A cheap chlorophyll *a* fluorescence imaging system

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Abstract

A cheap chlorophyll (Chl) *a* fluorescence imaging system was developed for measuring leaf areas of 30×45 cm. Uniform saturating irradiances were created using $CuSO_4$ filtered radiation from stroboscopes. The system was tested using maize leaves treated with diuron. Comparison was made with a small-area-measuring pulse amplified modulation Chl fluorometer.

Additional key words: diuron; *Zea mays*.

Introduction

Chlorophyll (Chl) *a* fluorescence has widely been used as a fast measuring method to assess stress and the photosynthetic properties of green plants. Research is often done with Chl fluorometers measuring leaf areas of approx. 1 cm^2 (see reviews of Horton and Bowyer 1990, Krause and Weis 1991, Mohammed *et al.* 1995, DeEll *et al.* 1999). Due to the variation of photosynthesis within a leaf (developmental and environmental factors - Rolfe and Scholes 1995, Šesták and Šiffel 1997), a lot of replications of the measurements has to be done before a Chl fluorescence parameter is sufficiently stable to detect differences between treatments. To overcome this problem, a camera can be used to take Chl fluorescence pictures. Björn and Forsberg (1979) already used a camera to image delayed light emission for detecting damage to the photosynthetic system. Several others followed, measuring prompt Chl *a* fluorescence and multi-colour fluorescence (Omasa *et al.* 1987, Daley *et al.* 1989, Balachandran *et al.* 1994, Lang *et al.* 1994, Rolfe and Scholes 1995, Lichtenthaler *et al.* 1996, Scholes and Rolfe 1996, Lichtenthaler and Miehé 1997, Buschmann and Lichtenthaler 1998) and using image analysis systems for measuring Chl *a* fluorescence for small or larger leaf areas. The aim was to detect the heterogeneity of Chl fluorescence in the leaf or to measure the fluorescence of a large leaf area. The limiting factor is always the amount and distribution of radiation for the Chl fluorescence measurement. The leaf area to be measured should be homogeneously irradiated and saturated with radiant energy. This is feasible for small leaf areas. Small radiation sources can easily be placed close to the measuring places. For measuring larger areas, a camera should be placed further away from the object. This also implements a greater distance between radiation source and object. To overcome this problem, expensive lasers are used (Lang *et al.* 1994, Lichtenthaler and Miehé 1997, Buschmann and Lichtenthaler 1998). Wenzel and Lichtenthaler (1998) used a Xenon flashing lamp instead of a Nd-Yad laser as a UV-containing radiation source. This paper describes the possibilities of using stroboscopes as a cheap alternative for measuring Chl *a* fluorescence of large leaf areas.

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Abbreviations: Chl: chlorophyll; F_0: initial level of Chl fluorescence; F_m: maximum level of Chl fluorescence in a dark-adapted leaf; F_v: variable fluorescence ($F_m - F_0$).

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Materials and methods

Chl fluorescence imaging: Measurements took place in a light-tight box (180×90×70 cm), painted white at the inside. This was done to insure reflection of the radiation and thus creating its more uniform distribution.

The irradiance (1.6 μmol m^{-2} s^{-1}) for the F_0 measurement was provided by a standard circular fluorescent lamp (*L22w/25c, Osram*, USA). Saturating irradiance was provided by 4 stroboscopes (*T.K.O. Strobe, Geni Electronics*, Taiwan) with a power output of 2 000 W each. Both radiation sources were filtered using a double clear glass plate filled with a saturated $CuSO_4$ solution in water. This ensured that only radiation up to 560 nm was used to excite the sample. Each saturation pulse reached 3 000 μmol m^{-2} s^{-1} for 7 ms at a flash rate of 12 flashes per s synchronised with the camera. This was measured using a PAR-sensor (Quantum sensor, *LI-COR*, USA) connected to an osciloscope (*Hungchang*, Taiwan). The irradiance was measured at a distance of 150 cm from the lamps. This was also the distance used for measuring the leaf samples.

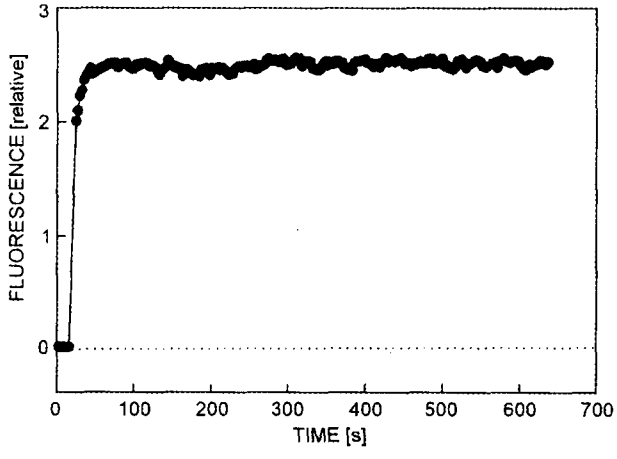

Fig. 1. Development of chlorophyll *a* fluorescence during the F_0 measurement. No Chl fluorescence induction kinetic is observed, indicating a true F_0 is measured.

Chl fluorescence was detected using a monochrome *CCD* camera (*Pulnix TM-765E*, Pulnix, USA). The lens (*Cosmicar Television Lens*, USA) used had a focal distance 12.5 mm (1.0 : 1.4). At a distance of 100 cm, the measuring area was 30×45 cm. The camera was placed below the $CuSO_4$ filter and was equipped with a 700 nm high pass filter (*40.5 E 092, B&W*, Germany). Thus we only measured the far-red fluorescence (730-740 nm). The image acquisition, camera, light control, and processing system was based around an *IBM* compatible personal computer running *Windows NT® 4.0* and *WiT® 5.2* image analysis software (*LogicalVision*, USA). A *Frame Grabber* card (*DT3155, Datatranslations*, USA) was used to grab the image from the camera. Measurements were automated using software written in *WiT®*. Images were taken at 760×575 pixels and an 8-bit resolution resulting in 437 000 individual Chl fluorescence measurements.

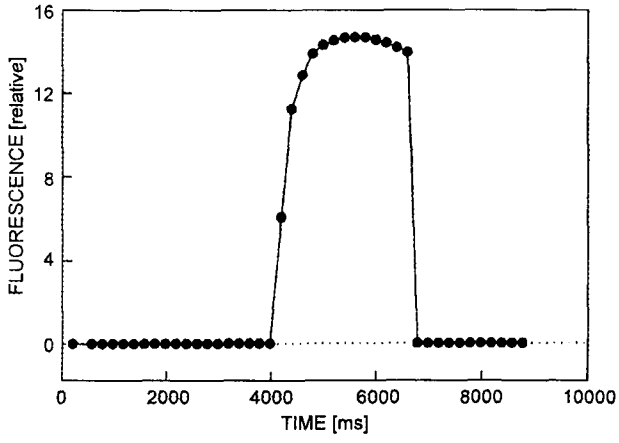

Fig. 2. Development of the chlorophyll *a* fluorescence during the F_m measurement. After 4 000 ms, the flashes were turned on. 1 600 ms later the F_m was reached. After 6 770 ms, the flashes were stopped.

At first, the F_0 was measured using the internal multiple frame storage mode of the camera. In our configuration, up to 64 images can be stored in the *CCD* and outputted as one frame. This enables to visualise the weak F_0 signal of the measured object. Then the camera is put in single frame mode for measuring F_m. The stroboscopes are activated. After saturation (approx. 1.6 s), the F_m image is captured. The measurement of F_0 was tested on its stability (Fig. 1). 20 s after turning on the light, a stable F_0 value was measured. The weak irradiance used for measuring F_0 did not induce a Chl fluorescence induction curve. A test was done to measure the time needed to saturate the maize leaf with radiant energy (Fig. 2). After 4 s, the saturation pulses started. It took about 1600 ms to reach the maximum value (F_m). The fluorescence value remained within the 95 % range of F_m until 1000 ms after F_m has been reached. The uniformity of radiation for the F_0 measurement and for the saturation flashes was tested by placing a blue plastic fluorescence standard (*Walz*, Effeltrich, Germany) systematically at different places in the measuring field. A maximal difference of 4.7 % was found. This difference was assumed to be negligible. Chl fluorescence was measured at the adaxial side of the leaf.

Application of diuron: The photosynthetic herbicide diuron (*Hermoo Diuron 800SC, Hermoo Belgium*, Belgium), which binds to the Q_B-protein, was applied at a concentration of 10 kg $m^{-3}(H_2O)$ (= 34.3 mM diuron) at the abaxial leaf side.

Plants: Maize (*Zea mays* L.) cv. Husar was grown outside on a sandy loam soil at the Department of Crop Husbandry and Ecophysiology of the Agricultural Research Centre nearby Gent, Belgium. Seeds were sown on 3 May, 1999. Mature leaves were harvested on 27 July, 1999 just before the measurements started.

Measurements of Chl fluorescence using a modulated fluorometer: As a reference, Chl *a* fluorescence (F_v/F_m) was measured (n = 15) at the adaxial leaf side with a modulated Chl fluorometer (*PAM-2000, Walz*, Germany), 30 min after the application of diuron.

Results

The development of F_0 due to the diuron application was compared with that of a non-treated leaf (Fig. 3). After 4 min, the effect of diuron was visible by the Chl *a* fluorescence image. The fluorescence image taken 30 min after application showed a strong difference between the treated and the control areas: the average F_v/F_m values for these areas were 0.464 and 0.768, respectively. Average F_v/F_m values measured as a reference using the *PAM-2000* were 0.594 and 0.791 for the same areas. Thus, both results corresponded. Minimum F_v/F_m values measured with the imaging system (lowest pixel value) and the *PAM-2000* (lowest value of 15 measurements) were 0.270 and 0.447, respectively. This indicates a larger dynamic range for the imaging system. The advantage of the image was that all the variation within the selected area, which was due to heterogeneity of the application of diuron or leaf inherent differences, was visualised and could be accounted for. If not enough samples are taken with a traditional (small area) *PAM* Chl fluorometer, the total variation present may not be detected.

Fig. 3. Chlorophyll fluorescence images of F_0 (*from left to right*: at 0, 4, and 30 min) from the adaxial side of two maize leaves. The lower half of the lower leaf was treated with 34.3 mM diuron. The diuron effect was detectable about 4 min after application.

Discussion

With our construction we are able to measure the far-red Chl *a* fluorescence in a cheap way. In comparison with the system described by Wenzel and Lichtenthaler (1998), we only measure the far-red fluorescence above 700 nm. Our system measures areas of 30×45 cm, which enables a measurement of many treatments and leaf samples at the same time. Both systems are non-invasive and are more precise than values obtained by conventional fluorometers.

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