Visualizing Chlorophyll *a* Fluorescence: A Practical Demonstration

Ali Ahmad – University of Granada 0000-0001-5530-7374
Santiago Atero Calvo – University of Granada 0000-0001-8446-5515
Begoña Blasco León – University of Granada 0000-0001-8061-5141
Safa Selmi – Association of Safeguard of Matmata - University of Gabes 0000-0002-3389-6806
Alessandro Candiani – DNAPhone SRL 0000-0002-6200-7705
Vanessa Martos Núñez – University of Granada 0000-0001-6442-7968

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Correspondencia a través de **ORCID**: Ali Ahmad **0000-0001-5530-7374**

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Abstract: Chlorophylls are the principal components of plants for light harvesting. They utilize the energy retrieved from solar radiations to carry out the process of photosynthesis and produce reduced organic compounds such as carbohydrates. However, all of the incident light is not used in photosynthesis process, it confronts two other fates. A part of it is dissipated as heat, whereas the other is emitted as fluorescence. These processes occur simultaneously and whether one or the other occurs to a greater or lesser extent will depend both on the physiological status of the plant and the environmental conditions it faces. Chlorophyll (Chl) fluorescence is inversely proportional to the yield of photosynthesis and therefore is of prime importance in plant physiology. Furthermore, there are a lot of studies where Chl *a* fluorescence has been used as a probe for estimating photosynthetic yield, drought, salinity, vigor, and environmental effects on crop production yield. Therefore, this study was undertaken to demonstrate the visualization of the light emitted from Chl, commonly known as Chl *a* fluorescence. Plant leaves were dark adopted for 20 minutes before their exposure to ultraviolet (UV) light. Red glasses were used to visualize the emitted red light (fluorescence) from the leaves. This study may instill further interest in the plant physiology students to deepen and expand their learning by undertaking simple demonstrations like this.

Keyword: Chlorophyll *a* Fluorescence

Visualización de la fluorescencia de clorofila a: Una demostración práctica

Resumen: Las clorofilas son los principales componentes de las plantas para recolectar la luz. Utilizan la energía procedente de la radiación solar para llevar a cabo el proceso de fotosíntesis y producir moléculas orgánicas reducidas como los hidratos de carbono. Sin embargo, el total de la utilización de la luz incidente no toda es aprovechada en el proceso de fotosíntesis, ya que esta se enfrenta a otros dos destinos. Una parte se disipa como calor, mientras que la otra se emite como fluorescencia. Estos procesos ocurren de forma simultánea y que se de uno u otro proceso en mayor o menor medida, va a depender tanto del estatus fisiológico de la planta como de las condiciones ambientales a las que se enfrente. La fluorescencia de la clorofila (Chl) es inversamente proporcional al rendimiento o la tasa de la fotosíntesis y, por lo tanto, tiene una importancia fundamental en los estudios de fisiología vegetal. Asimismo, hay muchos estudios en los que la fluorescencia de Chl *a* se ha utilizado como sonda para estimar el rendimiento fotosintético, la sequía, la salinidad, el vigor y los efectos ambientales en la producción y el rendimiento de los cultivos. Por lo tanto, este estudio se realizó para demostrar la visualización de la luz emitida por la Chl, comúnmente conocida como fluorescencia de Chl *a*. Las hojas de las plantas fueron adoptadas en la oscuridad durante 20 minutos antes de su exposición a la luz ultravioleta (UV). Se utilizaron gafas rojas para visualizar la luz roja emitida (fluorescencia) de las hojas. Este estudio puede infundir más interés en los estudiantes de fisiología vegetal para que profundicen y amplíen su aprendizaje realizando demostraciones sencillas como esta.

Palabra clave: Fluorescencia de clorofila *a*

Introduction

Chlorophylls are the principal components of plants for light harvesting. They utilize the harvested light to produce sugars through the process of photosynthesis. However, all the incident light is not absorbed by chlorophyll (Chl) and a part of it is dissipated (Maxwell & Johnson, 2000). More precisely, the incident light on Chl encounters the following three fates (Figure 1):

1. Absorbance for photosynthesis process (also known as photochemistry)
2. Dissipation in the form of heat
3. Re-emission in the form of light (known as Chl fluorescence)

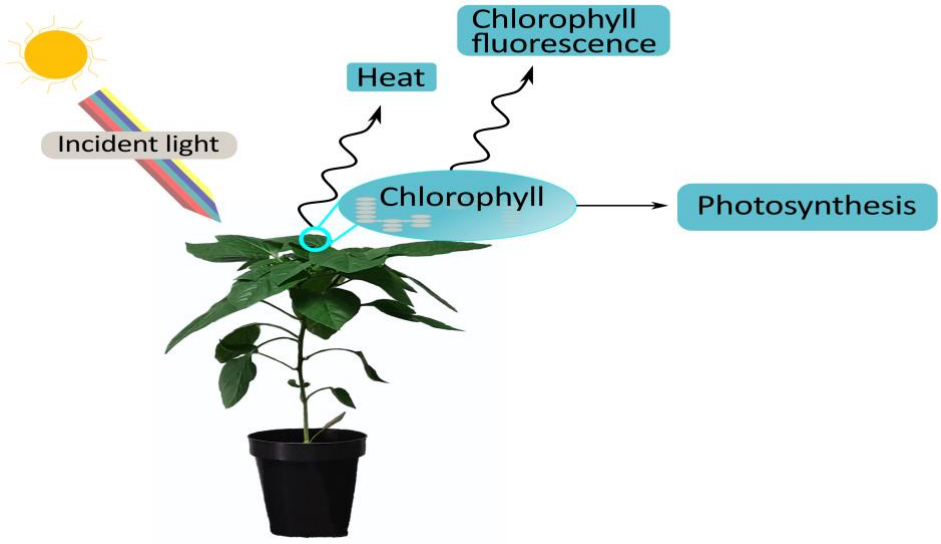

Figure 1. The incident light on plant leaf (chlorophyll) confronts three fates. A part of it is used in photosynthesis process, while a part of it is emitted as heat and other part is emitted as fluorescence.

Photosynthesis, Chl *a* fluorescence, and heat dissipation all of these processes occur simultaneously. The solar energy is absorbed by electron accepter of photosystem II (PSII), namely plastoquinone A (Q_A), which deliver it to photosynthetic apparatus. Once Q_A has accepted an electron it cannot accept another electron unless it transfers that to another electron accepter. Reaction center which accepts electron is referred to as "closed" when Q_A cannot accept another electron. Consequently, light is emitted in the form of fluorescence and heat; thereby resulting in lower rate of photosynthesis. Therefore, estimating the yield of one process could provide useful information about the other processes (Cosgrove & Borowitzka, 2010). For instance, Chl fluorescence yield would suggest the yield of photochemistry and heat emission. Consequently, this would indicate the efficiency of CO_2 assimilation in photosynthesis process. The intensity or yield of Chl fluorescence is inversely proportional to yield of photosynthesis and dissipative heat energy (Duysens, 1963; Krause & Weis, 1991). Hence, it is of paramount importance in plant physiology studies.

It has been reported that Chl fluorescence accounts for only a very small portion of absorbed light i.e., 0.5 to 10% (Barber et al., 1989; Brody & Rabinowitch, 1957; Latimer et al., 1956; Porcar-Castell et al., 2014). Similarly, almost all of the Chl fluorescence derives from PSII accounting for nearly 90—95% (Cosgrove & Borowitzka, 2010). The spectrum of Chl fluorescence is different from light absorption spectrum i.e., the energy emitted as Chl fluorescence has a higher wavelength than absorbed (Maxwell &

Johnson, 2000). Kautsky and co-authors were the first to report Chl fluorescence changes in 1960 (Kautsky et al., 1960). They reported an increase in the Chl fluorescence over a short period of time when dark adopted plants were exposed to light. The present study is meant to practically demonstrate this process of increased Chl *a* fluorescence for dark adopted leaf.

Importance of Chl a Fluorescence

The implication of studying Chl *a* fluorescence is multifold. Its yield provides useful information about various photosynthetic parameters including non-photochemical quenching (NPQ), JIP test, dark-adaptation kinetics of OJIP transients, statistic aspects of the measurements of parameters, the actinic light wavelength dependence of photosynthesis, rapid light curves (RLCs), Chl *a* fluorescence and 820nm transmission, electron transport rate (ETR), Chl *a* fluorescence and delayed fluorescence, flash-induced fluorescence, imaging, Chl *a* fluorescence and photoacoustic spectroscopy, and quenching analysis (Bukhov et al., 2001; Bukhov et al., 1997; Buschmann & Kocsányi, 1989; Bussotti et al., 2011; de Wijn & Van Gorkom, 2001; Goltsev et al., 2012; Gorbe & Calatayud, 2012; Hideg & Schreiber, 2007; Horton & Hague, 1988; Ioannidis et al., 2000; Kalaji et al., 2012; Kalaji et al., 2017; Klughammer & Schreiber, 1994; Krall & Edwards, 1990; Lichtenthaler et al., 2007; Nedbal & Whitmarsh, 2004; Ralph & Gademann, 2005; Schansker et al., 2003; Schansker et al., 2005; Schreiber et al., 2012; Schreiber et al., 1986; Snel et al., 1990; Strasser et al., 2004).

Plant Chl content is highly depictive of its vigor and is prone to changing environment (Ahmad, del Moral Garrido, et al., 2022). Therefore, any change affecting Chl would result in an alteration in Chl *a* fluorescence. Consequently, it could be implied to draw valuable information about various plant biological processes. In this regard, a summary of Chl *a* fluorescence with respect to various physiological parameters and its eco-physiological applications is provided in Table 1.

Table 1. A summary of few representative research articles on the application of chlorophyll a fluorescence in various agronomic and environmental studies.

Assay or Condition	Reference
Fungicide stress	(Ahmad, Navarro-León, et al., 2022)
Heat stress	(Brestic et al., 2012)
UV stress	(Guidi et al., 2011)
Salt stress	(Ahmad, Blasco, et al., 2022)
Photoinhibition	(Matsubara et al., 2011)
Drought stress	(Banks, 2018)
Urban tree conditions	(Swoczyna et al., 2010)
Environmental pollution	(Oláh et al., 2021)
Sulfur-deprivation	(Duan et al., 2019)
Water quality	(Sang et al., 2020)

Methodology

Plant material and growth conditions

The experiment was conducted on a laboratory grown lettuce (*Lactuca sativa*) plants that were 40 days old. Plants were grown in 13cm × 13cm x 12.5 cm plastic pot containing peat and vermiculite. They were irrigated twice a week with tap water. The growth chamber had a relative humidity of 60-80%, photosynthetically active radiation (PAR) of 350 µmol m^{-2} s^{-1}, and a photoperiod of 14-10 hrs with the subsequent temperature of 22 °C at night and 18 °C during the day.

Resources

An ultraviolet (UV) torch light, red color glasses, and a piece of paper to block light was used for this experiment. Similarly, Nikon-D5300 digital camera was used to record the video.

Visualization of Chl a fluorescence

Plants were dark adopted for 15-20 minutes (Figure 2) before their exposure to UV light, in order to record the high Chl *a* fluorescence (Kautsky et al., 1960). Subsequently, a small piece of paper was placed on a leaf along, whereas the rest of the leaf was continuously exposed to UV. After, few seconds the piece of paper was removed and a high fluorescence in the form of red light was recorded.

Figure 2. Dark adopted plant for 15-20 minutes, where all processes of energy entrapment and emission halt.

Results and Discussion

The dark adopted leaves when exposed to UV light emitted a strong signal of Chl *a* fluorescence, which was observed with the help of red glasses (Figure 3).

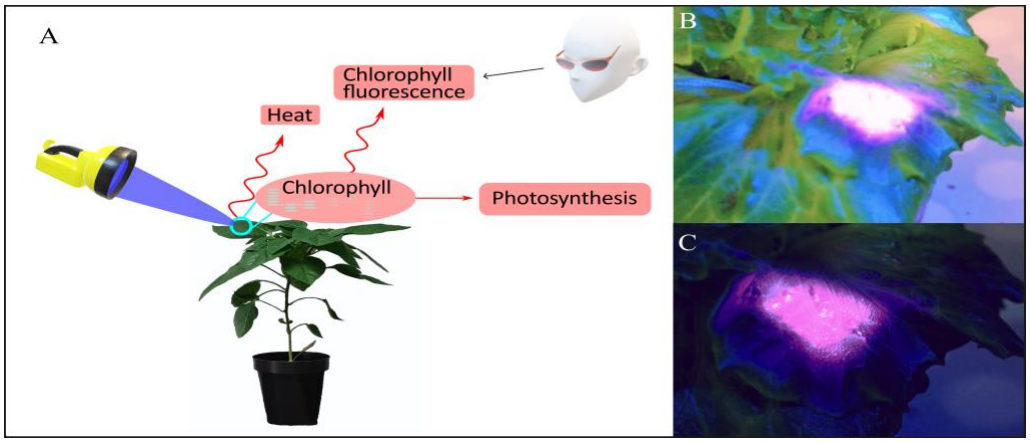

Figure 3. An illustration to observe chlorophyll a fluorescence under UV light with the help of red glasses (A). Visualization of red fluorescence from dark adopted leaf under UV light without red glasses (B) and with red glasses (C). Images captured through Nikon D-5300 digital camera.

This increase in Chl *a* fluorescence over a short period of time when dark adopted leaves were exposed to light had been previously documented by Kautsky and co-authors (Kautsky et al., 1960). The explanation of this lies in the fact that whenever a dark adopted leaf is brought to light, plastoquinone (electron acceptor of PSII) captures an

electron for its further transport. At this stage, the reaction center of Chl *a* is termed as "closed", which means no further electron can be accepted by plastoquinone. Consequently, the incident light is emitted back in the form of fluorescence and heat, leading to lower photosynthetic yield.

Conclusion

The incident light on plant leaves is emitted as fluorescence. This Chl *a* fluorescence can be visualized using a simple demonstration. The present study demonstrates the basic process of Chl *a* fluorescence using UV light and red glasses. Red glasses assist in visualizing the emitted red signal of fluorescence when the reaction centers are closed. This study may instill further interest in the plant physiology students to deepen and expand their learning by undertaking simple demonstrations like this.

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