The Influence of Dark Adaptation Temperature on the Reappearance of Variable Fluorescence following Illumination

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ABSTRACT

The effect of chilling temperatures (5°C) on chlorophyll fluorescence transients was used to study chilling-induced inhibition of photosynthesis in plant species with differing chilling sensitivities. A Brancker SF-20 fluorometer was used to measure induced fluorescence transients from both attached and detached leaves of chilling-sensitive cucumber (Cucumis sativus L. cv Ashley) and chilling-resistant pea (Pisum sativum L. cv Alaska). The rate of reappearance of the variable component of fluorescence (F), following a period of illumination at 25°C, was dependent on the temperature at which the leaf was allowed to adapt in chilling-sensitive cucumber, but not in chilling-resistant pea. In cucumber, dark adaptation at 25°C following illumination resulted in a much faster return of F, than dark adaptation at 5°C following illumination. However, F, reappearance during the dark adaptation period in chilling-resistant pea was temperature independent. The difference in the temperature response of F, following illumination correlated with temperature sensitivity of these two species. The process responsible for the difference in F, may represent a site of chilling sensitivity in the photosynthetic apparatus.

Chlorophyll fluorescence has been used to monitor changes in the photosynthetic metabolism of plants and algae (5, 10). The Chl fluorescence emitted upon illumination following a dark period (induced Chl fluorescence) (3) is altered when the tissue has been exposed to chilling temperatures (0–15°C). Generally, F, increases as temperatures are lowered to 0°C, if the chilling takes place in the dark (1, 2, 6–8, 12). However, chilling stress in conjunction with light causes F, to decrease compared to nonstressed controls (2, 9).

An induced fluorescence trace is usually measured after the tissue has ‘dark adapted’ for a standard amount of time. In whole tissues, this time is generally 30 min to 1 h, or after the fluorescence trace has stabilized. However, we found that the kinetics of the reappearance of the induced fluorescence trace to a stable F, value appeared to be influenced by the temperature of the dark recovery period itself. To ascertain the nature of the temperature effect on the rate of reappearance of F,, induced fluorescence was measured at regular time intervals during the course of the dark adaptation period.

The experiments reported in this paper show that chilling temperatures during the dark adaptation period alone delayed the reappearance of F, following illumination in cucumber. The return of F, in chilling-resistant pea was not affected by chilling during the dark adaptation period.

MATERIALS AND METHODS

Pea (Pisum sativum L. cv Alaska) and cucumber (Cucumis sativus L. cv Ashley) were grown in growth chambers in the Duke University Phytotron (4) under 25°C day/18°C night conditions and a 15 h photoperiod. Two to 4-week-old plants were used for all treatments. Whole pea and cucumber plants were chilled by dropping the growth chamber temperature to 5°C day/night, 1 to 2 h after the beginning of the photoperiod.

Fluorescence measurements were made on intact plants with a Brancker SF-20 fluorometer (Richard Brancker Research, LTD, 27 Monk St., Ottawa, Canada, K1S 3Y7). The plants were removed from the chilled growth chamber, and immediately placed in a dark room at 25°C. The fluorescence probe was mounted on a cantilever arm so that it could be brought in contact with leaves without damaging the plants. A magnet was placed under the leaf so that the leaf was sandwiched between the magnet and the probe, assuring good contact for fluorescence measurements.

In these whole plant experiments, leaves received different amounts of illumination, depending on their position on the plant, and the angle of the leaf relative to the light source. To insure that each leaf received the same amount of light in later experiments, leaves were detached and placed on a flat surface for treatment. Detached leaves or leaf discs (23 mm diameter) were positioned on a moistened sponge within a metal pan, and covered with a thin, transparent plastic film (‘Gladwrap,’ CO2 permeable), to reduce dehydration. The leaves or leaf discs were illuminated for 2 h at 350 μmol m−2 s−1 and either 25°C or 5°C, and then dark adapted for one hour at either 25°C or 5°C. A plastic template was placed over the leaves, so that a hole having the same diameter as the fluorometer probe was over each leaf (11). This allowed subsequent fluorescence measurements to be taken from the same area of a leaf as the original measurement, thereby reducing variability.

Induced Chl fluorescence was measured over a 5 s excitation period with a Brancker SF-20 fluorometer attached to a Tracor Northern recording oscilloscope. Groups of at least five leaves were treated at a time, and measurements of fluorescence were made at least every 2 min during the dark adaptation period. Leaves were measured in sequence so that any individual leaf was not illuminated longer than 5 s for every 10 min of darkness. This amount of illumination at the exciting irradiance (11 μmol m−2 s−1) did not interrupt the dark adaptation period. Fluorescence traces from leaves measured under this regime versus leaves kept in total darkness were identical throughout the 1 h dark adaptation. Results were expressed as the ratio of the variable (Fv) over the initial (Fo) fluorescence. Fv, as used in this paper refers to the peak of fluorescent light emission during irradiation.
at 11 μmol m\(^{-2}\) s\(^{-1}\). An example of actual traces recorded during a dark adaptation period is shown in Figure 1.

RESULTS AND DISCUSSION

Fluorescence is reported here as the ratio of \(F_v/F_o\), as demonstrated in Figure 1. Since \(F_o\) changed very little within a single experiment, an increase in this ratio signifies an increase in \(F_v\).

When detached cucumber leaves were illuminated at 25°C for 3 h, and then dark adapted at 25°C, the \(F_v\) component of the fluorescence trace fully reappeared after 10 min of darkness (Fig. 2).

The rates of reappearance of cucumber \(F_v\) from leaves of whole plants chilled in the light are shown in Figure 3. The magnitude of \(F_v\) decreased over time until the reappearance of \(F_v\) was completely inhibited after 25 h of chilling in the light. The time required for \(F_v\) to reach a maximum after the beginning of the 25°C dark adaptation period remained constant (~10 min) throughout the chilling treatment.

Further experiments showed that the reappearance of \(F_v\) could be delayed in detached cucumber leaves by maintaining chilling temperatures throughout the dark adaptation period. If leaves were illuminated at 5°C for 3 h, and maintained during the dark adaptation period at 5°C, \(F_v\) did not reappear even after 60 min of dark adaptation (Fig. 4). Reappearance of \(F_v\) of detached cucumber leaves illuminated at 5°C for 3 h and then dark adapted at 25°C was identical to the whole plant response after four hours chilling in the light shown in Figure 3.

In an effort to determine if the temperature of the dark adaptation period rather than the temperature during illumination was responsible for the delay in reappearance of \(F_v\) in cucumber, leaf discs were illuminated for 3 h at 25°C and immediately placed in the dark at 5°C. Leaf discs were used to minimize the time required to cool the entire tissue for dark
adaptation. Figure 5 shows that cucumber Fv, reappeared more slowly during the 5°C dark adaptation than 25°C (30 min versus 10 min) indicating that the temperature of the dark adaptation period was responsible for the delay in reappearence of Fv.

Low temperatures alone do not cause a decrease in cucumber Fv. A period of illumination (at 25°C or 5°C) must first quench Fv, before chilling temperatures can delay Fv reappearanee during dark adaptation. In the absence of a pre-illumination period, dark adaptation at 5°C increased the Fv component in cucumber leaves (Fig. 6), rather than delaying the reappearance of Fv. Dark chilling induced increases in Fv of this kind have been reported previously (1, 6–8, 12).

Complete recovery of Fv of detached pea leaves illuminated at 25°C and dark adapted at 25°C was slower than in cucumber, requiring 30 min for complete reappearance of the variable component (Fig. 7). In contrast to cucumber, pea Fv, reappeared at the same rate following 5°C illumination and 5°C dark adaptation as during the 25°C treatment. These results show that the reappearance of pea Fv was insensitive to chilling temperatures.

The results show that the temperature of the dark adaptation period is critical in the restoration of Fv of cucumber, regardless of the illumination temperature. This is the first report that the temperature of the dark adaptation period can influence the reappearance of Fv. This result may allow a more focused approach towards analyzing the complex problem of chilling-sensitivity by isolating (in time) a chilling-responsive component in cucumber photosynthesis.

Some process(es) of Fv, quenching in cucumber was maintained for a longer period of time during chilling temperatures than at 25°C. Although a number of different mechanisms of Fv, quenching have been described, such as photochemical quenching, pH (energy) quenching, or state I-state II quenching (for a review, see Krause and Weis [5]), the methods used in this report were not able to distinguish which one(s) was chilling-responsive. In addition to the recognized Fv quenching mechanisms, chilling temperatures may have delayed the restoration of Fv in cucumber by slowing metabolic processes or by affecting membrane fluidity.

Pea does not exhibit a similar chilling-responsive process during dark adaptation. This lack of responsiveness in pea correlates with its general chilling resistance relative to cucumber. A number of other agonistic species studied also demonstrated a chilling-responsive process during dark adaptation that correlated with their photosynthetic chilling sensitivity (results not shown). Further study of the chilling-responsive component of cucumber Fv, quenching, especially in comparison with nonresponsive pea, may provide valuable information about the mechanisms of chilling sensitivity.

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LITERATURE CITED