Early detection of drought stress in tomato plants with chlorophyll fluorescence imaging –practical application of the speaking plant approach in a greenhouse–


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Abstract: The chlorophyll fluorescence imaging technique is useful for evaluating photosynthetic functions of plants without actually touching the plant. In our previous study, we developed a chlorophyll fluorescence imaging system for tomato plants cultivated in greenhouses. This imaging system measures the chlorophyll fluorescence induction phenomenon, a dynamic change in chlorophyll fluorescence intensity induced by illuminating a dark-adapted leaf with a stable intensity excitation light, and analyzes the shape of the induction curve, i.e., the temporal course of chlorophyll fluorescence intensity during this phenomenon. The shape of the induction curve is characterized by an initial maximum peak (P), subsequent transient dip (S), and secondary small peak (M). We defined an index, the photosynthetic function index (PFI; fluorescence intensity of P divided by the average fluorescence intensity from S to M), to evaluate the shape of the induction curve. In this study, we applied this system to detect drought stress in tomato plants cultivated in a semi-commercial greenhouse. PFI was clearly lower in stressed plants than in healthy plants. The decreased PFI in stressed plants is probably attributable to photosynthetic dysfunction in these plants.

Keywords: image sensors; diagnosis; plants; photosynthesis; speaking plant approach

1. INTRODUCTION

As a highly sophisticated strategy for environmental control in greenhouses, the concept of the speaking plant approach (SPA) has attracted a great deal of attention. Originally, the SPA indicated that optimal crop cultivation conditions should be based on the physiological status of the plants (Udink ten Cate 1978; Hashimoto 1980, 1989). The first and most important step in the SPA is to obtain physiological information from a living plant and then to judge whether the plant is healthy. Various imaging techniques have been intensively investigated for this purpose.

Chlorophyll fluorescence imaging, originally developed by Omasa et al. (1987) and Daley et al. (1989), has been used to evaluate the heterogeneous distribution of photosynthetic activities over a leaf surface and has thus been applied to the detection of photosynthetic dysfunctions caused by biotic and abiotic stress factors (Omasa and Takayama, 2003; Takayama et al., 2003). However, most studies have utilized the chlorophyll fluorescence imaging technique for detailed analysis of photosynthetic function from the perspective of plant physiology.

In order to use the chlorophyll fluorescence imaging technique as a diagnostic tool in practical situations of agricultural production, we developed a chlorophyll fluorescence induction imaging system for the tomato canopy in our previous study (Takayama et al., 2010). In the present study, we applied this system for early detection of drought stress in full-size tomato plants cultivated in a semi-commercial greenhouse.

2. MATERIALS AND METHODS

2.1 Chlorophyll Fluorescence Induction Imaging for Plant Diagnosis

Chlorophyll Fluorescence Emission

Figure 1 shows a schematic of light energy distribution in chlorophyll a pigment, representing the main photosynthetic pigment in green leaves.

[Diagram of energy distribution in chlorophyll a pigment]
Plant leaves utilize light energy absorbed by photosynthetic pigments for photosynthetic photochemical reactions. However, plant leaves cannot use all the absorbed light energy for photosynthetic reactions. The residual light energy not used for photosynthetic reactions is dissipated as heat or re-emitted as red light. This red light emission is the chlorophyll fluorescence (Krause and Weis, 1991). Chlorophyll fluorescence intensity thus varies with the photosynthetic reactions and heat dissipation process, even if the excitation light intensity is stable. Accurate measurement of chlorophyll fluorescence emission thus allows the evaluation of photosynthetic functions, as both photosynthetic photochemical reactions and the status of heat dissipation processes, without any need for physical contact with the plant.

Chlorophyll Fluorescence Induction Phenomenon

As mentioned above, chlorophyll fluorescence intensity varies with the photosynthetic reactions and heat dissipation process under stable light intensity conditions. By illuminating a dark-adapted leaf with a stable intensity excitation light, dynamic changes in chlorophyll fluorescence intensity are observed. This is called the “chlorophyll fluorescence induction phenomenon” (Govindjee, 1995; Omasa and Takayama, 2002). The temporal course of chlorophyll fluorescence intensity during the induction phenomenon, as plotted along a logarithmic time axis, is called the “chlorophyll fluorescence induction curve”. Figure 2 shows a schematic of a typical chlorophyll fluorescence induction curve. The typical induction curve has characteristic inflection points of I, D, P, S, M and T (Govindjee, 1995; Omasa and Takayama, 2002). The fast phase (from I to P) takes a few seconds after the commencement of light excitation. In general, the fast phase is closely related to the photochemical reaction of photosystem (PS)II (Krause and Weis, 1991; Govindjee, 1995). In contrast, the slow phase (from P to T) requires a couple of minute and varies depending on various reactions, including both photochemical and non-photochemical reactions (e.g., heat dissipation processes such as the xanthophyll cycle) (Krause and Weis, 1991).

**Definition of Photosynthetic Function Index**

The shape of the induction curve varies depending on the status of photosynthetic function (Govindjee, 1995; Omasa and Takayama, 2002; Takayama et al., 2009). In most cases, the induction curve of a photosynthetically dysfunctional plant becomes relatively flat (Govindjee, 1995). To evaluate the shape of induction curve, we defined the photosynthetic function index (PFI) as (1):

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PFI = \frac{[P]}{Ave([S]-[M])}
\]

where \([P]\) is the maximum chlorophyll fluorescence intensity (i.e., chlorophyll fluorescence intensity at inflection point P), \(Ave([S]-[M])\) is the average chlorophyll fluorescence intensity between inflection points S and M (Fig. 2). The time points of these inflection points (P, S and M) vary depending on cultivars and growing seasons. PFI is low when the induction curve is relatively flat.

Chlorophyll Fluorescence Induction Imaging System

Figure 3A shows a schematic of the chlorophyll fluorescence induction imaging system for tomato plants cultivated in a greenhouse. Figure 3B shows the imaging system in a greenhouse. A 60 × 60 cm blue light-emitting diode (LED) panel (SE-LP60; Senecom, Kawaguchi, Japan) provides excitation light (\(\lambda < 520 \text{ nm}\)) irradiating the leaves around the shoot apex of the tomato plant. The duration of excitation light irradiation was set at 30 s. The photosynthetic photon flux density (PPFD) of the excitation light at the plant surface ranged from 30 to 100 \(\mu\text{mol m}^{-2}\text{s}^{-1}\).

**Fig. 2. Schematic of a typical chlorophyll fluorescence induction curve.**

**Fig. 3. The chlorophyll fluorescence imaging system for full-size tomato plants in a greenhouse (A, schematic; B, photo).**
A charge coupled device (CCD) camera (Stingray F145B ASG; Allied Vision Technologies, Stadtroda, Germany) with a long-pass filter (λ >640 nm, SC 66; Fujifilm, Tokyo, Japan), fixed in the middle of the LED panel, captured the chlorophyll fluorescence emission induced by the excitation light. The imaging system was about 0.6 m from the target plant. Temporal resolution of the imaging was 15 fps and the captured image was recorded onto the hard disk of a laptop computer in BMP format at 1024 (W) × 768 (H) pixels. Captured images were analyzed using Visual Basic 6.0 software we developed in-house. This software selected an image of the maximum chlorophyll fluorescence intensity from the 450 captured images and determined the plant area. Average pixel values within the determined plant area were calculated for all captured images and a chlorophyll fluorescence induction curve was made. All imaging was performed under dark conditions at night.

2.2 Plant Materials and Drought Stress Treatment

Tomato (*Lycopersicon esculentum* Mill. ‘TY Momotaro Sakura’) seeds were sown in spongy material on September 10, 2009, and transplanted into rockwool cubes (10 cm [W] × 10 cm [H] × 5 cm [D]) after germination, then the rockwool cubes were set onto rockwool slabs (100 cm [W] × 20 cm [H] × 5 cm [D]) on October 19, 2009, in a semi-commercial greenhouse in the Faculty of Agriculture at Ehime University. Four rockwool cubes were set on a rockwool slab at 0.25-m intervals, with each cube supplied nutrient water (A-type recipe, Otsuka House Solution; Otsuka Chemical, Osaka, Japan) through a dripper. In the greenhouse, 1000 tomato plants were cultivated on 12 cultivation gutters, aligned in parallel along the North-South axis, in a 400 m² (20 × 20 m) cultivation area. About 80 tomato plants were grown on each cultivation gutter. The distance between two adjacent cultivation gutters was 1.6 m. Plants were grown for 10 weeks before the experiment and professional growers performed all crop management.

Four healthy tomato plants grown on different rockwool slabs were used for the experiment. Two plants (Stress-1 and Stress-2) were exposed to drought stress by stopping nutrient water supply to the rockwool slabs for 17 days. Drought stress treatment was started on January 2, 2010. The other two plants (Control-1 and Control-2) were cultivated normally during the 17 days as controls. Total stem length of the tomato plants was about 2.5 m and the height of the leaf layer was about 1.5 m at the time of the experiment.

2.3 Measuring Parameters and Procedures

**Chlorophyll fluorescence induction imaging for PFI**

Using the chlorophyll fluorescence induction imaging system (Fig. 3), chlorophyll fluorescence imaging of leaves around shoot apexes of the four experimental plants was conducted under dark conditions at night, from 20:00 to 22:00, on days 1 and 17 of drought stress treatment. PFIs were calculated from the measured chlorophyll fluorescence induction curves.

**Visual appearance of tomato plant**

To monitor changes in the visual appearance of tomato plants, photographs of shoot apexes of the plants were taken on days 1 and 17 of drought stress treatment using a digital still camera.

**Water content of rockwool slab**

Water content of the rockwool slabs was measured days 1 and 17 of drought stress treatment, using a water content meter (WCM-control; Grodan, Roermond, The Netherlands). Water content was measured at five different sites on a rockwool slab and the average value was calculated.

**Leaf water potential**

Leaf water potential is one of the most general numerical indices to evaluate the water status of plants (Boyer, 1995). The leaf water potential of healthy and non-stressed tomato plants cultivated in greenhouse ranges from -0.3 MPa to -0.7 MPa. Leaf water potential decreases when the plant is exposed to drought stress. In this experiment, five single leaflets were sampled from leaves around the shoot apex of each experimental plant and water potential was measured using a pressure chamber (PMS Instrument Company, Albany, USA) on days 1 and 17 of drought stress treatment.

3. RESULTS AND DISCUSSION

3.1 Chlorophyll Fluorescence Induction Imaging and Determination of Inflection Points

Figure 4 shows a set of chlorophyll fluorescence intensity images at the inflection points I-D, P and S-M and the resulting chlorophyll fluorescence induction curve.
Inflection points I-D and P were clearly recognized in the induction curve, but inflection points S and M were unclear because the decrease in chlorophyll fluorescence intensity from P to S-M was very gentle. Such a chlorophyll fluorescence induction curve, with no clear inflection points for S and M, is usually observed in tomato plants during winter. The time points corresponding to inflection points S and M were thus manually determined and set at 13 s and 23 s after commencing irradiation with the excitation light, respectively. Average chlorophyll fluorescence intensity between 13 and 23 s after commencement of irradiation was calculated and substituted into Equation 1.

3.2 Changes in Visual Appearance of Tomato Plants

Figure 5 shows control (Control-1 and Control-2) and stressed (Stress-1 and Stress-2) plants on days 1 and 17 of drought stress treatment. No obvious changes in visual appearances were seen in control plants during the 17 days. Conversely, Stress-1 showed slight wilting and Stress-2 showed severe wilting. Wilting in both plants was obvious but proved reversible when water supply was restarted.

3.3 Changes in Water Content of Rockwool Slabs

Figure 6 shows the water content of rockwool slabs on days 1 and 17 of drought stress treatment. At the beginning of drought stress treatment, water contents of all rockwool slabs were within 60-80%. However, slab water content of Stress-1 and Stress-2 decreased to <5% by day 17 of drought stress treatment. This means that little nutrient water was present in the slabs of stressed plants after stopping water supply for 17 days.

3.4 Changes in Leaf Water Potential

Figure 7 shows leaf water potential for control (Control-1 and Control-2) and stressed (Stress-1 and Stress-2) plants on days 1 and 17 of drought stress treatment. Leaf water potential of the four plants ranged from -0.7 MPa to -0.5 MPa, with no significant difference between control and treated plants at the beginning of drought stress treatment. The leaf water potential of stressed plants had significantly decreased to -1.3 MPa by day 17 of drought stress treatment, although the leaf water potential of control plants remained about -0.4 MPa. This result shows that Stress-1 and Stress-2 were exposed to drought stress caused by a lack of water supply for 17 days.

Fig. 5. Changes in visual appearances of tomato plants.

Fig. 6. Changes in water content of rockwool slabs.

Fig. 7. Changes in leaf water potential.
3.5 Changes in shape of the chlorophyll fluorescence induction curve and PFI

Figure 8 shows chlorophyll fluorescence induction curves of control (Control-1 and Control-2) and stressed (Stress-1 and Stress-2) plants on days 1 and 17 of drought stress treatment. The chlorophyll fluorescence intensity of an induction curve was normalized to the maximum chlorophyll fluorescence intensity of the induction curve (P), in order to enhance the differences in shapes of the chlorophyll fluorescence induction curves. At the beginning of the drought stress treatment, shapes of all induction curves were similar, but a clear difference in the shapes of induction curves of control (Control-1 and Control-2) and stressed (Stress-1 and Stress-2) plants was recognized on day 17 of drought stress treatment. In particular, the inflection points of S-M for Stress-1 and Stress-2 were relatively higher, i.e., a small decline was seen from P to S-M, compared with those for Control-1 and Control-2 (arrow in Fig. 8).

The increase in PFIs of control plants was due to the extended decline from P to S-M on the induction curves. The decline from P to S-M is mainly caused by activation of heat dissipation processes such as the xanthophyll cycle (Krause and Weis, 1991). This result thus implies that the heat dissipation ability of leaves around the shoot apices of control plants increased during the 17 days of the experiment. Generally, the heat dissipation ability of tomato leaves is higher in summer compared with winter. This slight increase in PFIs of control plants might therefore be interpreted as a seasonal change in photosynthetic function of tomato plants in greenhouses. Conversely, PFIs of treated plants (Stress-1 and Stress-2) decreased to less than 1.1. This was due to the small decline from P to S-M on the induction curves, meaning that activation of heat dissipation processes was inhibited by drought stress. This result indicates that drought stress in tomato plants is detectable as a decrease in PFI. Of course, the wilting may have affected PFIs of treated plants. This possibility should be discussed in another study.

4. CONCLUSION

In this study, we applied a developed chlorophyll fluorescence induction imaging system, which was designed to evaluate photosynthetic functions of plants grown in greenhouses, for early detection of drought stress in tomato plants under practical tomato production greenhouse conditions. The imaging system successfully detected changes in photosynthetic function of drought-stressed plants as a decrease in PFI. This suggests that chlorophyll fluorescence induction imaging may represent a powerful tool for plant health monitoring in commercial greenhouses in the near future. In our research project at Ehime University, we have been developing an autonomous moving cart loaded with several sensors, including the chlorophyll fluorescence imaging system, for acquisition of plant physiological information to establish an integrated plant diagnosis for SPA-based agricultural production in greenhouses.
REFERENCES


