



Proximal Remote Sensing of Herbicide and Drought Stress in Field Grown Colocasia and Sweet Potato Plants by Sunlight-Induced Chlorophyll Fluorescence Imaging

Sadasivan Nair Raji¹ · Geetha Nair Aparna¹ · Changatharayil N. Mohanan¹ · Narayanan Subhash^{1,2}

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Abstract Chlorophyll fluorescence is an indicator of plant photosynthetic activity and has been used to monitor the health status of vegetation. Several studies have exploited the application of red/far-red chlorophyll fluorescence ratio in detecting the impact of various types of stresses in plants. Recently, sunlight-induced chlorophyll fluorescence imaging has been used to detect and discriminate different stages of mosaic virus infection in potted cassava plants with a multi-spectral imaging system (MSIS). In this study, the MSIS is used to investigate the impact of drought and herbicide stress in field grown crop plants. Towards this control and treatment groups of colocasia and sweet potato plants were grown in laterite soil beds and the reflectance images of these crop plants were recorded up to 14-days of treatment at the Fraunhofer lines of O₂ B at 687 nm and O₂ A at 759.5 nm and the off-lines at 684 and 757.5 nm. The recorded images were analyzed using the Fraunhofer Line Discrimination technique to extract the sunlight-induced chlorophyll fluorescence component from the reflectance images of the plant leaves. As compared to the control group, the chlorophyll fluorescence image ratio (F_{687}/F_{760}) in the treatment groups of both the plant varieties shows an increasing trend with increase in the extent of stress. Further, the F_{687}/F_{760} ratio was found to correlate with the net photosynthetic rate (Pn) and stomatal conductance (g_s) of leaves. The correlation coefficient (R^2) for the relationship

of F_{687}/F_{760} ratio with Pn were found to be 0.78, 0.79 and 0.78, respectively for the control, herbicide treated and drought treated colocasia plants, while these were 0.77, 0.86 and 0.88, respectively for sweet potato plants. The results presented show the potential of proximal remote sensing and the application F_{687}/F_{760} fluorescence image ratio for effective monitoring of stress-induced changes in field grown plants.

Keywords Chlorophyll fluorescence imaging · Drought stress · FLD · Herbicide stress · Proximal remote sensing · Red/far-red fluorescence image ratio (F_{687}/F_{760}) · Sunlight-induced fluorescence imaging

Introduction

Plants are adversely affected by several biotic (bacteria, virus etc.) and abiotic stresses (water stress, nutrient deficiency, chemicals etc.), which in turn affects the plant growth and yield. Under normal conditions, most of the light falling on the leaves is absorbed and used for the photosynthetic activities in plants. Upon stress, the physiological changes occurring in plants alter the absorption, reflectance and fluorescence properties of plants, which eventually influence the photosynthetic activity and carbon fixation. Chlorophyll fluorescence occurring in the visible spectrum consists of two bands, one in the red region near 690 nm and the other in the far-red region near 735 nm, arising from the photosystem II and photosystem I (PS II and PSI), respectively (Plascyck 1975). Previous studies have proved that the F_{690}/F_{740} ratio, which reflects the PS II and PS I activities, can be used as a tool to assess the performance of photosystem PS II and PS I (Subhash and Mohanan 1994; Gitelson

✉ Narayanan Subhash
subhashnarayanan@gmail.com

¹ National Center for Earth Science Studies,
Akkulam, Thiruvananthapuram, Kerala 695031, India

² Sascan Meditech Pvt. Ltd, CIME, Department of Medical
Electronics, BMS College of Engineering, Bull Temple
Road, Basavanagudi, Bangalore, Karnataka 560019, India

et al. 1999; Subhash et al. 2004). Fluorescence is one of the means for releasing excess energy from chlorophyll. The amount of chlorophyll fluorescence emitted by a leaf under ambient sunlight is less than 3 % of the total reflected light at a specific wavelength, which makes it difficult to extract the fluorescence component from the total reflected light (Plascyck 1975; Plasyck and Gabriel 1975). The magnitude of fluorescence emission on exposure to light is governed by the chlorophyll concentration and photosynthetic activity.

Studies suggest a strong inverse relationship between red to far-red fluorescence ratio and chlorophyll content in plants under stress, where the intensity of F_{690} increased with stress as compared to the F_{730} intensity (Subhash and Mohanan 1997). The ratio of the red to far-red (F_{690}/F_{730}) fluorescence intensity is particularly sensitive to environmental changes and permits an early detection of damage to the photosynthetic apparatus or stress conditions in plants (Subhash et al. 2004). Since the chlorophyll fluorescence at 690 nm falls within its absorption band there is a partial reabsorption of the emitted red chlorophyll fluorescence (F_{690}). Therefore, the observed intensity of fluorescence at 690 nm is dependent on the Chl concentration. Furthermore, the far-red emission is apparently less responsive than the red emission to the state of the electron transport mechanisms at normal physiological temperatures (Govindjee 1995; Buschmann and Lichtenthaler 1998). Several studies have shown chlorophyll fluorescence as an index of plant physiology as it is directly related to the plant photosynthesis (Rascher et al. 2009; Chaerle and Van Der Straeten 2001).

The past decades have seen laser-induced fluorescence (LIF) spectroscopy involving point monitoring of chlorophyll fluorescence using fiber optics probes in the conduct of stress related plant studies. Introduction of multi-spectral imaging modality has made monitoring of multiple plants under identical conditions possible. When compared to point monitoring techniques of spectral measurements, imaging techniques yield images that contain the information of several pixels covering a larger area in a single capture. With developments in the chlorophyll fluorescence measurement techniques, sunlight-induced chlorophyll fluorescence (SICF) imaging has emerged as a powerful and potential tool for studying the photosynthetic functioning of outdoor plants. For the SICF measurement of vegetation, one of the most useful frequency ranges is located in the atmospheric O_2 B absorption band. Importance of this band in chlorophyll fluorescence studies was motivated by its position, which is next to the maximum of the red fluorescence band, and by the advantage of it having a reduced reflectance signal. Furthermore, the molecular oxygen absorption of the solar radiation influences chlorophyll fluorescence minimally in this spectral window. Absolute measurements of chlorophyll

fluorescence are very difficult to obtain and need careful calibration (Mazzoni et al. 2008). Therefore ratiometric analysis of chlorophyll fluorescence emission assumes great significance.

Although the chlorophyll fluorescence represents measurable quantities of light, it is very low in comparison with the amount of light energy reflected from the leaf surface. Therefore, under conditions of day light, plant fluorescence is not observed without the help of equipments specially designed for the purpose. SICF imaging is an effective method for fluorescence measurement as it uses the Fraunhofer Line Discrimination (FLD) technique (Plascyck 1975; Plasyck and Gabriel 1975). Tanaka et al. (2009) investigated the applicability of SICF imaging using FLD technique on sampled leaves and live vegetation to study the relationship between chlorophyll fluorescence emission and intensity of green coloration present. SICF is extracted from the whole reflectance signal by using the FLD technique. Fraunhofer lines are spectral lines which have very low levels of incident solar irradiance. In these lines the stronger reflectance background is significantly reduced so that the weak fluorescence signal can be extracted from the reflected signal. FLD principle involves using the sun as an excitation source and compares the depth of the line in the solar irradiance spectrum to the depth of the line in the radiance spectrum. When a target is exposed to the sun, the infilling of a Fraunhofer line (increased radiation at the central wavelength of the Fraunhofer line) can be attributed to fluorescence. In the Fraunhofer band, two of the spectral lines (O_2 B and O_2 A positioned at 687 and 760 nm, respectively) overlap with the red and far-red chlorophyll fluorescence emission spectrum of plants (centered at 687 and 735 nm) and hence, these Fraunhofer lines are used for the measurement. Thus, the FLD method allows estimation of the SICF emission at the Fraunhofer lines of O_2 molecule (the A-band around 760 nm, and B-band around 687 nm), where the amount of incoming radiation is comparable to the fluorescence emission. This provides an opportunity for optical remote sensing of plant stress by monitoring changes in the emitted radiation from plants. Using multispectral imaging techniques, images of vegetation are acquired at these narrow bands and at adjacent wavelengths where Fraunhofer lines do not have absorption, which can be used for continuous monitoring of the plant physiological status. FLD technique has paved way to carry out several significant studies in the process of quantification of chlorophyll fluorescence using remote sensing measurements (Damm et al. 2014).

It is seen that, several vegetation assessment studies are ongoing in the evaluation of fluorescence signals using ground and space borne devices (Rossini et al. 2015). An intermediate scale observation which bridges the gap between the localized point monitoring devices and high

resolution space borne imaging devices is a critical requirement. Recently, we have carried out SICF studies to detect mosaic virus disease in potted cassava plants by developing a multi-spectral imaging system (MSIS) with narrow band interference filters centered at the Fraunhofer lines of O₂ B, O₂ A and at adjoining wavelengths (Raji et al. 2015). Until now, most of the studies were carried out in the laboratory on excised leaves, or in potted plant under controlled environments (Saito et al. 2007). The primary objective of the present study was to extent the capability of SICF imaging to field grown plants and to test its ability for detection of the impact of drought and herbicide stress in colocasia and sweet potato plants cultivated in outdoor environment using the red/far-red fluorescence image ratio (F_{687}/F_{760}). The net photosynthetic rate (Pn) and stomatal conductance (g_s) of the control and treatment group of plants were measured during various stages of stress and correlated with the fluorescence image ratio and the results are presented.

Materials and Methods

Plant Cultivation and Growth

Sri Rasmi variety of Colocasia (*Colocasia esculenta*) and Kanjangad variety of sweet potato (*Ipomoea batatas*), which are edible tuber crops, were selected for the study. These crop plants have large leaves that make it easier for plant physiological and spectral measurements. Moreover, these plants have a thick canopy formation with good ground coverage so that less amount of soil gets exposed during SICF imaging from an aerial platform. The field chosen for the study was made up of laterite soil with ample sunlight. The soil was tilled initially to loosen the soil texture and to remove stones and other debris. A large bed was prepared in laterite soil of uniform consistency, which was sub-divided into smaller beds of dimension 1 m × 1 m each for cultivating control, and herbicide/drought treated plants, with three replicates per treatment per plant variety.

During germination and early stage of growth, all the plants were watered daily and uniformly. Study was initiated after 2 months of planting when closed canopies were seen and soil beds were not visible from above. In the treatment group of plants, herbicide (15 ml Glyphosate diluted in 1 l of water) was sprayed using a sprayer underneath the leaves and drought condition was induced by withholding water supply. The measurements were carried out prior to initiation of treatment and were repeated daily until 14 days of treatment. As drought and herbicide stress caused considerable damage to the plants, measurements were not carried out beyond 14 days. Visual inspection of crop plants indicated wilting and

partial collapse of herbicide treated canopies by day-14. The ground coverage of herbicide and drought treated canopies started shrinking by day-7. Some of the treated plant leaves had senescence, while others were in dry state or experienced leaf loss at the end of the measurement period. Along with the recording of sunlight-induced reflectance images, laser-induced chlorophyll fluorescence spectrum (LICF), Pn and g_s were also measured as detailed below.

SICF Imaging

The MSIS used for fluorescence imaging of outdoor plants (Raji et al. 2015) consists of an Electron Multiplying CCD (EMCCD) monochrome camera (1024 × 1024 pixels, Model: LUCA-R-DL-604, Andor Technology, UK), coupled to a Nikon AF 35–70 mm zoom camera lens and interference filters (1 nm bandwidth) attached to a rotatable filter mount (Fig. 1). The imaging system was mounted on top of a building with a view angle of 45°, approximately 15 m above the ground level and 20 m away from the plot. Images were recorded on each day between 11.30 and 13.30 h under clear sky conditions under full sunlight through interference filters centered at 687 and 759.5 nm (matching the O₂ A and B bands) and at their offline wavelengths of 684 and 757.5 nm, respectively. During this time of the day, the sun would be at the zenith, so that the transmission path for solar irradiance and the effect of ground reflection is minimized (Carter and Miller 1994). The image processing steps for fluorescence measurement consisted of: (1) applying correction on the raw image for non-uniform solar illumination using a reflectance standard, (2) applying correction for lateral shifts in the image frame during interchange of filters, (3) extraction of fluorescence image using FLD method, and (4) derivation of red to far-red fluorescence image ratio.

The effect of variations in solar radiation are eliminated by taking a ratio of the radiance in the line center to that of the continuum adjacent to the Fraunhofer line (Tanaka et al. 2009). A reference reflectance standard (SRS-99-010-Labsphere, USA) was placed horizontally on the target site and imaged for each of the selected wavelengths to normalize the captured images and to correct for any spatial sensitivity characteristics within the viewed images. The resulting reflectance image R for each wavelength λ was calculated as follows:

$$R_{\lambda} = \left[\frac{I_{\lambda}}{r_{\lambda}} \right] K \quad (1)$$

where R_{λ} is the reflectance image of the target at the specified wavelength, I_{λ} is the raw image of the target at the specified wavelength, r_{λ} is the raw image of the reflectance standard at the specified wavelength, and K is

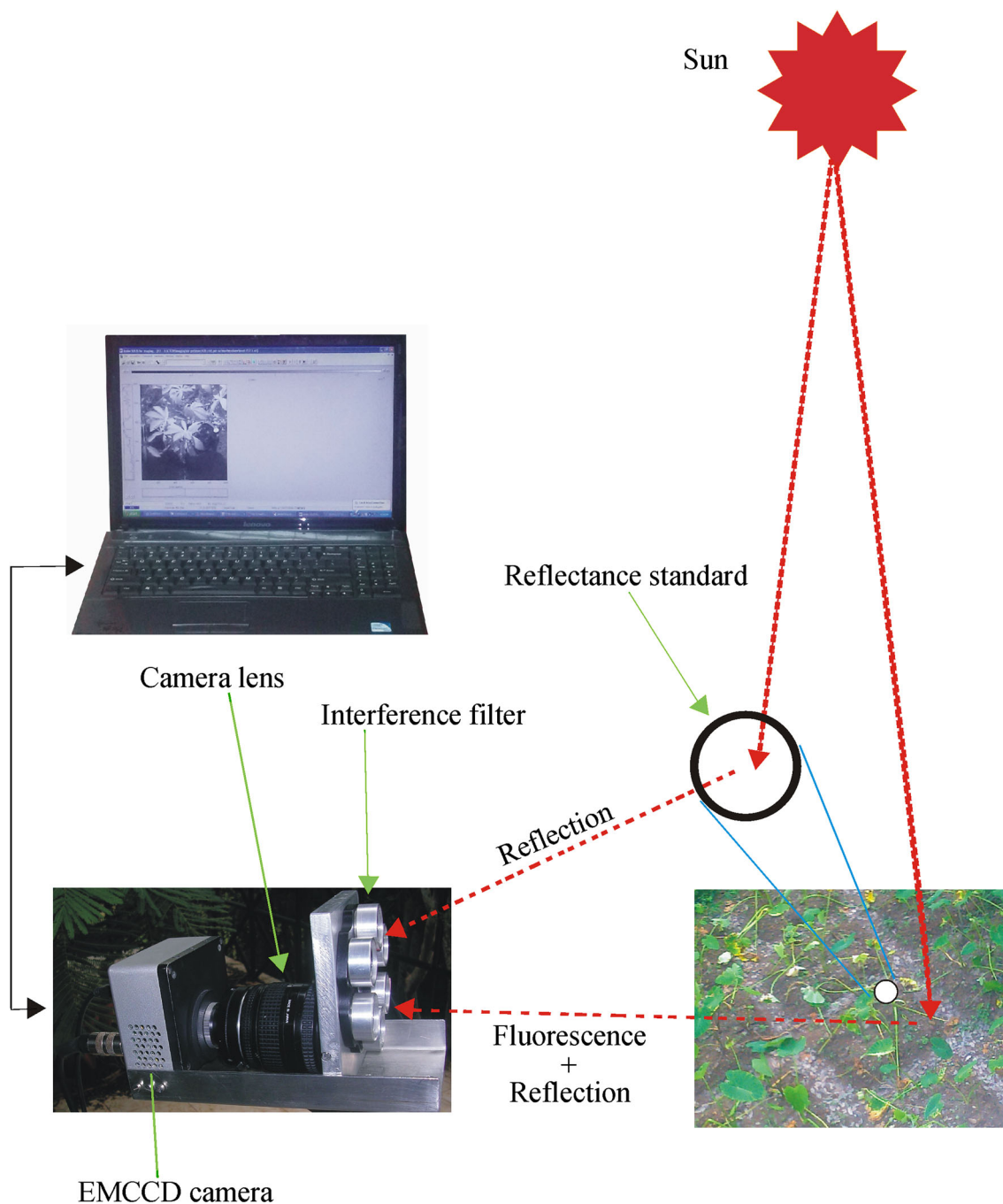


Fig. 1 Multi-spectral imaging system for sunlight-induced fluorescence imaging of outdoor plants

the reflectance factor of the standard as specified by the manufacturer at each wavelength. During fluorescence measurements, filters were changed manually, which could cause a slight shift in position of the images taken at two different bands. In order to correct this shift, a data processing program was coded and executed in Matlab Ver. 7.0 on the acquired images.

The SICF image intensity (F) at the chlorophyll bands is derived from images at the on and off bands of the

Fraunhofer line using the FLD method (Saito and Suzuki 2003) and is given by:

$$\text{Fluorescence image intensity, } F = \frac{d - c \left(\frac{b}{a}\right)}{1 - \left(\frac{b}{a}\right)} \quad (2)$$

where a is the reference intensity at Fraunhofer line, b is the reference intensity at the adjacent line, c is the target intensity at Fraunhofer line, and d is the target intensity at the adjacent line.

The captured images were normalized by applying Eq. (1), and then fluorescence image intensity at any specific band was derived by applying Eq. (2). The red/far-red fluorescence image intensity ratio (F_{687}/F_{760}) was determined arithmetically by dividing the corresponding images (F_{687} and F_{760}) using the SOLIS software (Andor, UK) to derive a monochrome ratio image. The fluorescence image ratio (F_{687}/F_{760}) values were determined from nine regions of interest (ROI), each region consisting of equal size of 20 pixels, randomly selected from each plant leaf subjected to treatment. Mean value of each ROI was taken. Fully grown leaves without senescence were used for measurements and care was taken to avoid leaf veins while selecting the ROI as the chlorophyll content could vary at such areas. The mean image ratio values were correlated with P_n and g_s values. This image ratio is then false-coloured (pseudo-color-mapped) for easy visualization of the difference between the healthy and stressed regions of plants. A colour bar was also provided which helped in understanding the extent of stress at image each point of the leaf surface. In the colour bar, lower ratio values are given in black or dark blue, which gradually changes to yellow and white, with increase in ratio value.

Chlorophyll Fluorescence Spectra

Experimental system used for intact measurement of LICF consisted of a diode laser emitting at 405 nm with a continuous wave (CW) output power of 50 mW and a fiber-optic spectrometer (Ocean Optics, Inc., Dunedin, FL; Model: USB 2000FL VIS–NIR) connected to the USB port of a laptop for recording of fluorescence spectra. The laser output was guided to the leaf through the central fiber of 3-m long bifurcated fiber-optic probe (Ocean Optics, Inc.; Model: ZR400—5-VIS/NIR) and the six surrounding fibers (400 mm diameter each) at the probe tip collect the fluorescence emission through a long-pass filter (Schott, USA, Model: GG420), which blocks the back-scattered laser light from entering the spectrometer. The LICF was recorded in the 500–800 nm spectral range with a resolution of 8 nm. Nine measurements each were recorded from fully grown leaves leaving the vein portion.

P_n and g_s Measurements

P_n and g_s were measured using a Hand-Held Photosynthesis measuring device (Model CI-340, CID Inc, USA) from well-developed plant leaves under a fixed external photosynthetically active radiation (PAR) of $1000 \mu\text{mol m}^{-2}$ from a LED source. The measurements were carried out between 10.00 and 13.30 h. Nine measurements were recorded from the uppermost fully-expanded leaves of each plant, with care taken to avoid larger veins, for the

control and treatment groups and the mean of these measurements were taken for further analysis.

Results

SICF and LICF Features

Typical SICF ratio images of colocasia and sweet potato plants under study are presented in Figs. 2 and 3, respectively. Fluorescence images F_{687} and F_{760} and corresponding ratio image F_{687}/F_{760} from control, herbicide and drought treated plants were obtained throughout the study period. Pseudo colour map (PCM) of the F_{687}/F_{760} fluorescence ratio image was generated for easy visualization of difference in the ratio value across the leaf surface. Figure 2a shows the F_{687}/F_{760} ratio images on day-0 prior to application of any stress on colocasia plants, while Fig. 2b shows the ratio image of the control group of colocasia on day-14. Figure 2c is the image ratio on day-14 of herbicide treated plant, whereas Fig. 2d shows the image of drought stressed colocasia on day-14 of treatment. Similarly, Fig. 3a refers to the image ratio on day-0 for sweet potato plants, Fig. 3b the image ratio on the control groups of sweet potato plants on day-14, Fig. 3c the image ratio of herbicide stressed sweet potato on day-14 and Fig. 3d shows the image ratio of drought stressed sweet potato on day-14 of treatment. It is seen from the images that on day-0 for all the treatment groups, the leaves were green and healthy and these leaves appear in blue or green colour in the PCM images. On day-14 of measurement, the control plants remain unchanged in colour and leaf structure, whereas, herbicide and drought stressed plants show visible symptoms associated with wilting and yellowing of leaves. It was observed that in each PCM image, the fluorescence image ratio was greater in the regions affected by yellowing, which is indicated in red or yellow, whereas healthy leaves are shown in blue or green. Image ratios of control plants on day-0 and day-14 appear in blue or green with almost constant values of 0.83 ± 0.05 in colocasia and 0.93 ± 0.17 in sweet potato plants, indicative of low chlorophyll fluorescence emission, whereas, on day-14 herbicide and drought stressed plants show more of red or orange on PCM, indicating higher fluorescence emission. Application of herbicide caused yellowing of leaves due to loss in chlorophyll pigment concentration, which could be seen in both colocasia and sweet potato plants. PCM ratio images showed a marked increase in ratio value from 0.89 ± 0.02 on day 0 to 1.89 ± 0.29 in colocasia and from 0.99 ± 0.08 to 1.53 ± 0.08 in sweet potato plants on day 14, as the plants were exposed to higher concentration of herbicides. With drought, the F_{687}/F_{760} ratio has increased from 0.85 ± 0.03 to 1.42 ± 0.21 in colocasia and from 0.94 ± 0.08 to 1.71 ± 0.09 in sweet potato over the treatment period.

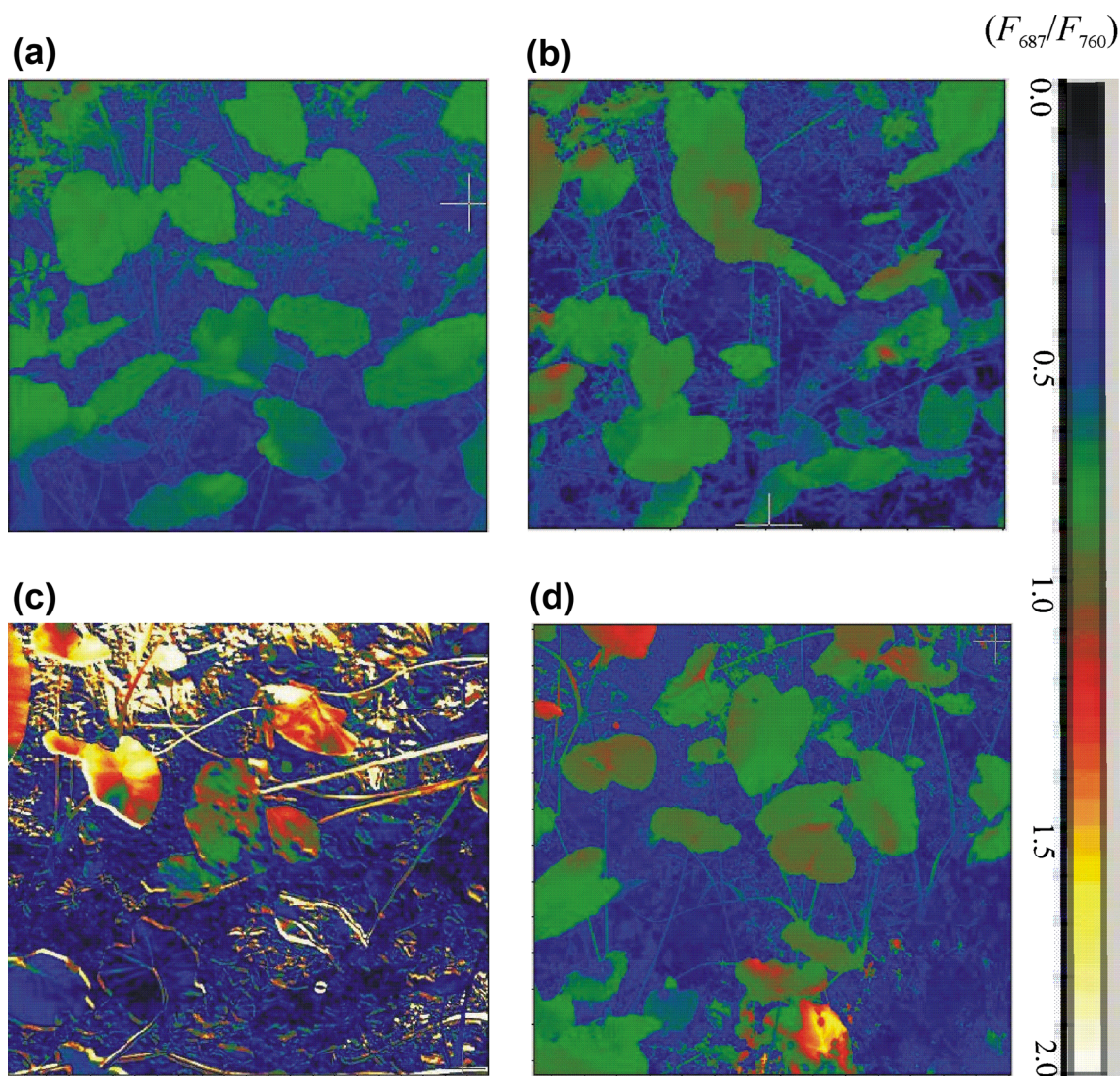


Fig. 2 Sunlight-induced fluorescence imaging of colocasia plants **a** PCM image F_{687}/F_{760} of untreated colocasia plants on day-0, **b** PCM ratio image F_{687}/F_{760} of control group of colocasia plants on

day-14, **c** PCM ratio image F_{687}/F_{760} of herbicide treated colocasia plants on day-14 and **d** PCM ratio image F_{687}/F_{760} of drought treated colocasia plants on day-14

LICF spectra of intact leaves were recorded for colocasia and sweet potato plants under herbicide, drought and control conditions, in the 500–800 nm spectral ranges, with the point monitoring fiber-optic probe. The results obtained from LICF studies were used to validate that herbicide and drought stress causes the chlorophyll fluorescence to increase, even though it has been proved from various studies. The red and far-red chlorophyll fluorescence peaks were observed at 684 and 738 nm, respectively for colocasia and at 684 and 737 nm, respectively for sweet potato plants. The LICF spectra of colocasia plants recorded on day-0, prior to the application of the stress and on day-14 after the application of the stress is plotted on Fig. 4a, b and that of sweet potato plants is plotted on Fig. 4c, d, normalized to the intensity of the 684 nm peak. At the start of the experiment, all the plant groups showed similar

LICF spectra, but as the study progressed, plants subjected to stress exhibited increase in the red/far-red fluorescence ratio as compared to the control plants. It was observed that, F_{684}/F_{735} ratio of control plants varied in the range 1.17 ± 0.23 for colocasia and 1.70 ± 0.20 for sweet potato plants over the whole study period. Application of herbicide has caused an increase of F_{684}/F_{735} ratio from 1.22 ± 0.21 to 2.04 ± 0.38 in colocasia plants. Similarly, the change in F_{684}/F_{735} ratio values in sweet potato was 1.56 ± 0.16 to 2.24 ± 0.18 . Water stressed plants showed an increase from 1.08 ± 0.19 to 1.92 ± 0.32 in colocasia plants and 1.68 ± 0.21 to 2.08 ± 0.24 in sweet potato plants. On day-14 of the experiment, there were noticeable differences for the red/far-red fluorescence ratio between control group of plants and herbicide and drought stressed plants (Fig. 4). The difference between control and

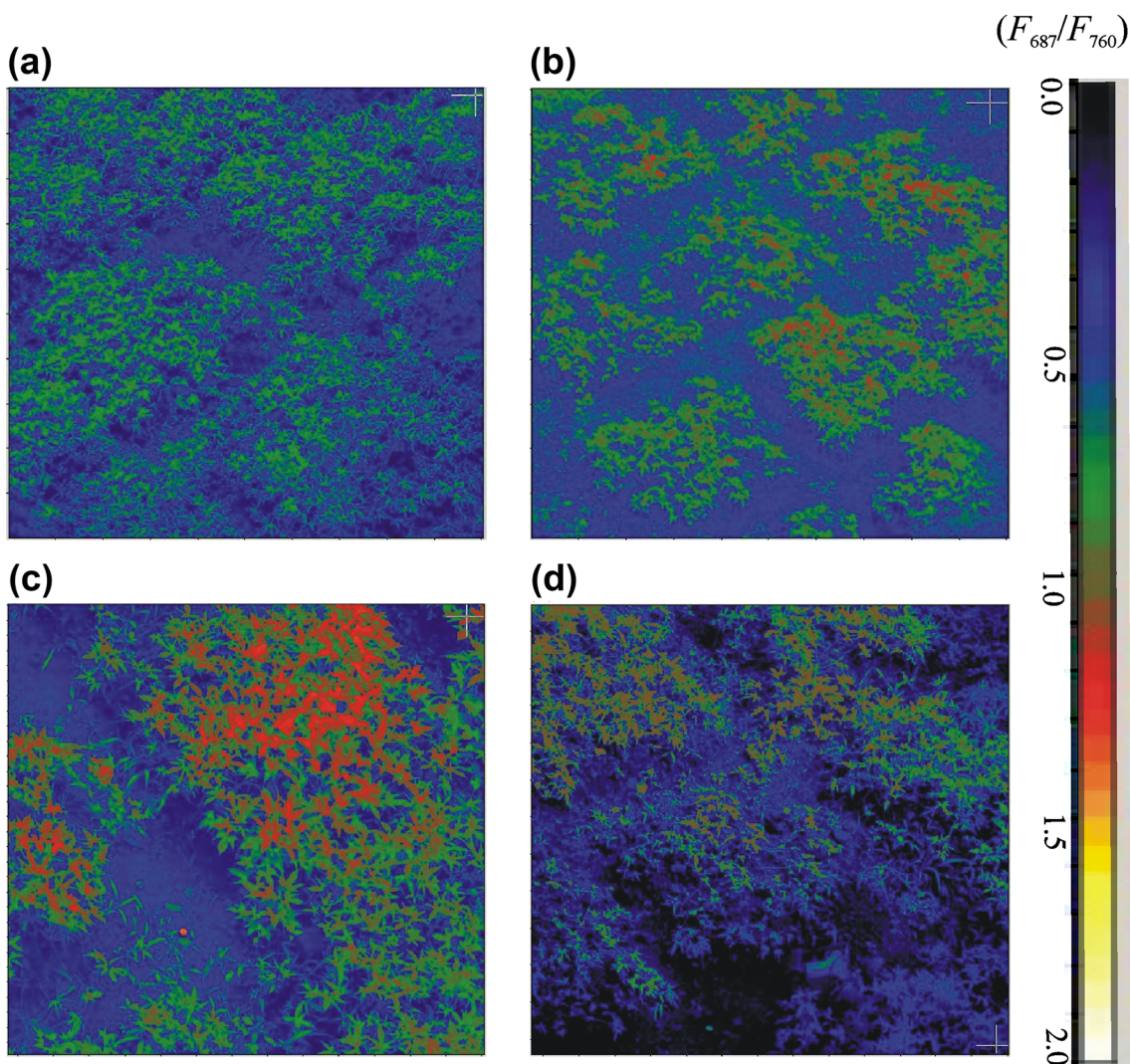


Fig. 3 Sunlight-induced fluorescence imaging of sweet potato plants **a** PCM image ratio F_{687}/F_{760} of untreated sweet potato plants on day-0, **b** PCM image ratio F_{687}/F_{760} of control group of sweet potato

plants on day-14, **c** PCM image ratio F_{687}/F_{760} of herbicide treated sweet potato plants on day-14 and **d** PCM image ratio F_{687}/F_{760} of drought treated sweet potato plants on day-14

treatment groups of plants progressively increased with increase in stress and on day-14, the red/far-red ratios of herbicide and drought stressed plants investigated by LICF were greater than the control plants by 70 and 60 %, in colocasia plants and 37 and 27 % in sweet potato plants, respectively.

Correlation of F_{687}/F_{760} Fluorescence Image Ratio with Pn and g_s

The Pn and g_s values remained nearly constant over the study period for the control group of plants, whereas for the stressed group of plants, the Pn decreased with increase in the number of days of treatment. The value of Pn in colocasia plants, which was $29.36 \pm 3.59 \mu\text{mol m}^{-2} \text{s}^{-1}$ prior to herbicide treatment started to decrease within a few days

of treatment and was $5.04 \pm 1.88 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-14. In comparison, for drought stressed colocasia plants, Pn decreased from $28.78 \pm 3.16 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-0 to $7.23 \pm 1.42 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-14. The value of g_s , which was $205.48 \pm 21.7 \text{mmol m}^{-2} \text{s}^{-1}$ on day-0, decreased to $98.58 \pm 19.86 \text{mmol m}^{-2} \text{s}^{-1}$ on day-14 for herbicide treated group, whereas for drought stressed plants the value of g_s decreased from $194.98 \pm 31 \text{mmol m}^{-2} \text{s}^{-1}$ on day-0 to $134.5 \pm 28 \text{mmol m}^{-2} \text{s}^{-1}$ on day-14. In sweet potato plants, Pn which was $35.4 \pm 2.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the start of the experiment decreased to $11.9 \pm 2.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-14, with herbicide treatment, whereas Pn which was $34.6 \pm 3.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-0 reduced to $10.7 \pm 1.54 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day-14 with drought stress. A similar reduction in value was observed in g_s from $199.8 \pm 33.5 \text{mmol m}^{-2} \text{s}^{-1}$ to

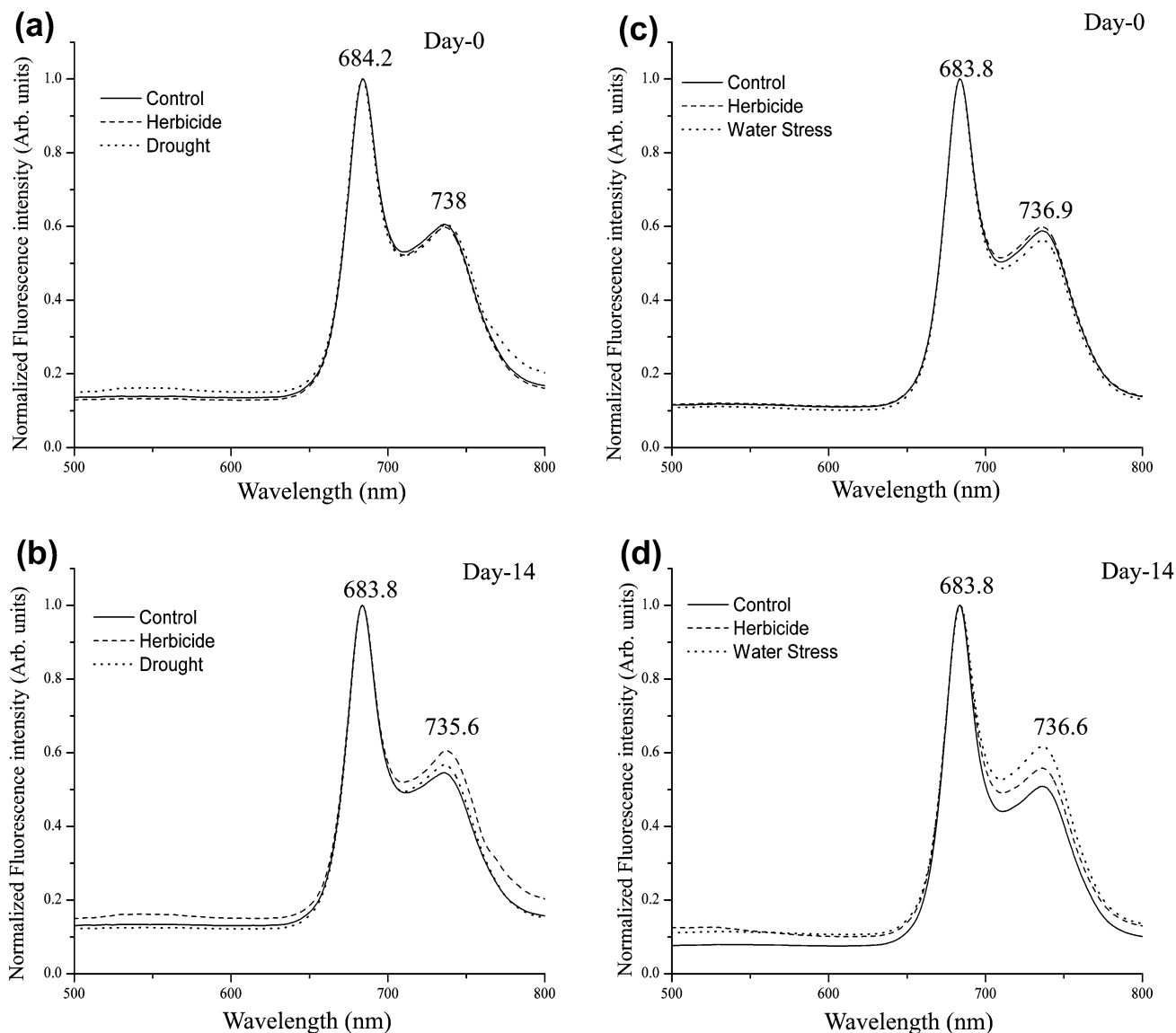


Fig. 4 Averaged normalized laser-induced chlorophyll fluorescence spectra of colocasia leaves measured on **a** colocasia leaves: day-0, **b** colocasia leaves: day-14, **c** sweet potato leaves: day-0 and **d** sweet

potato leaves: day-14 of the experiment under herbicide and drought treatments, compared against control plants

$92.4 \pm 22.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ with 14 days of herbicide treatment and from $236.3 \pm 35.7 \text{ mmol m}^{-2} \text{ s}^{-1}$ to $95.4 \pm 19 \text{ mmol m}^{-2} \text{ s}^{-1}$ with 14 days of drought treatment. Table 1 represents the measured values of red/far-red fluorescence ratio, Pn and g_s of control, herbicide stressed and drought stressed groups of colocasia and sweet potato plants. The continued reduction in Pn and g_s values during herbicide and drought stress corresponded with gradual induction of senescence and yellowing of leaves.

Dependence of F_{687}/F_{760} fluorescence ratio with Pn was determined and plotted for both the treatment and control groups of plants. Nine sets of data were taken on each day for plants under treatment. It was observed that the

fluorescence ratio F_{687}/F_{760} has a significant negative linear correlation with Pn and g_s Fig. 5a, b. A correlation coefficient of $R^2 = 0.78$, $R^2 = 0.79$ and $R^2 = 0.78$ were found for fluorescence image ratio F_{687}/F_{760} with Pn in control, herbicide and drought stressed colocasia plants, respectively. Whereas, in sweet potato plants, the F_{687}/F_{760} had correlation coefficients of $R^2 = 0.77$, $R^2 = 0.86$ and $R^2 = 0.88$ with Pn for control, herbicide and drought stressed plants, respectively (Fig. 5a–c). A similar negative correlation of $R^2 = 0.79$, $R^2 = 0.77$ and $R^2 = 0.79$ was observed between F_{687}/F_{760} fluorescence image ratio and g_s in colocasia and $R^2 = 0.75$, $R^2 = 0.85$ and $R^2 = 0.77$ in sweet potato plants (Fig. 5d–f).

Table 1 Variance of F_{687}/F_{760} image ratio, F_{684}/F_{735} spectral ratio, net photosynthetic rate Pn and stomatal conductance g_s for herbicide and drought treated with control group (a) colocasia plants, (b) sweet potato plants

| Time (days) | Treatment | F_{687}/F_{760} (%) | F_{684}/F_{735} (%) | Pn ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (%) | g_s ($\text{mmol m}^{-2} \text{s}^{-1}$) (%) |
|--------------------------------|-----------|-----------------------|-----------------------|---|--|
| <i>(a) Colocasia plants</i> | | | | | |
| Day 0 | Control | 0.83 ± 0.03 | 1.17 ± 0.23 | 31.79 ± 4.03 | 223.29 ± 19.14 |
| | Herbicide | 0.89 ± 0.02 (7) | 1.22 ± 0.21 (4.27) | 29.36 ± 3.59 (-7.64) | 205.48 ± 21.70 (-7.98) |
| | Drought | 0.85 ± 0.03 (2) | 1.08 ± 0.19 (-7.69) | 28.78 ± 3.16 (-9.47) | 194.98 ± 30.77 (-12.68) |
| Day 3 | Control | 0.85 ± 0.04 | 1.23 ± 0.22 | 30.63 ± 3.49 | 255.01 ± 23.80 |
| | Herbicide | 1.16 ± 0.08 (36.47) | 1.47 ± 0.26 (19.51) | 17.63 ± 4.27 (-62.03) | 152.5 ± 18.3 (-40.19) |
| | Drought | 0.93 ± 0.10 (9.41) | 1.40 ± 0.19 (13.82) | 23.82 ± 4.01 (-42.44) | 190.53 ± 21.90 (-25.29) |
| Day 7 | Control | 0.78 ± 0.02 | 1.08 ± 0.29 | 33.95 ± 3.68 | 201.96 ± 29.23 |
| | Herbicide | 1.71 ± 0.20 (119.23) | 1.58 ± 0.32 (46.29) | 7.01 ± 2.07 (-79.35) | 138.45 ± 28.5 (-31.45) |
| | Drought | 1.09 ± 0.18 (39.74) | 1.63 ± 0.28 (50.93) | 14.68 ± 2.19 (-56.76) | 155.6 ± 32.46 (-22.96) |
| Day 10 | Control | 0.79 ± 0.05 | 1.16 ± 0.30 | 34.62 ± 3.99 | 215.75 ± 26.73 |
| | Herbicide | 1.80 ± 0.22 (127.85) | 1.81 ± 0.26 (56.03) | 6.58 ± 2.70 (-80.99) | 105.92 ± 28.32 (-50.91) |
| | Drought | 1.21 ± 0.24 (53.16) | 1.78 ± 0.17 (53.45) | 11.35 ± 2.32 (-67.22) | 144.7 ± 21.63 (-32.93) |
| Day 14 | Control | 0.82 ± 0.06 | 1.20 ± 0.29 | 32.51 ± 4.90 | 246.03 ± 42.36 |
| | Herbicide | 1.89 ± 0.29 (130.49) | 2.04 ± 0.38 (70) | 5.04 ± 1.88 (-84.49) | 98.58 ± 19.86 (-59.93) |
| | Drought | 1.42 ± 0.21 (73.17) | 1.92 ± 0.32 (60) | 7.23 ± 1.42 (-77.76) | 134.5 ± 28.21 (-45.33) |
| <i>(b) Sweet potato plants</i> | | | | | |
| Day 0 | Control | 0.93 ± 0.06 | 1.70 ± 0.20 | 36.7 ± 3.26 | 211.1 ± 29.1 |
| | Herbicide | 0.99 ± 0.08 (6.45) | 1.56 ± 0.16 (-8.24) | 35.4 ± 2.40 (-3.54) | 199.8 ± 33.5 (-5.35) |
| | Drought | 0.94 ± 0.08 (1.07) | 1.68 ± 0.21 (-1.18) | 34.6 ± 3.01 (-5.72) | 236.3 ± 35.7 (11.94) |
| Day 3 | Control | 0.89 ± 0.09 | 1.74 ± 0.18 | 39.1 ± 1.80 | 254.1 ± 39.0 |
| | Herbicide | 1.06 ± 0.09 (19.1) | 1.81 ± 0.25 (4.02) | 28.7 ± 3.30 (-26.6) | 171.1 ± 27.14 (-32.66) |
| | Drought | 1.02 ± 0.08 (14.61) | 1.77 ± 0.15 (1.72) | 28.7 ± 2.14 (-26.59) | 156.8 ± 22.63 (-38.29) |
| Day 7 | Control | 1.01 ± 0.08 | 1.71 ± 0.19 | 40.4 ± 1.63 | 222.8 ± 40.1 |
| | Herbicide | 1.16 ± 0.08 (14.85) | 1.98 ± 0.12 (15.79) | 22.3 ± 2.07 (-44.8) | 137.1 ± 25.3 (-38.46) |
| | Drought | 1.20 ± 0.11 (22.77) | 1.85 ± 0.23 (8.19) | 20.1 ± 1.75 (-50.25) | 123.8 ± 22.7 (-44.43) |
| Day 10 | Control | 0.95 ± 0.09 | 1.67 ± 0.18 | 39.8 ± 1.38 | 216.1 ± 39.7 |
| | Herbicide | 1.28 ± 0.10 (34.74) | 2.02 ± 0.20 (20.96) | 14.4 ± 1.20 (-63.82) | 118.2 ± 25.4 (-45.3) |
| | Drought | 1.63 ± 0.09 (71.58) | 1.93 ± 0.18 (15.57) | 15.1 ± 2.85 (-62.06) | 115.4 ± 27.6 (-46.59) |
| Day 14 | Control | 0.97 ± 0.09 | 1.64 ± 0.23 | 33.5 ± 3.41 | 253.7 ± 31.2 |
| | Herbicide | 1.53 ± 0.08 (57.73) | 2.24 ± 0.18 (36.59) | 11.9 ± 2.18 (-64.48) | 92.4 ± 22.1 (-63.58) |
| | Drought | 1.71 ± 0.09 (76.29) | 2.08 ± 0.24 (26.83) | 10.7 ± 1.54 (-68.06) | 95.4 ± 19.4 (-62.39) |

Values given are mean ± standard deviation of 9 data sets. Data given in parenthesis indicates percentage variance; (+) indicates increase in value and (-) indicates decrease in value, as compared to corresponding control values

Discussion

A clear distinction in colour is seen in the PCM fluorescence image ratio (F_{687}/F_{760}) between the healthy and stressed plant leaves. Regions of stress in leaves are represented in PCM image by lighter colours, indicating higher values for the chlorophyll fluorescence ratio. The spectral measurements by point monitoring also give a higher fluorescence ratio values for herbicide and drought stressed plant leaves. The increase in red/far-red

fluorescence ratio with stress is due to the variation in chlorophyll content and can be related to the optical properties of the leaf chlorophyll (Van Wittenberghe et al. 2015). Portions of the stressed plant leaf that gets affected by stress starts showing symptoms of yellowing (senescence). These leaves contain less amount of chlorophyll and hence, reflect more light and have a higher fluorescence ratio value as compared to healthy plant leaves. The increase in fluorescence ratio intensity could be related to the inhibition of photosynthetic activity due to stress and

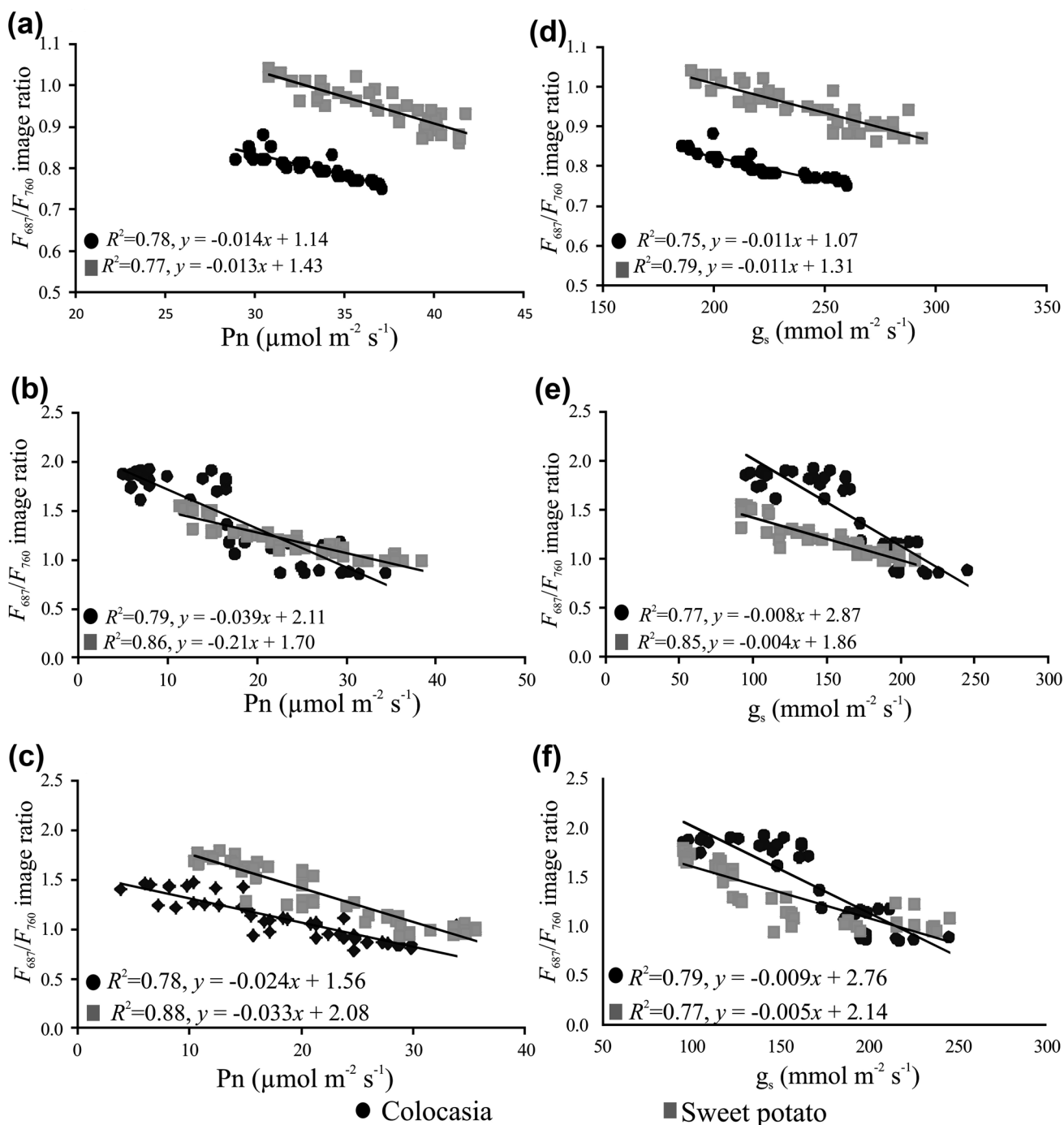


Fig. 5 Relationships between F_{687}/F_{760} image ratio with **a** net photosynthetic rate (Pn) for control plants, **b** Pn for herbicide treated plants, **c** Pn for drought stressed plants, **d** stomatal conductance (g_s) for control plants, **e** g_s for herbicide treated plants and **f** g_s for drought stressed plants

hence, the impinging light energy is not efficiently utilized for photosynthetic activities. Partial reabsorption of the emitted red chlorophyll fluorescence F_{684} occurs under normal conditions while under stressed conditions, there is loss of chlorophyll, which decreases the amount of reabsorption of 684 nm fluorescence (Buschmann and Lichtenthaler 1998). As the total chlorophyll content increases,

the F_{684} intensity maximum decreases, whereas intensity of F_{735} remains almost unaffected. Consequently, the red/far red fluorescence ratio becomes an inverse indicator of the total leaf chlorophyll content of the leaves (Rinderle and Lichtenthaler 1988; Freedman et al. 2002). The red/far-red fluorescence ratio F_{690}/F_{735} is proved to be linearly proportional to the chlorophyll content and hence could be

used as one of the best indicators of chlorophyll content in leaves (Gitelson et al. 1999). With increase in applied stress, chlorosis also increases, which results in higher F_{690}/F_{740} fluorescence ratio (Lichtenthaler 1987). Increase in red/far-red fluorescence indicates lower chlorophyll content and reduction in photosynthetic activity (Ndao et al. 2005). The decrease in chlorophyll content in leaves may be due to a decrease in the rate of chlorophyll accumulation due to stress factors.

The increasing loss in photosynthesis has been observed in herbicide and drought stressed plants as compared to the control set of plants. In drought stressed plants, decrease in Pn could be attributed to the closure of the stomata, which reduces the amount of carbon dioxide necessary for photosynthesis (Subhash et al. 2004; Reddy et al. 2004; Ibaraki et al. 2010). Stomatal closure in response to water deficit stress leads to limitation of carbon uptake by the leaves (Chaves 1991; Cornic and Massacci 1996). Studies have shown that in field grown plants, with the progress of drought, stomatal closure occurs and consequently, reduced stomatal conductance is observed (Tenhunen et al. 1987). In drought stressed plants, reduction in g_s occurs, which eventually results in reduction of photosynthesis (Yordanov et al. 2003). Studies on relationship between photosynthesis and water stress has reported that the reduction in photosynthetic responses in water stressed plants is associated with lower stomatal conductance (Vitale et al. 2012).

Reduction in net photosynthetic rate in herbicide treated plants could be due to the fact that herbicide strongly induces inhibition of photosynthetic activity in plants, which adversely affects the photosynthetic mechanism (Bigot et al. 2007). On applying herbicide Glyphosate considerable amount of stomatal and epidermal damage occurs, which leads to reduction in photosynthetic activity of the plants (Huang et al. 2012). High rate of net photosynthesis is attributed to high stomatal conductance, whereas, low stomatal conductance impairs plant photosynthetic productivity (Niinemets et al. 2009). It is seen from Table 1a, b that the percentage variance of F_{687}/F_{760} image ratio and F_{684}/F_{735} spectral ratio increased within days of herbicide and drought stress treatment in both colocasia and sweet potato plants, indicating increase of the ratio values in stressed conditions as compared to control values. Whereas, percentage variance of Pn and g_s with control showed negative variance indicating decrease in values under stressed conditions. The application of herbicide and drought stress has impaired the photosynthetic mechanism and stomatal conductance, which is evident from the corresponding values, and consequently increased the red/far-red fluorescence ratio values (Bigot et al. 2007). Since the study focuses on detection of herbicide and drought stress in field grown plants using

red/far-red fluorescence image ratio, less significance was given on difference between observed values among the two plant groups.

Conclusion

In this study, we have investigated the relationship of chlorophyll fluorescence image ratio F_{687}/F_{760} with Pn and g_s in herbicide and drought stressed colocasia and sweet potato plants. The correlation coefficient of F_{687}/F_{760} fluorescence image ratio with Pn and g_s in healthy and stressed leaves was found to be statistically significant with high R^2 values. F_{687}/F_{760} ratio yielded R^2 values of 0.93, 0.96 and 0.94 with Pn and values of 0.79, 0.89 and 0.92 with g_s in control, herbicide and drought treated colocasia plants, respectively. A similar trend was observed in sweet potato also, with R^2 values of 0.84, 0.87 and 0.93 for correlation of F_{687}/F_{760} ratio with Pn and R^2 values of 0.80, 0.90 and 0.81 for F_{687}/F_{760} with g_s in control, herbicide and drought treated colocasia plants, respectively. Our results reflect that with the increase in stress, plant photosynthetic parameters like net photosynthesis and stomatal conductance decreases and consequently, the red/far-red fluorescence ratio increases.

This study was carried out as an extension of our previous work on proximal monitoring of cassava mosaic virus infection on potted cassava plants. In this experiment, we have used SICF imaging system for early detection of herbicide and drought impact on field grown colocasia and sweet potato plants. The imaging system was placed at a height of 15 m above the ground level and at a distance of 20 m from the plot to record sunlight-induced Chl fluorescence images of plant leaves and extract the chlorophyll fluorescence emission from the reflectance signal using the FLD technique. Results obtained in this study on herbicide and water stressed colocasia and sweet potato plants grown under field conditions suggest that F_{687}/F_{759} fluorescence image ratio determined using SICF could be effectively used to monitor various types of stresses in other crop plants cultivated in different environments. With increase in distance between the sensor and target plants, this technique allows for a larger scale monitoring of vast areas of vegetation. The scope of the study can be extended further to applications like assessment of vegetation health using sensors set up in a helicam or drones, which could be controlled remotely from a ground station, thus facilitating continuous monitoring of stress-prone vegetation on demand basis.

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