Measurement of Heat Sensitivity in Cucumber Leaves by Chlorophyll Fluorescence

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Plants exposed to extreme ranges of temperatures become injured and their growth is often retarded. As cucumbers are cultivated in all seasons, they are often grown under unfavorable conditions; low or high temperatures during the winter and the summer seasons, respectively. Assessment of temperature sensitivity is necessary to analyze the physiological mechanisms and select resistant plants. Although a number of methods have been reported for assessing stress injuries, quantification of stress in a plant is usually difficult. Visual symptoms, such as growth retardation which often appears as a result of physiological injury cannot be easily estimated quantitatively. Physiological analyses including fatty acid composition in the thylakoid membrane, ethane production, ethylene production, alanine accumulation are likely to be too laborious for the screening of resistant cultivars. Electrolyte leakage is unable to compare chilling sensitivity between species, because the structural differences of the leaves affect the rates of leakage[7].

Although chlorophyll fluorescence emission kinetics at 77K or with DCMU is known to be useful for analyzing photosynthetic electron flow systems[6], this method has been applied at room temperature to analyze injuries, associated with various stresses including chilling, freezing, heat, high light intensity, water stress and air pollution. Such stresses result in a decrease of the chlorophyll fluorescence induction which is considered to be related to the damage of the photooxidizing side of PS II.

In this report, effects of heating on chlorophyll fluorescence were investigated, and usefulness of the chlorophyll fluorescence measurement at room temperature to detect and quantify heat sensitivity was examined.

Materials and methods

1) *Heat stress*

Seedlings of cucumber (*Cucumis sativus* L.) were grown in polyvinyl plastic cups with soil (26°C day/21°C night, 14 hr-photoperiod at a photosynthetic photon flux density, PPFD of 400 μmole $m^{-2}s^{-1}$, 80% relative humidity). In an experiment on growth temperatures, cucumber seedlings were grown under a low temperature regime (20°C day/15°C night) or a high temperature regime (26°C day/21°C night). Seedlings with 2 to 3 true leaves or detached leaves placed in polyethylene bags were heated at 38–48°C for 5–40 min in darkness. Relative humidity was kept at 100%.

2) *Chlorophyll fluorescence measurement*

The kinetics of the induced chlorophyll fluorescence rise was measured with a portable fluorometer (type SF-20, Richard Branker Research Ltd., Canada). Before and after heat treatments, seedlings or leaf discs were adapted to darkness for 30 min at room temperature. The measuring probe of the fluorometer was placed directly over the surface and the leaves were illuminated for 5–10 sec with red light (670 nm, 25 μmole $m^{-2}s^{-1}$) and relative fluorescence yield was recorded directly or coupled with an XY recorder (type 3025, Yokogawa Ltd., Japan).

3) O_2 gas exchange measurement

O_2 gas exchange rates (OER) were measured with an Oxygen electrode (Rank Brothers, England) as described before[1]. OER was measured at 25°C, at a PPFD of 800 μmole $m^{-2}s^{-1}$ and in 25 mM sodium bicarbonate. Each measurement was repeated 4 times and the average of OER was compared with that of untreated seedlings and expressed as the percentage of the remaining* OER.

4) Leaf growth measurement

To analyze the effects of heat on early leaf growth, the heat-treated seedlings were cultured for further 3 days in a growth chamber (26°C day/21°C night, 14 hr-photoperiod at a PPFD of 400 μmole $m^{-2}s^{-1}$, 80% relative humidity). The length and width of the true leaves were measured and the product of the two parameters was referred to as apparent leaf area. The relative increase of the apparent leaf area in the heat-treated seedlings was compared with that of the untreated seedlings and expressed as the percentage of the remaining apparent leaf area.

Chlorophyll fluorescence measurement

As shown in Fig. 1, there was a first rise up to an inflection point, I, followed by a slow increase to a maximum, P. The first rise to I is considered to be due to the reduction of Q_a, while the latter transitory rise, from the inflection point I to the maximum P, depends on the further reduction of Q_a and plastoquinone pool. Although some calculation methods were reported to enable to estimate the activity of photosystem II, the method of Downton[1] was applied with a slight modification as the use of an XY recorder or an oscilloscope was not necessary. In the current study, Fr, the difference between I and P levels which was further normalized by I level, (P-I)/I was measured.

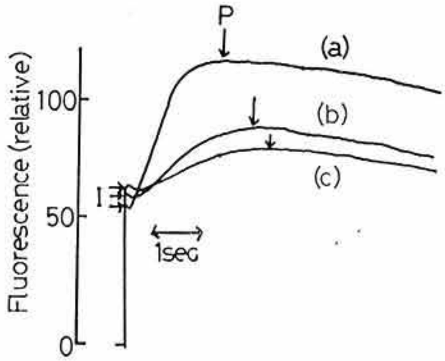

Fig. 1. Kinetics of induced chlorophyll fluorescence rise in cucumber cv., Sagamihanjiro leaves
Seedlings were dark-adapted for 30 min at room temperature and illuminated with red light for 10 sec.
I: Inflection point,
P: Maximum.
Seedlings were heated at 46°C for 5 min (b), 10 min (c) or not treated (a).

Each measurement was repeated at least 9 times. The average of Fr was compared with that of nontreated seedlings of leaf discs and expressed as the percentage of the remaining Fr.

By the heat treatment (Fig. 1), the P value decreased and the P appearance time was delayed. P could be detected within 4–5 sec after the illumination even under more drastic treatments (46°C, 10 min). Although a 10 sec-illumination was sufficient to measure Fr, in most cases, 5 sec-illumination was used.

Since chlorophyll fluorescence rise depends on the redox state of the primary electron acceptor (Q_a) of photosystem II, the leaves must be adapted to darkness until Q_a is fully oxidized. Based on the experiments analyzing the effects of dark-adaptation time, it was considered that a period of 30 min of dark-adaptation at room temperature was sufficient to measure Fr.

Moreover, dark-adaptation was dependent on the temperature. The maximum Fr for dark-adapted leaves at low temperatures was almost the same as that for the dark-adapted leaves at room temperature in lettuce and pea,

* Remaining OER (or Fr, etc.) means the OER (or Fr, etc.) value remaining after the heating treatment.

but not in cucumber[3,8]. Therefore, it is necessary that leaves become adapted to darkness at a relatively high temperature.

In addition to the temperature dependency of the dark-adaptation, the illumination intensity is considered to influence the chlorophyll fluorescence; the stronger the illumination intensity, the earlier the P appearance time and the lower Fr was[3]. Thereby, in this study, the illumination intensity was fixed at 25 μmole $m^{-2}s^{-1}$.

Heat sensitivity measured by chlorophyll fluorescence

Effects of the heating temperature and exposure time on Fr were examined using seedlings and detached leaves of cucumber (Fig. 2-a, b). The Fr value had already decreased when the seedlings and detached leaves were heated at 38°C for 10 min and a temperature dependency of the Fr decrease was revealed (Fig. 2-a). Although the Fr value also decreased depending on the exposure time to heating at 42° and 45°C, the Fr value markedly decreased for an exposure time of 5 min at 48°C (Fig. 2-b). The Fr decrease was more severe in detached leaves than in seedlings. As for the effect of leaf age, a small difference was obtained in the seedlings but not in the detached leaves. Water stress might influence the different sensitivities between leaf ages.

When the heated seedlings were returned to normal growth conditions (26°C day/21°C night, 14 hr-photoperiod), the Fr decrease was almost reversed in the following day (24.2 to 103.1%, 8.8 to 95.2%). However, the leaf growth of cucumber continued to be depressed after the alleviation of the Fr decrease (when the apparent leaf area in control seedlings increased 9 fold in three days). In Fig. 3, the remaining apparent leaf growth was referred to as a function of the remaining Fr, which was measured immediately after the heat treatment. These two parameters were found to be positively correlated with each other in a quadratic curve. In chilling experiments, the decrease of growth was also positively correlated with the chlorophyll fluorescence in maize[5] and with OER in cucumber[2]. These results indicate that there is a close parallel relationship between the Fr and the growth decrease induced by stresses, and the physiological characteristics as well as photosynthetic abilities can be monitored by the Fr measurement.

Cucumber seedlings were grown under a

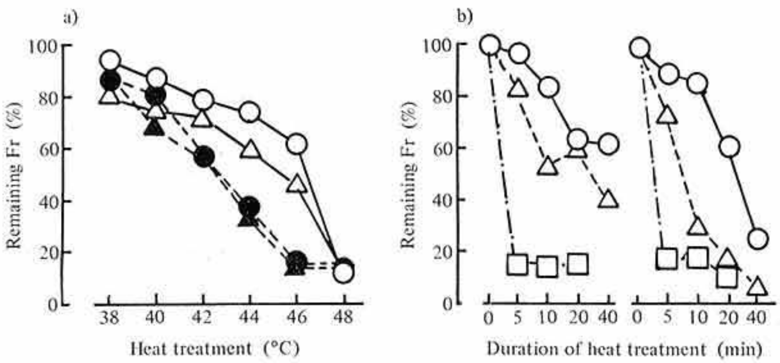

Fig. 2. Effects of heat treatment on Fr in cucumber cv., Sagamihanjiro seedlings and detached leaves

a) : Seedlings (○, △) and detached leaves (●, ▲) were heated at 38—48℃ for 10 min. Circles: First leaves, Triangles: Second leaves.

b) : Seedlings (left) and detached leaves (right) were heated at 42℃ (○), 45℃ (△) and 48℃ (□) for 5—40 min.

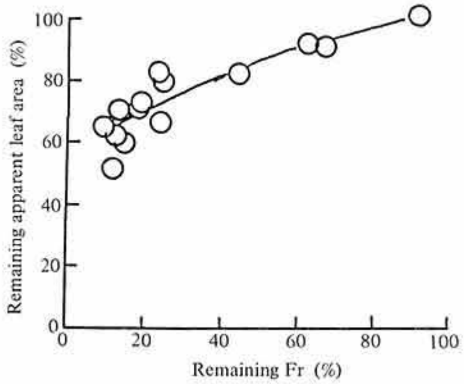

Fig. 3. Relationship between the remaining Fr and the remaining apparent leaf area in heated cucumber cv., Sagamihanjiro seedlings

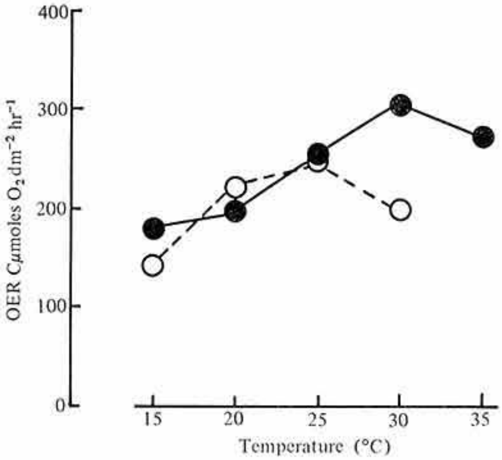

Fig. 4. Temperature-dependent OER changes in cucumber cv., Sagamihanjiro

○ : LT-seedlings (20℃ day/15℃ night)

● : HT-seedlings (26℃ day/21℃ night)

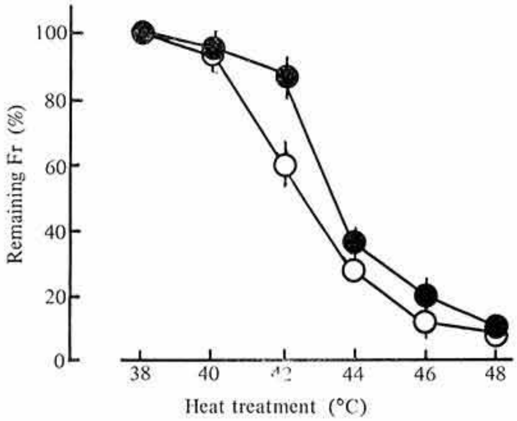

Fig. 5. Effects of heat treatment on Fr in the LT- and the HT-seedlings of cucumber cv., Sagamihanjiro

LT-seedlings (○) and HT-seedlings (●) were heated at 38—48 ℃ for 5 min.

Table 1. Effects of heat treatment on the leaf growth and Fr in LT- and HT-seedlings of cucumber cv., Sagamihanjiro

Growth	Heat treatment[1] (℃)	Remaining Fr (%)	Remaining apparent leaf area[2] (%)
LT-seedlings	42	61.0±0.7	91.0±0
20℃ day/	45	18.5±1.6	72.1±1.1
15℃ night	48	11.2±1.0	50.5±3.0
HT-seedlings	42	90.0±1.4	102.5±2.7
26℃ day/	45	22.8±2.6	81.5±0.5
21℃ night	48	14.4±1.6	58.6±3.1

1) : Seedlings were heated at each indicated temperature for 5 min.

2) : After the heat treatment, seedlings were grown at 26℃ day/21℃ night (14 hr-photoperiod) under a PPFD of 400 μmole m^{-2} s^{-1} for further three days.

low temperature regime (20°C day/15°C night, LT-seedlings) or a high temperature regime (26°C day/21°C night, HT-seedlings). The OER of the HT-seedlings peaked at a higher temperature than that of the LT-seedlings (Fig. 4). Using these seedlings differing in their adaptation to temperature changes, the effects of heating on Fr and leaf growth were examined (Fig. 5 & Table 1). When the heating temperature changed from 38° to 48°C for 5 min, the remaining Fr of the HT-seedlings was higher than that of the LT-seedlings (Fig. 4). In three temperature treatments (Table 1), the decrease of the Fr value and leaf growth was less pronounced in the HT-seedlings. These results

Table 2. Effects of heat treatment on the leaf growth and Fr in seedlings of heat-resistant cultivar, Rensei and heat-sensitive cultivar, Tachibana

Varieties	Heat treatment[1)] (℃)	Remaining Fr (%)	Remaining apparent leaf area (%)
Rensei	42	72.9±3.3	74.7±3.4
	45	23.7±3.3	55.9±1.2
	48	11.0±1.2	dead
Tachibana	42	58.1±2.9	71.6±1.7
	45	13.4±2.5	51.6±1.7
	48	10.7±1.2	dead

1) : Seedlings were heated at each indicated temperature for 5 min.

Table 3. Effects of heat treatment on Fr in seedlings of cucumber varieties

Varieties	Remaining Fr (%)
Rensei	38.9±2.0
Tsuken-2	35.4±2.0
Shindome	33.1±1.7
Nisshiaonagafushinari	27.5±1.4
Tachibana	24.2±1.1

Seedlings were heated at 43℃ for 10 min.

suggest that the seedlings adapted to higher temperatures were heat tolerant.

Effects of heating on Fr were measured using cucumber varieties with different heat-sensitivities (according to personal communication from Dr. T. Kanno). Table 2 shows the effects of heating on the Fr value and leaf growth in a heat-sensitive cultivar, Tachibana and a heat-resistant cultivar, Rensei. The decrease of the Fr value and leaf growth was lower in Rensei than in Tachibana, although the leaf growth difference was negligible. Varietal differences in heat sensitivity were further examined (Table 3). The value of the remaining Fr was highest in Rensei, followed by Tsuken-2 and Shindome. On the other hand, the value of the remaining Fr of Nisshiaonagafushinari was low and that of Tachibana was the lowest. These results suggest that heat sensitivity can be estimated by the Fr measurement.

Heat sensitivities among cucumber varieties (Table 3) as well as the seedlings differing in their adaptation to temperature changes (Table 2) were quantitatively assessed by the Fr measurement. The usefulness of the Fr measurement, especially, lies in its rapidity, and a non-destructive character as well as the use of intact plants.

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