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Factors Affecting the Measurement of Chlorophyll a Fluorescence in Cucumber Leaves

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Abstract

Recently, the measurement of chlorophyll a fluorescence has been applied extensively to detect environmental stresses in plants. However, methods of measurement applicable to higher plants at room temperature have not been fully developed. The present study was conducted to analyze the factors affecting the measurement of chlorophyll a fluorescence in cucumber plants. The results suggested that higher values of Fv/Fm could be obtained by using lower intensities of actinic light. The light intensity irradiated on plants immediately before dark adaptation influenced the Fv/Fm values of chlorophyll a fluorescence. However, a period of 2 hr dark adaptation was long enough at a photosynthetic photon flux below 200 μmol/m^2/s. Plants grown at 25°C showed the largest value of Fv/Fm compared with those grown at 15 and 35°C. Since differences in plant growth temperature may affect the value of Fv/Fm, it is likely that the dark adaptation period required to obtain stable values of Fv/Fm depends on the light intensity during plant growth, and not on the temperature. Leaves with partial dark adaptation showed almost the same values as leaves with full dark adaptation. It appears that partial dark adaptation with an attachment for the fluorometer may enable to detect stresses in plants in open field under sunshine conditions.

Discipline: Horticulture/Experimental apparatus and methods
Additional key words: actinic light, dark adaptation, Fv/Fm

Introduction

To detect various kinds of stresses in plants, photosynthetic activity, plant growth and cell destruction have been investigated. Recently, the measurement of chlorophyll a fluorescence has been applied extensively as a rapid and non-destructive method to determine the effect of environmental stresses on the photosynthetic activity.

Chlorophyll a fluorescence is measured to analyze the changes in the fluorescence intensity referred to as chlorophyll a fluorescence induction or Kautsky effect. Fluorescence intensity from chlorophyll a shows the time course indicated in Fig. 1, which reflects the redox reactions of photosystem II (PSII), mainly regulated by the state of the primary electron acceptor of PSII (Q_A). Since environmental stresses can readily affect PSII, the measurement of chlorophyll a fluorescence was applied to the detection of stresses, such as low and high temperatures[1], water stress[3], salt excess[5], photoinhibition[4].

The measurement of chlorophyll a fluorescence is a useful tool to detect stresses in plants. However, the methods of measurement for

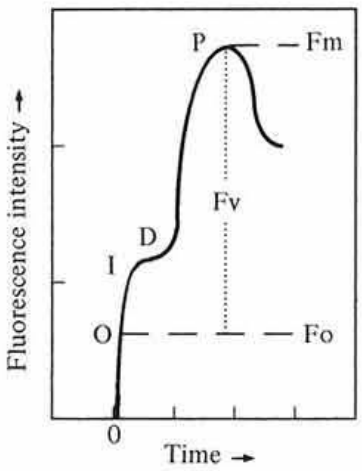

Fig. 1. Time course of chlorophyll a fluorescence induction
OI: Photoreduction of Q_A, ID: Reoxidation of Q_A by electron transfer, DP: Photoreduction of Q_A, Fo: Initial fluorescence from chlorophyll a molecules in the antennae of PSII, Fm: Maximum fluorescence, Fv: Variable part of the fluorescence.

higher plants at room temperature and analysis of fluorescence parameters have not yet been developed. In recent years, an attachment that keeps a small part of a leaf partially dark without the use of a dark room has become available commercially. It is thus necessary to determine the effectiveness of the attachment and develop a measuring method.

In this study, we investigated the following aspects: 1) effect of the growth temperature and light on the induction of chlorophyll a fluorescence, 2) effect of the intensity of actinic light on specific measurements and 3) effect of partial dark adaptation of a leaf on the measurement of induced chlorophyll a fluorescence using cucumber plants.

Materials and methods

Seeds of cucumbers (*Cucumis sativus* L. cv. Nankyoku No. 2) were sown in plastic pots on 6 February 1993. They were grown in a greenhouse at 23 ± 10°C.

Chlorophyll a fluorescence was measured with a fluorometer (CF-1000, Morgan Instruments, Andoval, USA) at room temperature. In the kinetics of induced chlorophyll a fluorescence there are various parameters for detecting stresses[2]. We used the ratio Fv/Fm (Fig. 1) that appears to be correlated linearly with the quantum yield of photosynthesis[6].

1) Exp. 1: Effect of actinic light intensity on Fv/Fm values

Seedlings that unfolded 2 leaves were transferred to a dark room on 23 February 1993. After the seedlings became adapted to darkness for 5 hr at room temperature, chlorophyll a fluorescence was measured using actinic light at intensities of 100, 120, 150, 200, 300, 400, 500, 600, 800 and 1,000 μmol/m^2/s for 3 sec. Chlorophyll a fluorescence was measured in the center of the first leaf except for the midrib. Samples consisting of 5 plants were used to measure the chlorophyll a fluorescence at each actinic light intensity.

2) Exp. 2: Effect of light intensity before dark adaptation on Fv/Fm values

Plants that unfolded 4 leaves were illuminated at light intensities of about 16 (low) and 200 μmol/m^2/s (high) for 4 hr and were transferred to a dark room on 3 March 1993. Plants treated with both light intensities were adapted to darkness for 1, 5, 30, 120 and 180 min. The center of the 2nd leaf was used for the measurement of chlorophyll a fluorescence except for the midrib at an actinic light intensity of 250 μmol/m^2/s for 3 sec. Five plants were used for the measurement of chlorophyll a fluorescence in each treatment.

3) Exp. 3: Effect of temperature before dark adaptation on Fv/Fm values

Plants that unfolded 5 leaves were transferred to the growth chambers at 15, 25 and 35°C in

light for 1 day. Conditions for measuring chlorophyll a fluorescence were the same as those described in Exp. 2.

4) Exp. 4: Possibility of partial dark adaptation

Leaves were subjected to partial dark adaptation (4 cm^2) for 3 hr with an attachment for the fluorometer under dark (control) and light (200 $\mu mol/m^2/s$) conditions. Dark adaptation period was 3 hr. The intensity of the actinic light was 250 $\mu mol/m^2/s$. Five plants were used in each treatment. Other conditions were the same as those described in Exp. 1.

Results and discussion

1) Effect of actinic light intensity on Fv/Fm values

After dark adaptation for full oxidation of Q_A, values of Fv/Fm at various intensities of actinic light are shown in Fig. 2. Chlorophyll a fluorescence was too low to be measured at an actinic light intensity of 100 $\mu mol/m^2/s$. Values of Fv/Fm could be measured at an intensity of more than 120 $\mu mol/m^2/s$ of actinic light using the fluorometer. Values of Fv/Fm decreased with increasing intensity of actinic light. The lowest value of Fv/Fm was obtained at an actinic light intensity of 100 $\mu mol/m^2/s$. Thus, in cucumber plants, it was considered that large values of Fv/Fm could be obtained at intensities of actinic light ranging from 120 to 250 $\mu mol/m^2/s$ in the present experiment and that it was necessary to keep a constant intensity of actinic light to obtain stable values of Fv/Fm.

2) Effect of light intensity before dark adaptation on Fv/Fm values

Light intensity before dark adaptation affected the values of Fv/Fm (Fig. 3). Immediately after the start of dark adaptation, the value of Fv/Fm was low (0.725) in plants grown at 200 $\mu mol/m^2/s$ light intensity and increased

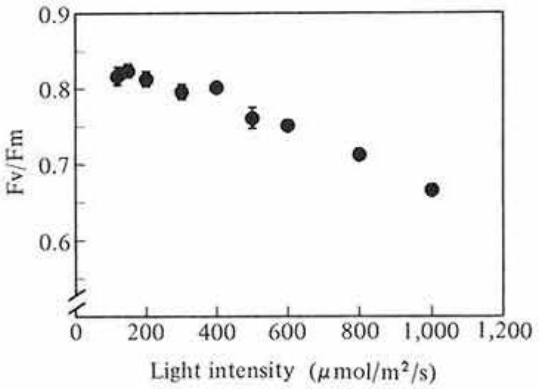

Fig. 2. Effect of actinic light intensity on Fv/Fm values of chlorophyll a fluorescence emitted from cucumber leaves
Vertical bars indicate standard deviations.

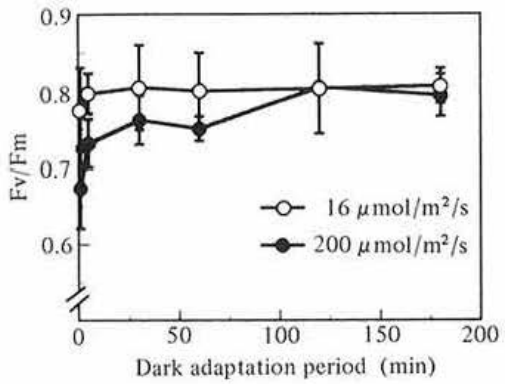

Fig. 3. Effect of light intensity before dark adaptation on Fv/Fm values of chlorophyll a fluorescence emitted from cucumber leaves
Vertical bars indicate standard deviations.

with the prolongation of the period of dark adaptation. The value became stable after 2 hr of dark adaptation.

On the other hand, in the plants grown at a low light intensity (16 $\mu mol/m^2/s$) the value of Fv/Fm was 0.78 after 5 min of dark adaptation and the value remained stable until 2 hr of dark adaptation. These results indicate that a longer adaptation period is necessary to obtain stable values of Fv/Fm when the light

intensity before dark adaptation is high, because an electron acceptor Q_A of PSII under high light intensity is more reductive and it takes much time to oxidize Q_A fully, compared with a low light intensity. Therefore, the higher the light intensity before dark adaptation, the longer the period of dark adaptation. Aoki et al.[1] considered that a dark adaptation period of 30 min was sufficient to measure chlorophyll a fluorescence at room temperature. However, 2 hr dark adaptation was necessary when plants were illuminated at a high light intensity before the dark adaptation.

3) Effect of temperature before dark adaptation on Fv/Fm values

Fv/Fm values of chlorophyll a fluorescence emitted from plants subjected to different growth temperatures are listed in Fig. 4. Stable values were obtained after 30 to 60 min of dark adaptation in each temperature treatment. Fv/Fm values of chlorophyll a fluorescence emitted from plants grown at 15°C and at 35°C were lower than those of plants grown at 25°C. These figures indicate that the photosynthetic ability is affected by the growth temperature and plants subjected to temperature

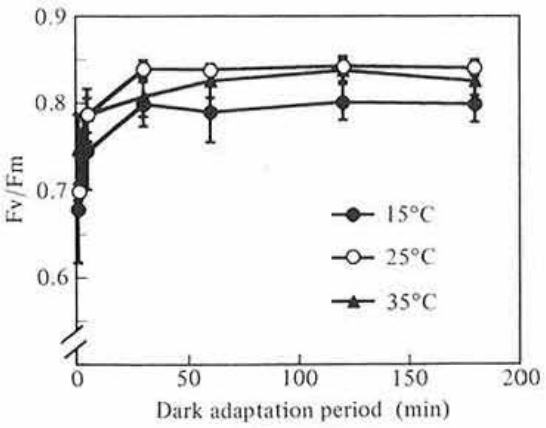

Fig. 4. Effect of temperature before dark adaptation on Fv/Fm values of chlorophyll a fluorescence
Vertical bars indicate standard deviations.

Table 1. Effect of partial or full dark adaptation on Fv/Fm values of chlorophyll a fluorescence emitted from cucumber leaves

Level of dark adaptation	Fv/Fm	Ratio (%)
Full	0.832±0.012[a]	100
Partial	0.842±0.070	99
t-test	NS[b]	

a): Mean±S.D.
b): Nonsignificant at 1% level.

stress do not recover during a short period of dark adaptation.

4) Possibility of partial dark adaptation in light

Fv/Fm values of chlorophyll a fluorescence emitted from leaves subjected to partial dark adaptation were very similar to those from leaves subjected to full dark adaptation (Table 1). These results indicate that the Fv/Fm values can be measured in light by partial dark adaptation with the use of an attachment.

Conclusion

Fv/Fm values that were correlated linearly with the quantum yield of photosynthesis changed depending on the light intensity and temperature immediately preceding dark adaptation. A longer period of dark adaptation was required to obtain stable values of Fv/Fm when plants were irradiated at higher light intensities. To analyze precisely stresses in plants based on the measurement of chlorophyll a fluorescence, an actinic light intensity of 120 to 250 μmol/m^2/s must be used. It appears that partial dark adaptation with an attachment for the fluorometer is suitable for the detection of stresses in plants in open field under sunshine conditions.

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