Communication

Cold-Induced Sudden Reversible Lowering of *in Vivo* Chlorophyll Fluorescence after Saturating Light Pulses

A Sensitive Marker for Chilling Susceptibility

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ABSTRACT

In chilling-sensitive plants (*Glycine max*, *Saintpaulia ionantha*, *Saccharum officinarum*) a sudden reversible drop in chlorophyll fluorescence occurs during photosynthetic induction immediately following saturating light pulses at low temperatures in the range 4 to 8°C. A comparison of two soybean cultivars of different chilling sensitivities revealed that this phenomenon, termed low-wave, indicates specific thresholds of low temperature stress. Its occurrence under controlled chilling can be regarded as a quantitative marker for screening chilling susceptibility in angiosperms.

*In vivo* Chl fluorescence has in many respects proved to be a sensitive and reliable method for the detection and quantification of chilling-induced disturbances (1, 2, 4, 5, 11, 12). A number of parameters of the photosynthetic induction transient may be employed for rating chilling susceptibility. Smillie and Hetherington (12) determine $F_{F_q}$ of induced Chl fluorescence of leaves exposed to a constant temperature of 0.5°C and take the time required for a 50% reduction of $F_F$ as a measure of chilling susceptibility. Havaux (1) estimates relative chilling sensitivity by measuring the $Q_2$ redox state under low temperature conditions. Larcher and Bodner (4) base their assessment of chilling susceptibility on the temperature at which the decrease in fluorescence, $vF_d = F_F - F_R$, is 50% of its highest value measured on the same leaf before cooling, and on the readiness and extent of recovery of photosynthetic function after rewarming.

While searching for additional warning signs of chilling-induced deviations from normal photosynthetic activity it was observed that at low temperatures the leaves of various chilling-sensitive plants exhibited a sudden undershoot in Chl fluorescence immediately after saturating light pulses. This phenomenon has proved to be a good diagnostic criterion for the onset of chilling stress.

MATERIALS AND METHODS

Plant Materials

*Glycine max* (L.) Merr. cv ‘Maple Arrow’ and cv ‘Evans’ were grown from seed in pots in a soil mixture of compost, litter, and clay (2:1:1) in the Botanical Garden of the University of Innsbruck. Second trifoliate leaves of well watered plants in the vegetative state, 38 d after seedling emergence, were used for the experiments. For additional check measurements, leaves were taken from greenhouse plants of *Saintpaulia ionantha* hybrids and from young developing shoots of *Saccharum officinarum*.

Chilling Treatments

For progressive cooling, attached leaves were placed with the abaxial surface down on a 165×95 mm aluminium plate mounted on the thermal sink side of a thermoelectric module (type 803-1008-01) controlled by a bipolar controller (type 809-3030) and a range extender (type 809-1019) manufactured by Midland-Ross Corp. (Cambridge, MA). After each fluorescence measurement the temperature was lowered at a rate of 4 K min$^{-1}$ in 8 steps from 20°C down to 0.9°C. For continuous long-term cooling at a preset constant temperature (3.5°C), attached leaves were fixed with a magnet to the surface of an insulated metal chamber, through which cold ethylene glycol was pumped. The temperature of the cooling fluid was regulated by means of a through-flow cooler (K11, Haake, Karlsruhe, FRG). The sample temperature was measured on the upper leaf surface using a copper-constantan thermoelement connected to a digital voltmeter (accuracy: ± 0.1 K).

Determination of Chlorophyll Fluorescence

Photosynthetic induction transients were recorded before and during cooling of the leaves. The leaves were predarkened

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2 Abbreviations: $F_{F_q}$, maximal rate of fluorescence rise; $F_F$, basic fluorescence; $F_F$, peak of the induction curve upon excitation with actinic light; $F_R$, stationary level of variable fluorescence; $F_0$, variable fluorescence at any given time during induction; $(F_D)_o$, minimal variable fluorescence at appearance of L-waves; $(F_D)_o$, maximal fluorescence of a dark-adapted leaf upon excitation with a saturating light pulse; $(F_D)_o$, minimal variable fluorescence at any given time during induction observed upon application of a saturation pulse; $qP$, photochemical quenching coefficient; $Q_2$, primary electron acceptor of PS II; $vF_d$, variable fluorescence decrease $(F_F - F_R)$; L-wave, sudden reversible lowering of *in vivo* Chl fluorescence after saturating light pulses.
RESULTS AND DISCUSSION

If short light pulses of saturating intensity are superimposed on a light-driven photosynthetic induction curve the enhanced fluorescence usually relaxes within seconds to the level reached shortly before application of the pulse (Fig. 1A). In each of the chilling-sensitive species tested, at a certain temperature level during cooling, the fluorescence intensity dropped within milliseconds after saturating light pulses below the previous level of the induction curve. Within a few seconds after the undershoot, the fluorescence gradually rose again (Fig. 1B). This reversible short-term decrease in fluorescence will be termed low-wave. If the temperature is progressively lowered, the L-waves first appear after several saturating light pulses. As cooling progresses, L-waves are seen after each saturating pulse and, in addition, the extent of the undershoot gradually increases.

If soybean leaves are cooled for a longer period (several hours) at a constant temperature at which L-waves begin to appear, the sudden reversible lowering of fluorescence steadily increases the longer the cooling continues (Fig. 2). If the relative amplitude of the L-wave in the region of the largest undershoots, \((F_v - [F_v])/(F_v)\), is plotted against the leaf temperature, the critical temperature at which the L-waves appear can be read from the curve (Fig. 3).

A functional phenomenon can be considered as a useful criterion of chilling stress if it is (a) specific, i.e. if it occurs at different critical temperatures in different chilling-susceptible species and varieties, and (b) if it is reliable, i.e. if it is invariably observed when the temperature drops below a certain low level but in no case at temperatures above a critical level.

(a) In order to test the specificity of the appearance of L-waves, fluorescence transients were measured in two soybean varieties, 'Evans,' known to be less sensitive, and 'Maple Arrow,' which is considered to be more sensitive (3), and in some other chilling-sensitive and chilling-tolerance angiosperm species during cooling down to 0°C. As Figure 3 shows, L-waves do indeed appear at lower temperatures in the variety 'Evans' than in the variety 'Maple Arrow.' In Saintpaulia ionantha L-waves were observed from a temperature of 9 to 10°C, and in Saccharum officinarum from 7 to 8°C. The sensitivities of these species and varieties were in the same order if \(F_v/F_m\) was calculated. The value of \(F_v/F_m\) at 4°C, as compared with that at 20°C, was lowered by 0.027 (2.9%) in 'Evans,' by 0.046 (5.3%) in 'Maple Arrow,' by 0.047 (5.6%) in S. officinarum, and by 0.142 (17.9%) in S. ionantha. The photochemical quenching coefficient \(qP\) under steady state conditions is lowered by 50% in 'Evans' at approximately 4°C, in 'Maple Arrow' at 5.5°C, in S. officinarum at about 4°C, and in S. ionantha at 9 to 10°C (Fig. 3). Thus our results are in good agreement with the responses of chilling-sensitive crop plants as reported by Havaux (1). A 50% depression of
may be connected with chilling-induced changes in the location of components of the thylakoid membrane (6). Furthermore, it was observed (Fig. 1B; Fig. 2) that the initial $(F_{m} - F_{o})$, before application of actinic light, was lower at low temperatures than at 20°C. The reduction in fluorescence yield in the first light pulse may be explained as a type of nonphotochemical quenching recently described by Schreiber and Neubauer (8, 10); they attribute this saturating light effect to donor side-dependent quenching.

For diagnostic purposes the L-wave phenomenon can, under controlled treatment with cold, and given a knowledge of the fluorescence response to saturating light pulses at room temperature, be regarded as a marker for thresholds of specific chilling susceptibility. Of the various criteria indicating abnormal alterations of vital functions at low temperatures in chilling sensitive species, the occurrence of low-waves appears to be particularly promising on account of the ease of recognition and sensitivity of the response. The nonintrusive nature of the measurements and the speed with which they can be carried out make the method particularly suitable for screening tests.

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**LITERATURE CITED**


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