as a purpose of time are then plotted using these average values.

D. Experimental protocols

The experimental techniques created for non-imaging fluorometry could often be easily converted to use with image fluorometers (Osmond *et al.,* 1998).

Table 1. Common Parameters of Chlorophyll Fluorescence

Note: The reader should check in-depth reviews of Baker in 2008 as this is simply presented to identify the most prevalent factors.

Fluorescence parameters	Definition
Fo	In the absence of any light-induced closure of PSII reaction centers, the lowest level of chlorophyll fluorescence intensity is detected in darkness.
Fm	The peak chlorophyll fluorescence intensity observed in the dark-adapted mode when applying a sopping light pulse.
F´o	In the light-adapted condition, the lowest chlorophyll fluorescence strength is measured.
F´m	The highest chlorophyll fluorescence strength calculated when the system is acquinted to light conditions and subjected to a saturating light pulse.
Fv	Variations in chlorophyll fluorescence (Fm-Fo) calculated under dark-adapted conditions with minimal interference from non-photochemical processes.
F´v	In the light-adapted condition, variable chlorophyll fluorescence (Fm-Fo) is monitored.
Fv/Fm	PSII photochemistry's maximum quantum yield recorded in the dark-adapted condition
Fo/Fv	Under dark-adapted conditions, the assessment involves calculating the proportion between minimum chlorophyll fluorescence intensity and the variable chlorophyll fluorescence.
ΦPSII	The efficient quantum yield of photochemical energy alteration in Photosystem II (PSII).
NPQ	Stern-Volmer non-photochemical quenching coefficient

The saturation pulse approach was applied to calculate chlorophyll fluorescence (adapted from Van Kooten and Snell, 1990):

- The satiating analysis of modulated fluorescence by the saturation pulse technique is the utmost practical as well as extensively utilised chlorophyll fluorescence methodology.
- Prior to the measurement, a leaf is kept in the dark for at least 15 minutes (depending on temperature).
- When a saturating flash is administered, fluorescence increases from its initial ground state value (Fo), which is established in the absence of light using a low-intensity measuring beam, to its peak value, Fm.

- The highest quantum proficiency of photosystem II (PSII) primary photochemistry, expressed as Fv/Fm, can be determined using this technique.

The Fv/Fm test is made to enable as much light energy to travel along the fluorescence route as possible. In general, the Fv/Fm is decreased and the plant stress increases when fewer open reaction centers are available. The approximate ideal Fv/Fm number for many plants is in the range of 0.79 to 0.84, with lower levels indicating plant stress.

Table 2. Utilization of CFIT reported in horticultural crops

Crop	Purpose	Fluorometer used	References
Apple	Spotting and diagnosis of scab induced stress in leaves	A portable device created within the Molecular and Physical Plant Physiology laboratory at the University of Hasselt.	Delalieux et al., 2009
Ber	Automatic sorting	MINIPAM (PAM-2000) fluorometer (Heinz Walz, GmbH, Effeltrich, Germany)	Zheng et al., 2010
Strawberry	To comprehend the influence of iron deficiency	Mini chlorophyll fluorometer–IMAG-MIN/B (Walz, Effeltrich, Germany)	Osorio et al., 2014
Tomato seedlings	Early determination of bacterial wilt	FluorCam 800-O/1010 (Photon System Instruments, Drasow, Czech Republic)	Kim et al., 2019
Tomato	Detection of chilling injury	IMAG-MAX/B (Walz, Effeltrich, Germany)	Dong et al., 2019
Cucumber	Impact of exogenic calcium application on fruit quality	Imaging-PAM fluorometer (Walz, Effeltrich, Germany)	He et al., 2018
Lemon	Early detection and localization of rind injury	FluorCam system (Photon System Instruments, Brno, Czech Republic)	Obenland & Heipp, 2005
Apple	Assessing quality and storage longevity	Transportable fluorescence imaging system (FIS) developed by Laboratory of Botany, Limburgs University, Belgium	Huybrchts et al., 2002
Lemon	Prediction of fruit quality	Kinetic Imaging Fluorometer (Photon System Instruments, Brno, Czech Republic)	Nedbal et al., 2000
Grape	Early discovery of *Plasmopara viticola* contamination in grapevine leaf before symptoms appear.	Commercial Kinetic Imaging Fluorometer (Open FluorCam 700 MF, Photons Systems Instruments, Ltd., Brno, Czech Republic)	Csefalvay et al., 2009

Conclusion

This review demonstrates the CFI's immense potential as a valuable technique for use in horticulture. This technique has ever since effectively used for various tasks, like identification of biological as well as nonbiological stressors in plants and its products, both before as well as after harvest. This review demonstrates how CFI can identify stresses before they develop as visible symptoms. This is crucial when testing diverse genotypes for forbearance to particular stresses since it reduces the amount of time needed to complete the work by identifying the stress at the earliest feasible stage. Furthermore, CFI is quick, non-destructive, and reasonably priced, and it can concurrently screen a lot of plants. The limitation of measuring only horticulture goods with at least modest chlorophyll content should be taken into account when utilizing CFI in the future. If not, there could be significant inaccuracies caused by a misreading of the fluorescence that is released. To ensure that the studies can be replicated, it would be wise to standardize and specify uniform protocols. Additionally, as has been mentioned in this analysis, it is typical for the crop to be subjected to multiple types of stresses at once in actual situations. While the method has been shown to be a reliable stress detector, it might be challenging to determine the specific sort of stress that is impacting the crop using CFI. Perhaps a thorough examination of the variables in question and how they are distributed among the leaves could provide some insight. To make it possible to test multiple larger plans simultaneously, CFI systems for genotype screening need to be further developed.

Acknowledgement

The authors would like to thank and acknowledge the researchers who have been tirelessly working on this area of research and make their findings easily accessible to the scientific community which had helped in the successful completion of this manuscript.

Author contributions

Lal Rinmawii drafted the manuscript and completed the necessary revisions. Corrections to the manuscript were made by Sukjai Dhar and Songthat William Haokip was responsible for the framework, revision and overall supervision.

Funding

No funding.

Conflict of interest

Every author of this manuscript affirms that they do not have any conflicts of interest to disclose.

Ethics approval

Not applicable

References

Baker, N.R., (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology*, *59*, 89-113.

Calatayud, A., Roca, D. & Martínez, P.F., (2006). Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging. *Plant Physiology and Biochemistry*, *44*(10), pp.564-573.

Cséfalvay, L., Di Gaspero, G., Matouš, K., Bellin, D., Ruperti, B. & Olejníčková, J., (2009). Pre-symptomatic detection of Plasmopara viticola infection in grapevine leaves using chlorophyll fluorescence imaging. *European Journal of Plant Pathology*, *125*, 291-302.

Delalieux, S., Auwerkerken, A., Verstraeten, W.W., Somers, B., Valcke, R., Lhermitte, S., Keulemans, J. & Coppin, P., (2009). Hyperspectral reflectance and fluorescence imaging to detect scab induced stress in apple leaves. *Remote sensing*, *1*(4), 858-874.

Dong, Z., Men, Y., Li, Z., Zou, Q. & Ji, J., (2019). Chlorophyll fluorescence imaging as a tool for analyzing the effects of chilling injury on tomato seedlings. *Scientia Horticulturae*, *246*, 490-497.

Ellenson, J.L. & Amundson, R.G., (1982). Delayed light imaging for the early detection of plant stress. *Science*, *215*(4536), 1104-1106.

Fenton, J.M. & Crofts, A.R., (1990). Computer aided fluorescence imaging of photosynthetic systems: Application of video imaging to the study of fluorescence induction in green plants and photosynthetic bacteria. *Photosynthesis research*, *26*, 59-66.

Guidi, L., Lo Piccolo, E., & Landi, M., (2019). Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 species? *Frontal of Plant Science*, *10*:174.

He, L., Yu, L., Li, B., Du, N. & Guo, S., (2018). The effect of exogenous calcium on cucumber fruit quality, photosynthesis, chlorophyll fluorescence, and fast chlorophyll fluorescence during the fruiting period under hypoxic stress. *BMC plant biology*, *18*(1), 1-10.

Heredia, P. & De Las Rivas, J., (2003). Fluorescence induction of Photosystem II membranes shows the steps till reduction and protonation of the quinone pool. *Journal of plant physiology*, *160(12):*1499-506

Huybrechts, C.J.G., Deckers, T. & Valcke, R., (2002). August. Assessing apple quality and storage capability by means of fluorescence imaging. In *XXVI International Horticultural Congress: Issues and Advances in Postharvest Horticulture 628*, 91-96.

Jensen, M. & Siebke, K., (1997). Fluorescence imaging of lichens in the macro scale. *Symbiosis (Philadelphia, PA)*, *23*(2-3), 183-196.

Kautsky, H. & Hirsch, A., (1931). Neue versuche zur kohlensäure assimilation. *Naturwissenschaften*, *19*(48), 964-964.

Kim, J.H., Bhandari, S.R., Chae, S.Y., Cho, M.C. & Lee, J.G., (2019). Application of maximum quantum yield, a parameter of chlorophyll fluorescence, for early determination of bacterial wilt in tomato seedlings. *Horticulture, Environment, and Biotechnology*, *60*, 821-829.

Nedbal, L. & Whitmarsh, J., (2004). *Chlorophyll fluorescence imaging of leaves and fruits* (Vol. 14, pp. 389-407). Dordrecht, The Netherlands: Springer.

Nedbal, L., Soukupová, J., Whitmarsh, J. & Trtílek, M., (2000). Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. *Photosynthetica*, *38*, 571-579.

Nedbal, L., Trtílek, M. & Kaftan, D., (1999). Flash fluorescence induction: a novel method to study regulation of photosystem II. *Journal of Photochemistry and Photobiology B: Biology*, *48*(2-3), 154-157.

Ning, Y.N., Wang, Z.P., Palmer, A.W., Grattan, K.T. & Jackson, D.A., (1995). Recent progress in optical current sensing techniques. *Review of Scientific Instruments*, *66*(5), 3097-3111.

Obenland, D. & Neipp, P., (2005). Chlorophyll fluorescence imaging allows early detection and localization of lemon rind injury following hot water treatment. *Hortscience*, *40*(6), 1821-1823.

Omasa, K. & Takayama, K., (2003). Simultaneous measurement of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant and Cell Physiology*, *44*(12), 1290-1300.

Osmond, C.B., Daley, P.F., Badger, M.R. & Lüttge, U., (1998). Chlorophyll fluorescence quenching during photosynthetic induction in leaves of Abutilon striatum Dicks. infected with Abutilon mosaic virus, observed with a field-portable imaging system. *Botanica Acta*, *111*(5), 390-397.

Osório, J., Osório, M.L., Correia, P.J., de Varennes, A. & Pestana, M., (2014). Chlorophyll fluorescence imaging as a tool to understand the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants. *Scientia Horticulturae*, *165*, 148-155.

Schreiber, U.S.C.H.L.I.W.A., Schliwa, U. & Bilger, W., (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis research*, *10*, 51-62.

Takayama, K., Konishi, A. & Omasa, K., (2003). Diagnosis of invisible photosynthetic injury caused by a herbicide (Basta) with chlorophyll fluorescence imaging system. *Agricultural Engineering International: CIGR Journal*.

Van Kooten, O. & Snell, J.F.H., (1990). Progress in fluorescence research and nomenclature for quenching analysis. *Photosynth Res*, *25*, 147-150.

Wang, P., Li, H., Jia, W., Chen, Y., & Gerhards, R., (2018). A fluorescence sensor capable of real-time herbicide effect monitoring in greenhouses and the field. Sensors 18:3771.

Weber, J. F., Kunz, C., Peteinatos, G. G., Santel, H. J., & Gerhards, R., (2017). Utilization of chlorophyll fluorescence imaging technology to detect plant injury by herbicides in sugar beet and soybean. *Weed Technology*, *31*, 523–535.

Yanase, D. & Andoh, A., (1992). Translocation of photosynthesis-inhibiting herbicides in wheat leaves measured by phytofluorography, the chlorophyll fluorescence imaging. *Pesticide Biochemistry and Physiology*, *44*(1), 60-67.

Zheng, H., Lu, H., Zheng, Y., Lou, H. & Chen, C., (2010). Automatic sorting of Chinese jujube (*Zizyphus jujuba* Mill. cv.'hongxing') using chlorophyll fluorescence and support vector machine. *Journal of Food Engineering*, *101*(4), 402-408.