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Remote detection of light tolerance in Basil through frequency and transient analysis of light induced fluorescence

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ABSTRACT

Artificial lighting control in industrial scale greenhouses has a large potential for increased crop yields, energy savings and timing in greenhouse production. One key component in controlling greenhouse lighting is continuous and accurate measurement of plant performance. This paper presents a novel concept for remote detection of plant performance based on the dynamics of chlorophyll fluorescence (CF) signals induced by a LED-lamp. The dynamic properties of the CF is studied through fitting a linear dynamic model to CF data. The hypothesis is that changes in photochemistry affects the fluorescence dynamics and can therefore be detected as changes in the model parameters and properties. The dynamics was studied in experiments using a sinusoidal varying light intensity (period 60 s) or step changes (step length 300 s). Experiments were performed in a controlled light environment on Basil plants acclimated to different light intensities. It is concluded that the capacity to use a certain light intensity is reflected by how fast and how complex the dynamics are. In particular, the results show that optimal model order is a potential indicator of light tolerance in plants that could be a valuable feedback signal for lighting control in greenhouses.

1. Introduction

Chlorophyll fluorescence (CF) is a widely used non-invasive probe of photosynthesis. The close relationship between CF and photosynthetic performance has made it an indispensable tool in studying photosynthesis in a wide range of applications on-leaf as well as remotely from satellites (Murchie and Lawson, 2013; Porcar-Castell et al., 2014). From lab-scale to ecosystem-level CF is used to detect stress caused by various types of stressors such as drought, nutrient deficiency, pests and excess light (Baker and Rosenqvist, 2004). Although at present not widely used in greenhouses, the potential of using CF for optimisation of greenhouse production has been pointed out by, for example, Baker and Rosenqvist (2004).

Photosynthesis in plants is driven by light energy absorbed by chlorophyll molecules within the photosynthetic apparatus. The absorbed light can be used to (i) drive photosynthesis (photochemistry), (ii) be dissipated as heat or (iii) be re-emitted as light (CF). These three processes compete for the absorbed light quanta and hence, fluorescence emission indirectly contains information about the quantum efficiency of photochemistry and heat dissipation (Murchie and Lawson, 2013). The heat dissipation is a protective mechanism induced in conditions when the excitation pressure on photosystem II (PSII) exceeds the rates of electron transport and carbon fixation. Thus, at low light conditions, the probability of fluorescence is negatively correlated with the probability of photochemistry, while at high light conditions it is turned into a positive correlation due to an increased probability of heat dissipation (van der Tol et al., 2009). The actual yields of photochemistry and heat dissipation, respectively, are generally sorted out from the fluorescence signal by so called fluorescence quenching analysis, described below.

CF has become a useful tool for studying photosynthesis mainly due to the invention of the pulse amplitude modulation fluorometer (PAM), which has the ability of extracting the yield of fluorescence (i.e., the probability of fluorescence) from noisy data in presence of background light (Schreiber, 2004). The PAM measures the CF response to short duration (micro seconds) light pulses and discriminates between CF induced by the excitation pulse from CF induced by ambient light through synchronous detection. The excitation light pulse is so weak that photochemistry and heat dissipation

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are considered unaffected during the pulse and, consequently, the yield of fluorescence can be determined by dividing the amplitude of the induced CF with the amplitude of the excitation pulse (Schreiber, 2004). Since the system is considered constant under the short interval of measurement, the frequency of modulation of the excitation light source does not affect the response (more than through increasing the integral of the excitation light). By measuring the fluorescence yield at different light intensities, (Fm) at short duration saturating light that transiently eliminate the photochemical yield, and (Fo) at extremely low light intensities where the photochemical yield is maximal, and comparing these through quotients, the rate constants for photochemistry, heat dissipation and fluorescence are sorted out and quantified (Govindjee, 2004). By doing so at different physiological conditions, such as dark adapted, light adapted or conditions caused by different types of stress, changes in heat dissipation and changes in photochemistry can be detected.

The PAM-technology has found numerous applications. Although best suited for on-leaf application, the PAM methodology has been adapted to remote sensing at different distances by using lasers for the fluorescence induction (Ounis et al., 2001; Moya and Cerovic, 2004). In remote sensing applications the main problem is to generate a saturating light for the measurement of Fm. Dark adaption for measuring the Fo level is another problem. In these applications, though, tracking the development of the steady state fluorescence yield has proven useful (Evain et al., 2004). The PAM technology has also been implemented in imaging systems for CF detection (Nedbal et al., 2000). These systems have found their use mainly for phenotyping (Gorbe and Calatayud, 2012). The PAM has also been modified for the use in commercial greenhouses. Schapendonk et al. (2006) have, with a modified PAM-equipment mounted fairly close to the plants, shown that fluorescence signals can be used for optimisation of light intensity in a greenhouse crop.

Photochemistry can also be studied based on the characteristic inflection points of the CF transient in dark-adapted leaves that are illuminated with continuous light. The inflection points on this transient are labelled OJIPSMT. During the initial fast phase of the induction curve (OJIP) chlorophyll fluorescence rises from the initial low origin level O, via the intermediate inflections J and I, to a peak level P in about 1 s, which is considered to reflect the successive reduction of the electron acceptor pool of PSII. During the subsequent slow phase (PSMT), the fluorescence declines to a terminal steady-state level T in the time-scale of minutes. The decline is often accompanied by a local, semi steady-state, minimum S and a local maximum M, and sometimes more than one pair of S and M inflections (oscillations) can be observed (Walker, 1992; Walker et al., 1983). The slow phase is more difficult to interpret as many different processes linked to photochemical quenching (qP) and non-photochemical quenching (NPQ) are known to influence the signal. The fast phase of this transient (OJIP) has been used for detecting drought in tomato plants remotely in a greenhouse by Takayama et al. (2011). However, their method requires dark adaption, and can thus only be used at night and not in daylight.

The aim with our research is ultimately to achieve an automatic and closed loop control of lighting in greenhouses, based on plant performance estimated from remotely sensed CF. For this we are aiming at a method for continuous estimation of plant health at daytime in a greenhouse. We propose a method that employs the dynamic properties of the remotely sensed light induced CF-signal. The dynamic properties of the CF is studied through the parameters in a linear dynamic model fitted to CF data. The hypothesis is that changes in photochemistry affects the fluorescence dynamics and can be detected as changes in the model parameters. Practically, we suggest this technique to be integrated into an advanced LED-lamp, where the LED-lamp induces fluorescence that is measured by a sensor in, or attached to the lamp. The sensor receives CF from the whole region of the plant canopy illuminated by the lamp, hence giving an aggregated and representative measure that could be used for feedback control of the LED lamp.

In the present work two different approaches to the study of CF dynamics are explored, namely frequency analysis and transient analysis. In the frequency analysis the fluorescence response to a sinusoidal varying light (period 60 s) was studied through linear analysis of the gain and phase shift. The frequency analysis has some similarities to analysis performed with a PAM. Similarly to the fluorescence yield measured by a PAM, the fluorescence gain discussed here is the quotient between the amplitude of the excited fluorescence divided by the amplitude of the excitation signal. However there are, two major differences between fluorescence yield and fluorescence gain. Fluorescence yield is excited by high frequency pulses of low amplitude, whereas fluorescence gain measured here is excited by low frequency harmonic modulations of high amplitude. The PAM method is assumed to maintain constant conditions under measurement (due to the weak excitation) such that no dynamics are exhibited in the fluorescence response, while the latter method do exhibit dynamics governing the gain as well as the phase. Furthermore, the dynamics imply that the frequency of modulation affects these values.

The dependency of the frequency of modulation is further explored through models of CF tranients. In the transient analysis the CF response to a step change in light intensity (step length 300 s) was analysed in terms of linear dynamic parametric black box models fitted to data. The analysis of the step responses constitutes a dynamic analysis of the PSMT transient in light adapted leaves.

Here we present some initial results based on light stress induction and recovery experiments performed on Basil plants acclimated to low, intermediate and high light. One of the key findings was that the intensity of the ambient light, in relation to the light intensity that the plants were acclimated to, determined how fast the plants responded to the step changes. Hence, light intensity shifted the plants' dynamic behaviour in the frequency domain. Even more interesting was that the complexity of the dynamics was decreased upon increased light intensity above the light intensity of acclimation. The complexity of the dynamics was also affected by light induced stress. The mechanisms behind these observations have the character of a buffer system with feedback, where the buffers are likely metabolite pools. These results were obtained from the analysis of black-box models of step responses. Interestingly, these results were also in agreement with the gain and phase shifts estimated from the experiments with sinusoidally varying light.

2. Materials and methods

2.1. Plant material and growth conditions

Ocimum basilicum (sweet basil cv. Nufar) was grown in five different growth units under different light settings. In three of the growth units the plants were grown under L4AS1 LED-lamps (Heliospectra, Sweden) with incident light intensity set to 80, 250 and 500 μmol photons m^{-2} s^{-1} respectively within PAR and with the spectral distribution presented in Fig. 1. In the two remaining growth units plants were grown under HPS-lamps (spectrum shown in Fig. 1) with the light intensity set to 80 and 500 μmol photons m^{-2} s^{-1} within PAR. These light intensities and spectral distributions were measured with a calibrated JAZ spectrometer (Ocean Optics, US) without plants in the units. The photoperiod was 16 h.