Vegetation Monitoring of Chilling Stress by Chlorophyll Fluorescence Ratio

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ABSTRACT

The chlorophyll fluorescence ratio F690/F730 was determined in bean seedling (Phaseolus vulgaris L., cv. Mondragone) unchilled and chilled at a low no freezing temperature of 4°C under low and high relative humidity. The two chlorophyll fluorescence emission maxima were monitored using the portable fluorometer LEAF (Laser Excited Automatic Fluorometer) developed by the Institute of Quantum Electronics. The ratio F690/F730 and the relative water content (RWC%) show a more significant decrease when the plants are chilled in light at low relative humidity, although the chlorophyll content does not change during chilling. A complete recovery of the ratio to the initial values (about 0.8-1.0) is evident after a few hours from the transfer of the plants at 20°C, while a slow increase of the relative water content was observed during the chilling treatment. The data indicate that the ratio F690/730 can be a sensitive probe to monitor chilling stress in higher plants, but it is also a useful tool to investigate the complex water relations during chilling stress. This technique, based on laser excited fluorescence spectra, could be an early stress indicator for plants and may be applied in remote sensing detection of vegetation utilizing existing fluorescence lidar systems.

INTRODUCTION

Chilling temperature (i.e.: 0-12 °C) is an important climatic factor affecting growth and development of green plants. The main features of chilling stress are the inhibition of photosynthesis and the disruption of several physiological processes concerning plant water balance, stomatal control of leaf transpiration and hormonal balance. This leads to a reversible or irreversible cellular dysfunction depending on the time of exposure to chilling temperature and on the capacity of the plant to recover when restored to higher temperatures.

The red "in vivo" chlorophyll fluorescence has been widely applied to investigate the photochemical mechanism underlying photosynthesis as well as to detect stress effects to the photosynthetic apparatus.

The present study investigates the possibility of chilling stress monitoring by means of laser induced chlorophyll fluorescence intensity at two peak wavelengths of the emission spectrum, around 690 and 730, measured on the upper leaf surface. Spectrally selective measurements are quite interesting for the detection of plant stresses, since the fluorescence spectral shape can be easily measured in remote sensing by the existing fluorescence lidar systems, both airborne or ground based. This technique, based on the measurement of the ratio of chlorophyll fluorescence intensity at the two peak wavelengths (F690/F730), is also useful in laboratory research since the measurement is much easier and faster than conventional chlorophyll fluorescence induction kinetics.

Highly chilling sensitive plants of Bush Bean (Phaseolus vulgaris L.) were used for this experiment. Plants were grown under controlled conditions and part of them exposed to a low (no freezing) temperature. The laser excited chlorophyll fluorescence ratio F690/F730 was recorded with the LEAF® fluorometer, as described in the next section.

1. METHODS

1.1 Plant material and physiological measurements

Bush Bean (Phaseolus vulgaris L. cv. Mondragone) seedling, 20 days old, were grown under greenhouse conditions in plastic pots filled with expanded clay, during the spring of 1991. The plants were placed in tanks in half strength Cooper nutrient solution continuously aerated. Each tank contained 6 l of nutrient solution for 15 plants. Plants at the stage of fully expanded primary leaves were used for the experiments.

Plants were chilled in a growth chamber at 4 ± 0.5 °C for 96 hr in moderate light intensity of 100 $\mu E/m^2/s$ under high

relative humidity (RH) (100%) and low RH (60%). The high RH treatment was performed enveloping the tank with a transparent plastic sheet. At the end of the experiment the plants were transferred at 20 ± 0.5 °C in same light conditions of chilled plants. Control plants were maintained at 20 ± 0.5 °C and 70 - 60 % RH, with the same light intensity of 100 μE/m²/s.

Throughout the experiment relative water contents and total chlorophyll concentration were measured at regular time intervals on sample leaves.

The relative water content was measured drying leaf discs (14 mm ϕ) punched from intact seedling, both in chilled and unchilled plants.

Chlorophyll concentration was measured with two different methods. The chlorophylls on sample leaves were extracted with 100% acetone and the pigment concentrations were determined according to. Chlorophyll concentration on leaves under fluorescence measurements was optically determined by a Minolta SPAD 502 chlorophyll meter, previously calibrated in comparison with the extraction method.

1.2 Fluorescence measurements

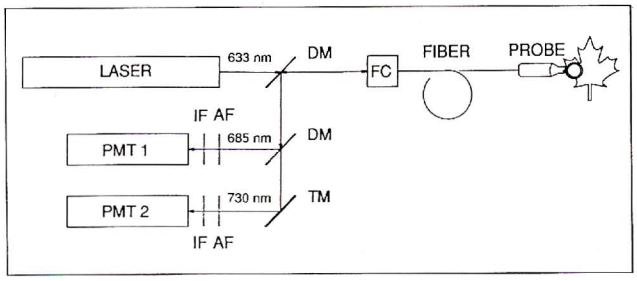

Fig. 1 - Schematic diagram of the LEAF fluorometer. DM are dichroic mirrors. TM is a totally reflecting mirror. FC is the fiber coupling optics. IF and AF are interference and glass absorption filters, respectively.

The red Chlorophyll Fluorescence ratio F690/F730 was measured at chilling temperature (4 ± 0.5 °C) and at room temperature (20 ± 0.5 °C) utilizing the laser-equipped portable two wavelength chlorophyll fluorometer LEAF® (Laser Excited Automatic Fluorometer) developed at IEQ. The LEAF fluorometer uses a HeNe laser (0.5 mW @ 633 nm) for exciting the fluorescence. The laser beam is carried to the leaf by an optical fiber and a suitable coupling optic (fig 1). The same fiber is used to bring back chlorophyll fluorescence, which is separated from the laser beam by a series of dichroic mirrors. The two detection wavelengths are 685 ± 5 nm and 730 ± 5 nm. The bands are selected by interference filters and broadband glass absorption filters placed directly in front of the detectors. The thin optical fiber probe allows a fast sampling of the fluorescence, making it easy to measure many points on each plants, in order to give a statistical significance to the data.

The instrument is small enough (37x29x11) cm to be put directly in the growth chamber. In spite of that it includes a microprocessor to store the acquired data, making a separate data logger unnecessary. The microprocessor also controls laser power supply and the measurement mode. Using the front panel keyboard the user can set the delay of the fluorescence measurement from the onset of the laser radiation and the time interval between successive measurements. The instrument can then perform a complete cycle of automatic measurements fully unattended.

In this experiment all data were sensed on the upper side of the leaf, with a laser pulse duration of 100 ms. The quantum intensity incident of the excitation light on the leaf surface was 600 μE/m²/s. The measurements were performed on fully expanded primary leaves at regular time intervals during the stress treatment and after a period of recovery at 20 ± 0.5 °C in chilled and unchilled plants. For the experiment in high RH conditions, with plants under a transparent plastic sheet, the measurement was done inserting the fiber optics probe under the plastic sheet. This was allowed by flexibility of the thin optical fiber of the LEAF fluorometer.

Two types of experimental protocols were used. At first the fiber optic probe was put on a leaf at the beginning of the chilling treatment and the instruments were set to sample fluorescence at 60 s time intervals, during all the thermal cycle. This is useful to give evidence to the changes in the fluorescence due to chilling alone, without any effect due to possible difference between different leaves and different plants. After that the measurement was repeated at longer time intervals, manually scanning the fiber optic probe on many leaves. For each leaf ten single measurements at random in all upper leaf surfaces were taken and repeated on 30 plants. Each experiment was carried out three times and the average and standard deviation were determined. This procedure simulates better a remotely sensed measurement, where the target is likely to be a random population of leaves of many plants.

2. RESULTS AND DISCUSSIONS

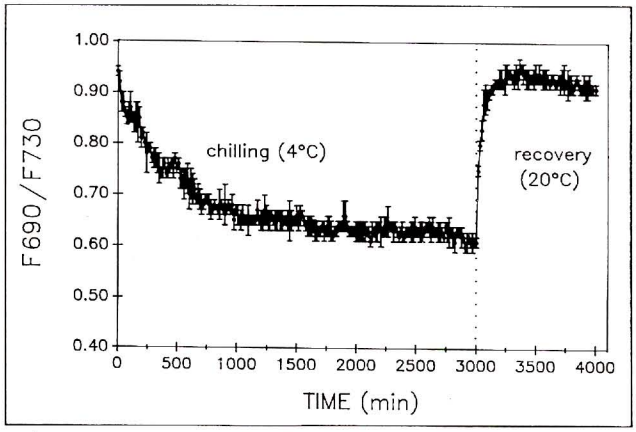

Fig 2 - Time course of chlorophyll fluorescence ratio F690/F730 measured in bean seedling(Phaseolus vulgaris L., cv. Mondragone) during chilling (4 °C) at low RH (60 %) and after a recovery period (20 °C). The ratio was determined by LEAF in auto-repeat mode every minute. Each point represents the average and standard deviation of ten measurements.

The shape of red-laser induced chlorophyll fluorescence emission spectra in intact green leaves shows two maxima around the 685-690 and 730-735 nm regions. The two fluorescence maxima measured at room temperature exhibit about equal intensity with values for fluorescence ratio of 0.85-1.1 when sensed from the upper leaf side.

Fig. 2 shows the variations of chlorophyll fluorescence ratio in a bean leaf during the complete stress cycle, including chilling and recovery. The measurement was performed with the LEAF fluorometer in auto-repeat mode, sampling the same point of the leaf once a minute. During chilling the chlorophyll fluorescence ratio decreases gradually in the first 16 hours, reaching a steady state after 24 hours of chilling. When the plants were transferred at 20 °C a fast increase of chlorophyll fluorescence ratio was evident. In a few hours the plants recover the initial values, as measured before the chilling treatment.

The same behaviour was observed averaging the F690/F730 ratio on many plants, all in the same conditions, both in plants chilled at low and high RH (Fig. 3). The chlorophyll fluorescence ratio decreasing was less marked when the plants were chilled at high RH.

This difference can be understood comparing fluorescence measurements with RWC (fig. 4).

In our experimental conditions, the bean seedlings chilled at low RH showed consistent water stress with loss turgor in the leaf extended in the first 20 hr. of the stress treatment. Then the RWC of the leaf increases again until the complete hydration of the tissues.

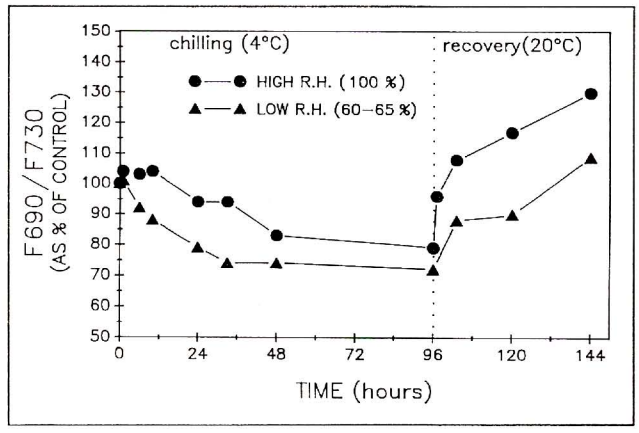

Fig. 3 - Variations of the chlorophyll fluorescence ratio F690/F730 in bean seedling exposed to low and high RH during chilling treatment (4°C) and after a recovery period (20°C). The values are averaged on many leaves and expressed as percentage of control (standard devation 6%).

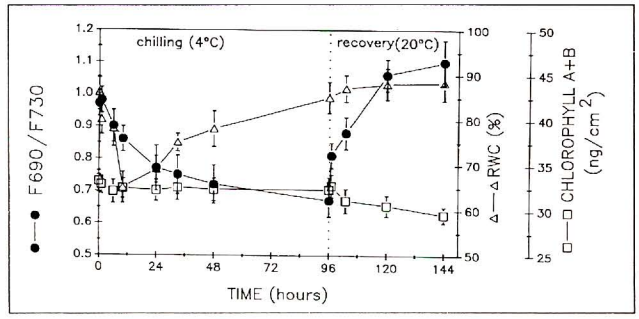

Fig. 4 - Changes of chlorophyll fluorescence ratio F690/F730, relative water content (RWC %), and chlorophyll content (a+b, ng/cm2) in bean seedling exposed to chilling treatment (4 °C) in low RH (60%) and after a recovery period (20 °C). Each point represents the average and standard deviation of measurements on 3 different plants, with 10 measurement per plant for fluorescence.

This effect does not occur in plants chilled at high RH conditions (fig. 5)

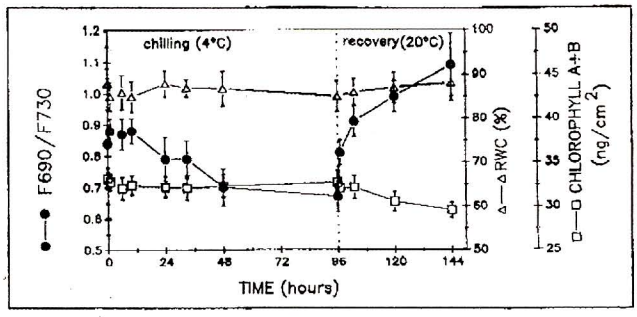

Fig. 5 - Changes of chlorophyll fluorescence ratio F690/F730, relative water content (RWC %), and chlorophyll content (a+b, ng/cm2) in bean seedling exposed to chilling treatment (4 °C) in high RH (100 %) and after a recovery period (20 °C). Each point was averaged as in the previous figure.

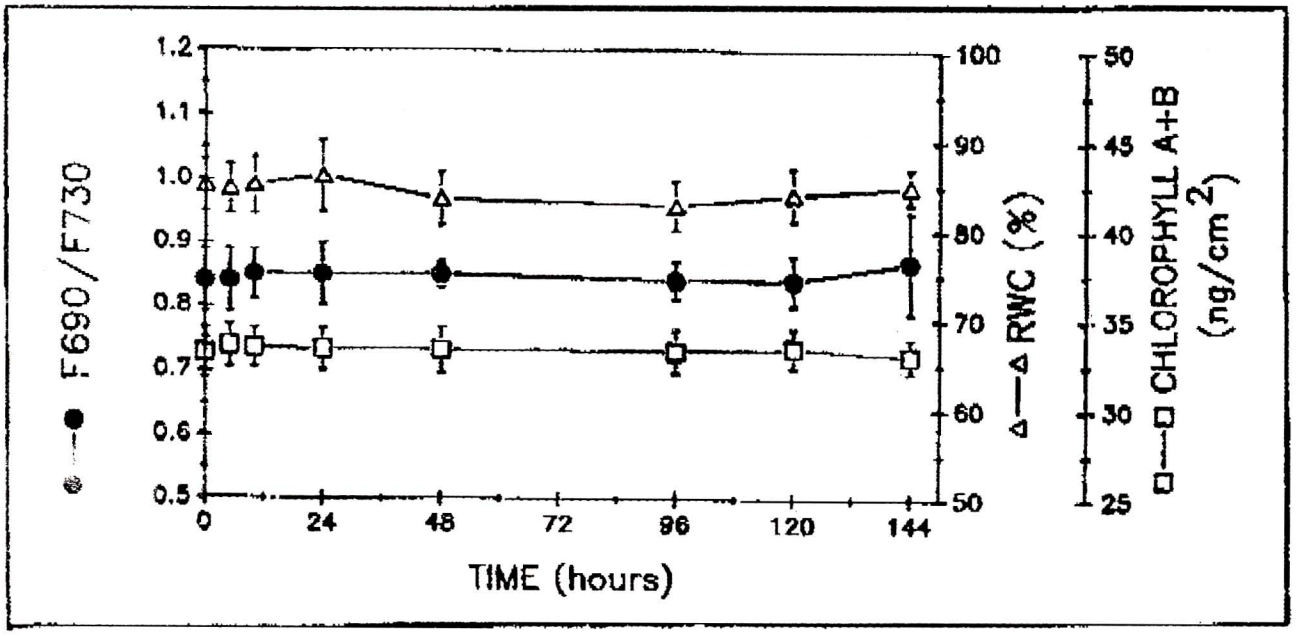

Fig. 6 Determination of chlorophyll fluorescence ratio F690/F730, relative water content (RWC %), and chlorophyll content(a+b, ng/cm²) in unchilled bean seedling grown at 20 ± 1 °C and 70-80% RH. Each point was averaged as in the previous figure.

On the other hand chlorophyll concentration remains constant, within errors, in both conditions (squares in fig. 4 and 5). All the values (fluorescence ratio, RWC and chlorophyll concentration) remain constant in the control plants (fig 6).

The comparison of the data reported in Fig. 4 and 5 indicates that the changes in the chlorophyll fluorescence ratio in chilled plants at low RH are not dependent on leaf water status, but seem to be a specific response of the plant to low temperature, which induced a marked reduction of the total fluorescence yield (data not shown) with the simultaneous decreasing of intensity in Chlorophyll Fluorescence emission spectra at two maxima 690 and 730 nm, although the chlorophyll content remained stable during chilling both low and high RH (Fig. 4, 5). As already reported in literature at low temperature (5 °C) the primary photochemical reaction in the PSI, like that in PSII, is trap limited. The oxidized pigments P-700 (like P-680) still act as a trap for excitation energy (which is dissipated as heat) and therefore the fluorescence yield remains low. On the other hand we observed an increase of the chlorophyll fluorescence ratio a few hours after the transfer of the plants to a higher temperature. Even though these temperature induced changes are presently not well understood, that response may be explained with a quick restoration of photosynthetic activity where the complex process of photosynthesis quantum conversion has been affected during chilling and then most of the excitation energy is dissipated as fluorescence.

According to the chlorophyll fluorescence emission spectrum was found dependent on chlorophyll content and the rate of photosynthetic activity. In our case the decrease of chlorophyll fluorescence ratio in bean seedlings under chilling temperature in low and high RH (Fig.3, 4, 5) cannot be attributed to a change in chlorophyll content but to a significant decline of the rate of photosynthesis as described in many reports. Chilling under moderate light inhibited photosynthesis by up 60-80 % in sensitive species but did not cause a loss of chloroplast pigments or damage to PSII. It is more likely that it involves some terminal component on the reducing side of PSI.

In conclusion the results indicate that the chlorophyll fluorescence ratio is a sensitive probe to monitor chilling stress in higher plants, but it is also a useful tool to investigate the complex water relations (i.e. effects of relative humidity) during chilling stress.

This method based on spectral measurements of laser excited fluorescence presents the advantage to require only a few milliseconds to detect fluorescence while all the kinetics methods have the disadvantage that they always need several minutes for fluorescence recordings in addition to the time for the leaf pre-darkened adaptation. For this reason the ratio F690/F730 is a suitable stress indicator for remote sensing detection of vegetation utilizing existing fluorescence lidar systems.

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