MEASUREMENT OF HEAT SENSITIVITY IN CUCUMBER LEAVES BY CHLOROPHYLL FLUORESCENCE METHOD

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ABSTRACT

Usefulness of the chlorophyll fluorescence measurement at room temperature (Fr) to detect and quantify heat sensitivity was examined. Seedlings with 2 to 3 true leaves, detached leaves placed in polyethylene bags or leaf discs floated on distilled water were subjected to 38-48°C temperatures for 5-40 min in the dark at 100% relative humidity. The Fr value decreased depending on the heating temperature and exposure time to heat. When leaves and roots of the seedlings were heated separately, the decrease of the Fr value was pronounced in the seedlings in which both leaves and roots had been subjected to heating, but not in those in which only roots had been exposed to heating. After the seedlings were returned to normal growth conditions, leaf growth continued to be depressed. The depression rates of the Fr value and leaf growth were positively correlated with each other in a quadratic curve. Cucumber seedlings grown under a high temperature (26°C day/21°C night), with a high optimal temperature for oxygen exchange rate, were resistant to heating with regard to Fr and leaf growth compared to those under a low temperature regime (20°C day/15°C night). Heat sensitivity was ranked in cucumber varieties. Based on these results, it was considered that heat sensitivity can be easily assessed by the Fr measurement.

Introduction

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 unfavourable conditions ; low or high temperatures during the winter and 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 does not enable to compare temperature sensitivity between species, because the structural differences of the leaves affect the rates of leakage (MacRae *et al.*, 1986).

Although chlorophyll fluorescence emission kinetics at 77K or with DCMU is known to be useful for analyzing photosynthetic electron flow systems (Hipkins and Baker, 1986), 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 (Bradbury and Baker, 1981 ; Gibbons and Smillie, 1980 ; Downton, 1983; Hetherington and Öquist, 1988 ; MacRae *et al.*, 1986., Omasa *et al.*, 1987 ; Peeler and Naylor, 1988 ; Schreiber and Berry, 1977 ; Smillie, 1979 ; Smillie and Hetherington, 1983;

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Van Hasselt and Van Berlo, 1980). 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. cv. Sagamihanjiro), maize (*Zea mays* L. cv. Honeybantam sigma), squash (hybrid of *Cucurbita maxima* Dough. x *Cucurubita maxita* Pang. cv. Just), netted melon (*Cucumis melo* L. cv. Natsukei-7), lettuce (*Lactuca sativa* L. cv. Grandrapid), spinach (*Spinacia oleracea* L. cv. Pioneer) and cabbage (*Brassica oleracea* var. capitrata L. cv. Kinkei-20) were grown in polyvinyl plastic cups with soil (26°C day/21°C night, 14h photoperiod at a photosynthetic photon flux density, PPED 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, detached leaves placed in polyethylene bags or leaf discs floated on distilled water were heated at 38-48°C for 5-40 min in the dark. Relative humidity was kept at 100%. In an experiment where leaves and roots were heated separately, cucumber seedlings were subjected to 25°C or 43°C for 60 min and 120 min in the dark at 100% relative humidity.

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, detached leaves or leaf discs were adapted to darkness for 30 min at room temperature. The measuring probe of the fluorometer was placed directly over the leaf surface and the leaves were illuminated for 5-10 sec with red light (670 nm, 25μ mole $m^{-2}s^{-1}$) and the relative fluorescence yield was recorded directly or coupled with a 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 earlier (Aoki, 1981). OER was measured at 25°C, at a PPFD of 800μ mole $m^{-2}s^{-1}$ 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 remaining Fr.

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, 14h photoperiod at a PPFD of 400μ mole $m^{-2}s^{-1}$, 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 remaining apparent leaf area.

Results and discussion

1 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 Qa, while the latter transitory rise, from the inflection point, I to the maximum, P depends on the further reduction of Qa and plastoquinone pool. Although some calculation methods were reported to enable to estimate the activity of photosystem II, the method of Downton (1983) was applied with a slight modification as use of a 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. Each measurement was repeated at least 9 times. The average of Fr was compared with that of untreated seedlings, detached leaves of leaf discs and expressed as the percentage of 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.

Because chlorophyll fluorescence rise depends on the redox state of the primary electron acceptor (Qa) of photosystem II, the leaves must be adapted to darkness until Qa 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, but not in cucumber (Aoki and Oda, 1988 ; Peeler and Naylor, 1988). Therefore, it is necessary that leaves become adapted to darkness at a relatively high temperature.

In addition to the temperature dependency on the dark-adaptation, the illumination intensity is considered to influence the chlorophyll fluorescence ; the stronger the illumi-

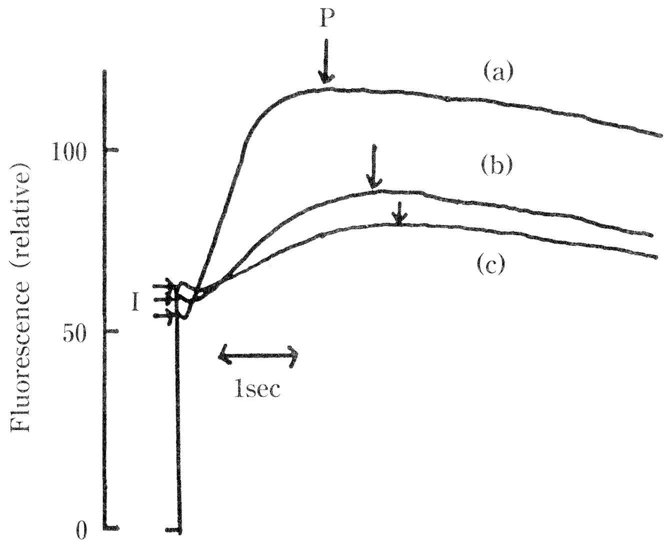

Fig. 1 Kinetics of induced chlorophyll fluorescence rise in cucumber, (Sagamihanjiro) leaves
Seedlings were dark-adapted for 30 min. at room temperature and illuminated with red light for 10 sec I, inflection point ; P, maximum. Seedlings were heated at 46°C for 5 min (b), 10 min (c) or not treated (a).

nation intensity was, the earlier the P appearance time was and the lower Fr (Omasa *et al.*, 1987 ; Aoki and Oda, 1988). Thereby, in this study, the illumination intensity was fixed at 25μ mole $m^{-2}s^{-1}$.

2 Effects of heat treament on chlorophyll fluorescence

Effects of the heating temperature and exposure time on Fr were examined using seedlings and detached leaves of cucumber (Fig. 2a, 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. 2a). Although the Fr value also decreased depending on the exposure time to heating at 42°C and 45°C, the Fr value markedly decreased for an exposure time of 5 min at 48°C (Fig. 2b). 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.

Leaves and roots of the cucumber seedlings were separately exposed to 43°C for 60 min and 120 min (Fig. 3). The Fr values decreased when leaves were heated and the Fr decrease was pronounced in the seedlings in which both leaves and roots had been subjected to heating. On the other hand, the Fr values did not decrease when leaves were not heated regardless of whether the roots were exposed to heating or not. These results indicate that the decrease of the Fr value induced by heating was due to direct damage to leaves.

When the heated seedlings were returned to normal growth conditions (26°C day/21°C night, 14 h-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. 4, 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 (Hetherington and Öquist, 1988) and with OER in cucumber (Aoki *et al.*, 1989).These results indicate that there is a close parallel relationship between the Fr and the growth decrease induced by

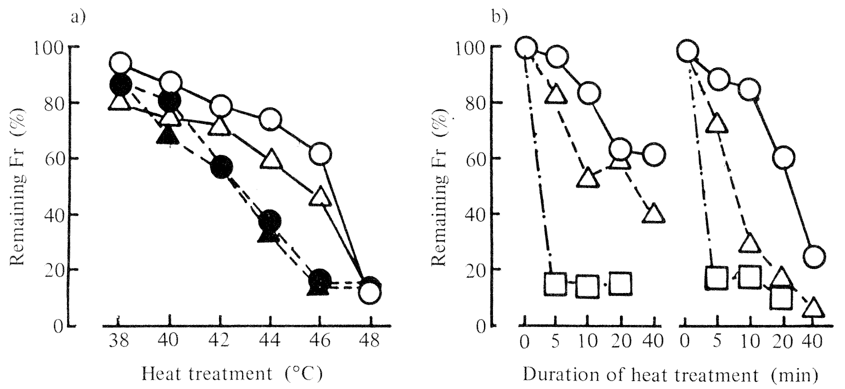

Fig. 2 Effects of heat treatment on Fr in cucumber, 'Sagami-hanjiro' seedlings and detached leaves
a : Seedlings (○, △) and detached leaves (●, ▲) were heated at 38-48°C for 10 min. Circles : first leaves ; triangles : second leaves.
b : Seedlings (left) and detached leaves (right) were heated at 42°C(○), 45°C(△) and 48°C(□) for 5-40 min.

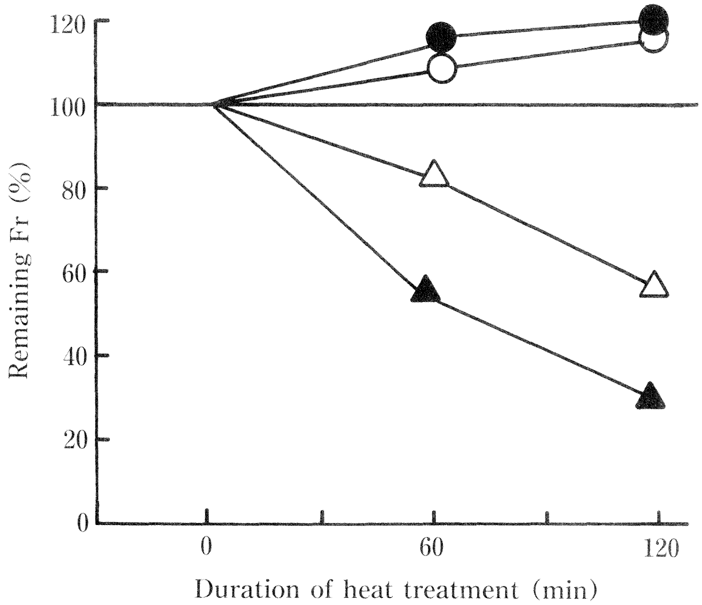

Fig. 3 Effects of heat treatment on leaves and roots of cucumber, 'Sagamihanjiro' seedlings
Treatment temperatures of leaves/roots were as follows, ○ ; 25/25°C, ● ; 25/43°C, △ ; 43/25°C, ▲ ; 43/43°C.

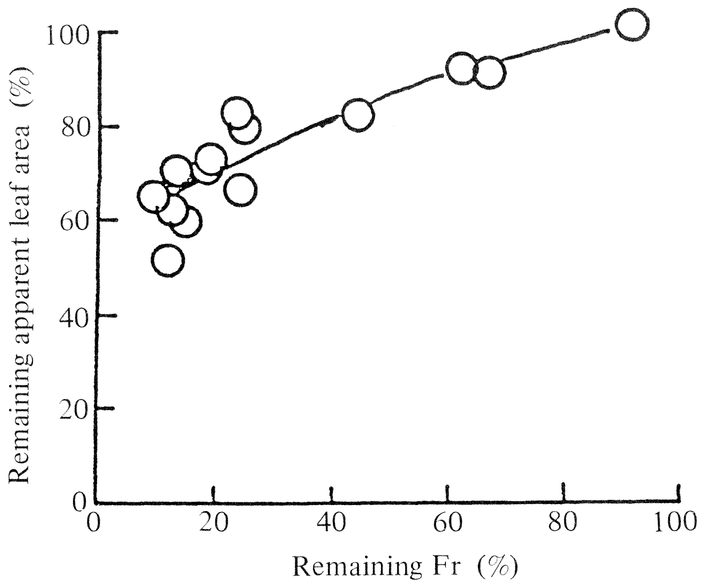

Fig. 4 Relationship between the remaining Fr and the remaining apparent leaf area in heated cucumber, 'Sagamihanjiro' seedlings

stresses, and the physiological characteristics as well as photosynthetic abilities can be monitored by the Fr measurement.

3 Heat sensitivity measured by chlorophyll fluorescence

Heat sensitivities of some plants were assessed by the determination of Fr (Table 1). Seedlings were heated at 42°C for 5 min, under which conditions the Fr value of the cucumber seedlings was slightly depressed. The value of remaining Fr was highest in maize followed by squash and netted melon. The corresponding values in spinach and cabbage were the lowest. Such a pattern of heat sensitivity seemed to resemble that shown in the field.

Cucumber seedlings were grown under a 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. 5). Using these seedlings differing in their adaptation to temperature changes, the effects of heating on Fr and leaf growth were examined (Fig. 6 and Table 2). When the heating temperature changed from 38°C to 48°C for 5 min, the remaining Fr of the HT-seed-

Table 1 Effect of heat treatment on Fr in various plants

Plants	Remaining Fr (%)
Maize	99.7±5.0
Squash	92.3±3.7
Netted melon	77.9±4.3
Lettuce	68.5±4.5
Spinach	31.3±1.9
Cabbage	31.3±3.4

Plants were heated at 42°C for 5 min.

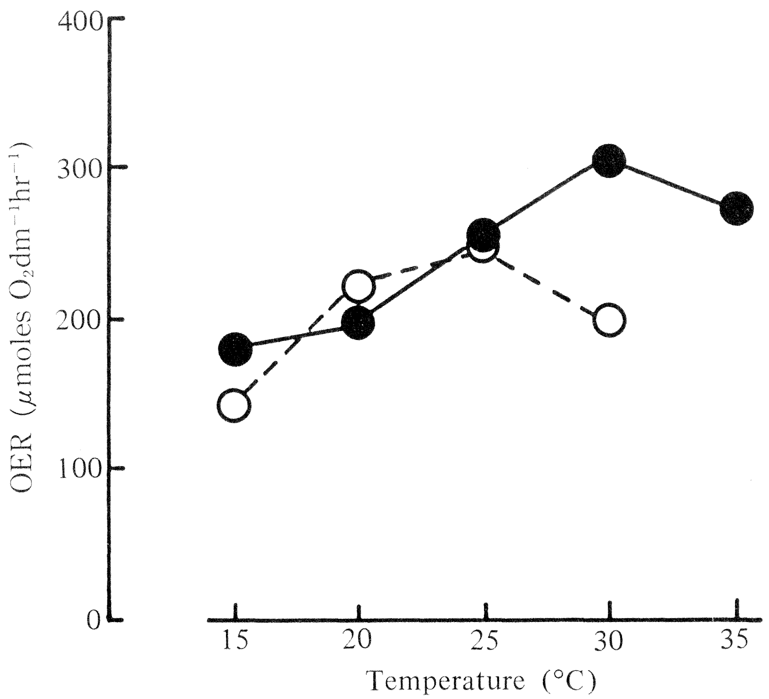

Fig. 5 Temperature-dependent OER changes in cucumber 'Sagamihanjiro', (○) ; LT-seedlings (20°C day / 15°C night). (●) ; HT-seedlings (26°C day / 21°C night).

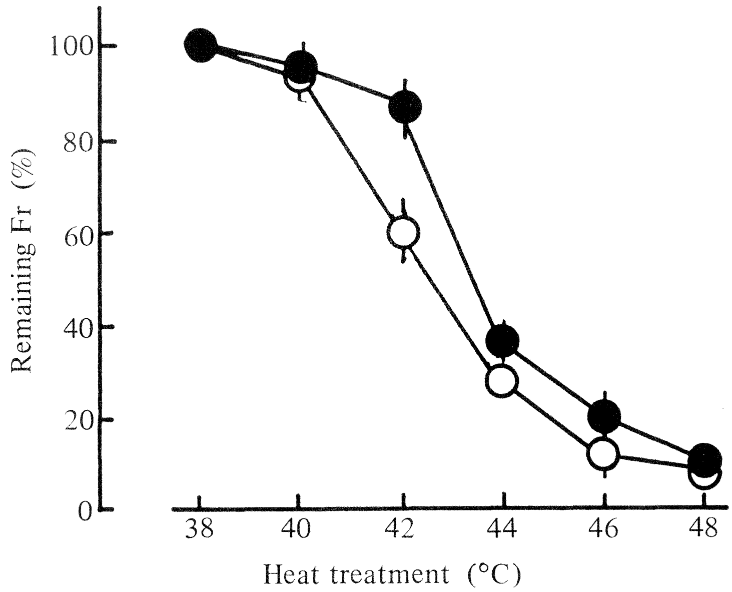

Fig. 6 Effects of heat treatment on Fr in the LT-and the HT-seedlings of cucumber, 'Sagamihanjiro'
LT-seedlings (○) and HT-seedlings (●) were heated at 38-48°C for 10 min.

Table 2 Effect of heat treatment on the leaf growth and Fr in LT-and HT- seedlings of cucumber, 'Sagamihanjiro'

Growth	Heat treatment[1)] (°C)	Remaining Fr (%)	Remaining apparent leaf area[2)] (%)
LT-seedlings	42	61.0±0.7	91.0±0
20°C day /	45	18.5±1.6	72.1±1.1
15°C night	48	11.2±1.0	50.5±3.0
HT-seedlings	42	90.0±1.4	102.5±2.7
26°C day /	45	22.8±2.6	81.5±0.5
21°C 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°C day / 21°C night (14h photoperiod) under a PPFD of 400 μmole $m^{-2}s^{-1}$ for further three days.

Table 3 Effect of heat treatment on the leaf growth and Fr in seedlings of a heat-resistant, 'Rensei' and a heat-sensitive, 'Tachibana' cucumber cultivars

Varieties	Heat treatment[1] (°C)	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 4 Effect 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°C for 10 min.

lings was higher than that of the LT-seedlings (Fig. 6). In three temperature treatments (Table 2), the decrease of the Fr value and leaf growth was less pronounced in the HT-seedlings. These results suggest that the seedlings adapted to higher temperatures were heat-tolerant.

Effects of heating on Fr were measured using cucumber varieties with known and different heat sensitivities (personal communication from Dr. T. Kanno). Table 3 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 4). 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 4) as well as the seedlings differing in their adaptation to temperature changes (Table 3) were quantitatively assessed by the Fr measurement. The usefulness of the Fr measurement, especially, lies in its rapidity, and non-destructive character as well as use of intact plants.

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Discussion

Paje, M. M. (Philippines) : In your experiment on cucumber seedlings, could you please explain why you selected the temperature regime 26°C day/21°C night ?
Are these temperatures high enough ? Why not 30°C or above ?

Answer : This temperature regime is commonly used in Japan. Although 26°C is slightly higher than the temperature suitable for cucumber growth, abnormal growth occurs when the temperature reaches or exceeds 30°C.