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Assessment of the Chilling Sensitivity of Korean Rice Cultivars by Chlorophyll Fluorescence Analysis

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葉綠素螢光 測定에 의한 水稻 耐冷性 檢定 方法

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ABSTRACT

The potential of the chlorophyll fluorescence technique in screening eleven rice varieties for chill-sensitivity was assessed by measuring the change in the rate of rise of variable fluorescence (F_R) and the maximum yield of variable fluorescence, F_{VAR} ($F_{MAX} - F_0$) after 8 days chilling at 10°C. The changes in both fluorescence parameters with chilling agreed well with visual assessments of injury carried out at the end of the chilling period. Furthermore, it was found that in the majority of the rice cultivars tested their chilling sensitivity agreed well with the rate of rise of chlorophyll fluorescence (F_R) measured at 25°C. The method of chlorophyll fluorescence was rapid, reproducible, sensitive and non-destructive and it should be a valuable new tool in screening large numbers of closely related rice varieties for chilling sensitivity.

INTRODUCTION

Quantitative assessment of visible symptoms of chilling injury such as water soaked patches, scald, necrosis and chlorosis is subjective and time consuming, especially as some of these symptoms only appear after prolonged chilling or a period of rewarming. Many problems exist with the use of physiological techniques such as electrolyte leakage[6], anatomy[5], vital staining and metabolic measurements[4] in quantifying chilling sensitivity. This report investigates the potential of chlorophyll fluorescence analysis as a new tool for the quantitative assessment of chilling injury as it is rapid and can detect damage to the chloroplast membranes before the appearance of visual symptoms of injury.

When a leaf is illuminated the chlorophyll in the membranes of the chloroplasts emits a red fluorescence of which a part, the variable chlorophyll fluorescence (F_R), is responsive to photosystem II activity. Thus any stress such as chilling which directly or indirectly affects photosynthetic metabolism is likely to change the yield of this fluorescence. The technique so far has been mainly applied to chilling stress but it is also considered to have considerable potential in quantifying other stresses such as drought[10] salinity [9] herbicides[1], heat[8], pollutants, and frost[2,3,8].

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MATERIALS AND METHODS

Growth conditions

Sixty-days old rice plants were grown under glasshouse conditions in July and August in Korea. Eleven rice varieties were sown in 1/5,000 a pots at a density of 20 seeds per pot.

Test varieties include:

1. Taebaeg-byeo (I/J)
2. Chungchung-byeo (I/J)
3. Pungsan-byeo (I/J)
4. Chupung-byeo (I/J)
5. Milyang #23 (I/J)
6. Hanganchal-byeo (I/J, Waxy)
7. Manseog-beyo (I/J)
8. Baegyang-byeo (I/J)
9. Chilseong-byeo (I/J)
10. Samnam-byeo (J)
11. Sangpung-byeo (J)

The letters I or J after each variety indicate whether it is an Indica or Japonica or a cross between the two.

Chilling conditions

The plants were chilled in the phytotron at the Crop Experiment Station, Suweon, Korea. Plants were chilled at 10°C, 85% RH and light intensity of 30,000 lux provided by a mixture of fluorescent and mercury lamps on a 12-hours day cycle.

Fluorescence measurements

Fluorescence measurements were made with the Plant Productivity Fluorimeter manufactured by Richard Brancker Research Ltd. (Model SF. 10)[7]. This machine uses a light emitting diode to provide the exciting illumination and the intensity of the illumination used in these experiments were approximately 3.2 μ E $M^{-2}S^{-1}$ for periods of approximately 4 seconds' duration. All readings were made in the dark using a dim green safe light. The probe was placed on the adaxial surface of the leaf. For each variety 5 flag leaves were selected at random and 3 readings made at the base, middle and tip of each leaf. The values presented are therefore the mean of 15 readings. Fluorescence measurements were made after approximately 5 hrs. dark acclimation. The fluorescence of the control was measured at 27°C and the fluorescence of the chilled plants was measured at 10°C. Both the rate of rise of chlorophyll fluorescence (F_R) and the maximum fluorescence yield F_{VAR} ($F_{MAX} - F_0$) were measured.

RESULTS

The changes observed in fluorescence emission with time when a dark adapted rice leaf is illuminated in shown in Fig. 1. Light caused the chlorophyll

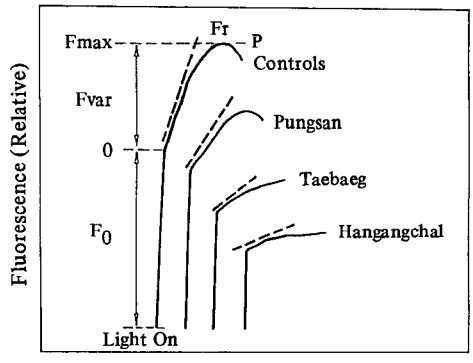

Fig. 1. Changes in the chlorophyll fluorescence kinetics of 3 varieties of rice leaves after 8 days chilling at 10°C, 85% RH. The results are the mean of 15 replicate measurements.

fluorescence to rise immediately to a and this initial level is termed F_0. There then follows a slower increase in fluorescence to a peak F_{MAX} which is attained at p. The increment in fluorescence yield above a represents the yield of induced or variable fluorescence, F_{VAR}. The rate of rise of the variable fluorescence (F_R) can be calculated by drawing a straight line to the linear part of the fluorescence rise as indicated by the dashed line (----) on Fig. 1. It can also be seen from Fig. 1 that both the rate of rise in chlorophyll fluorescence (F_R) and the maximum fluorescence yield (F_{VAR}) is reduced after 8 days chilling in the varieties

Pungsan-byeo (3), Taebaeg-byeo (1) and Hangangchal-byeo (6).

Fig. 2 compares the changes in chlorophyll fluorescence (as a percentage of the control at 27°C) after 8 days chilling at 10°C with the visual estimation of chilling damage made on a 1 to 9 scale at the end of chilling. It can be clarly seen that the Japonica varieties Samnam-byeo (10) and Sangpung-byeo (11) were the most chill tolerant. The fluorescence of these varieties increased by 23 to 34 per cent during 8 days chilling at 10°C.

In contrast the Indica/Japonica varieties Taebaeg-byeo (1), Chilseong-byeo (9), Hangangchal-byeo (6) and Chupung-byeo (4) were the most susceptible to chilling and the decrease in F_R in comparison to the controls was 77, 89, 95 and 98% respectively. The more cold tolerant Indica/Japonica varieties tested showed smaller decreases in chlorophyll fluorescence which agreed well with the visual observations of injury (Fig. 2).

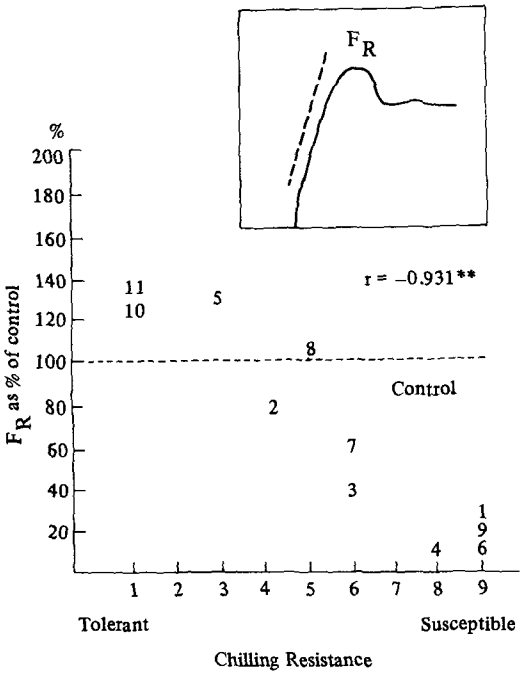

Fig. 2. A comparison of the changes in the rate of rise of varliable fluorescence (F_R) of the leaves of 11 rice varieties (expressed as a percentage of the control at 27°C) after 8 days chilling at 10°C, 85% RH with a visual estimate of injury on a 1 to 9 scale.

Fig. 3 shows that there is also good agreement between chilling injury symptoms and the maximum fluorescence yield (F_{VAR}) of the 11 varieties tested. There is a good correlation between F_{VAR} and F_R readings.

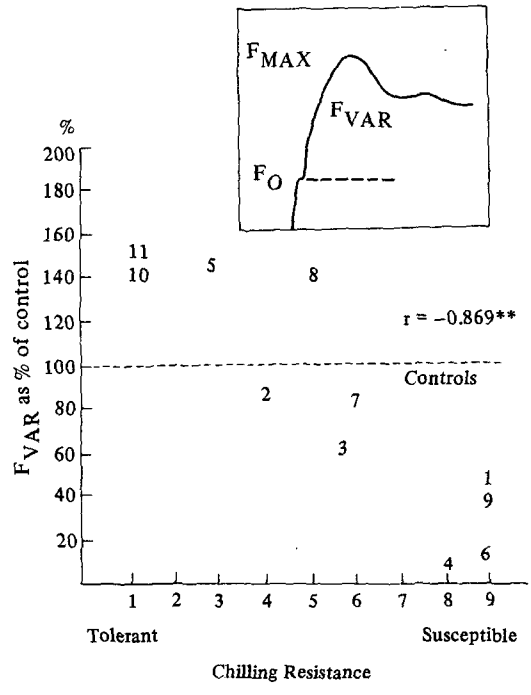

Fig. 3. A comparison of the changes in the yield of variable fluorescence (F_{VAR}) of the leaves of 11 rice varieties (expressed as a percentage of the control at 27°C) after 8 days chilling at 10°C, 85% RH with a visual estimate of injury on a 1 to 9 scale.

It was noticed that the rate of rise of the variable fluorescence (F_R) of the controls at 27°C agreed well with the chill tolerance of the varieties (Fig. 4). The more chill tolerant varieties Samnam-byeo (10) and Sangpung-byeo (11) had higher F_R values than the more chill sensitive varieties. However, the very chill sensitive varieties Taebaeg-byeo (1) and Hangangchal0byeo (6) had high F_R values. The agreement between F_R of the controls and chill sensitivity is therefore not as good as the change in F_R as a percentage of the control after chilling which is shown in Fig. 2.

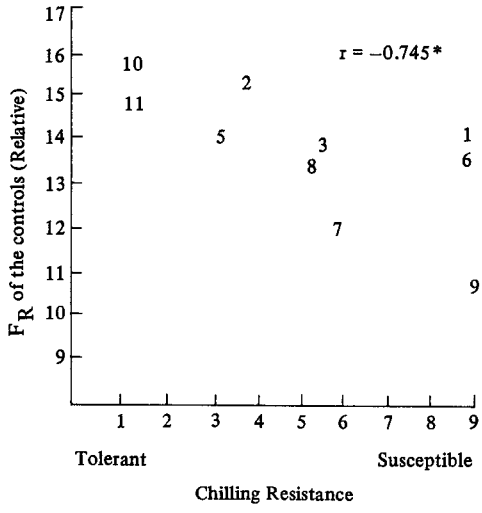

Fig. 4. F_R values of the controls at 27°C compared to the visual assessment of chilling injury (on a 1 to 9 scale) of the 11 rice varieties after 8 days at 10°C, 85% RH.

DISCUSSION

It was possible to rank the varieties tested in order of their chill sensitivity on the basis of either the change in F_R or F_{VAR} values. The increases in the F_R and F_{VAR} values of the more cold tolerant varieties Milyang 23 (5), Baegyang-byeo (8), Samnam-byeo (10) and Sangpung-byeo (11) after chilling at 10°C are unusual and have not been reported previously. These increases can be interpreted as indicating acclimation to low temperatures and may be caused by increased chlorophyll content of the leaves at 10°C. Other research workers may not have detected increases in fluorescence as usually much lower temperatures (5 or 0°C) have been used for the chilling treatments. These temperatures are too low for the hardening or acclimation processes of tropical plants[10].

There has been a need for many years for a quantitative assay of cold tolerance for use by plant breeders that is rapid, reproducible, sensitive, nondestructive and cost effective. Measurement of chlorophyll fluorescence appears to be a very sensitive method for screening large number of closely realted varieties for chilling sensitivity and it fulfills many of the criteria for a good chilling tolerance assay[3]. Furthermore it may be useful in monitoring acclimation processes in the different varieties at low temperature between 10 and 15°C. The ability to detect chill damage to the thylakoid membranes by chlorophyll fluorescence analysis should not only be a useful tool for plant breeders but it should also provide plant physiologists with a valuable insight into the causes and machanisms of chilling injury.

摘　　要

葉綠素螢光測定에 의한 水稻 耐冷性檢定의 可能性을 檢討하기 위하여 耐冷性이 多樣한 11個 品種을 1/5000a pot에 圓型直播하여 育成된 材料들을 穗孕期에 10℃의 低溫에 8日間 處理하여 低溫處理前後의 可變螢光量(F_{VAR})과 螢光增加速度(F_R)의 變化를 比較하였다. 耐冷性이 弱한 品種들에서 低溫處理에 의한 螢光特性들의 減少가 커서 各 品種들의 低溫障害程度와 可變螢光量 및 螢光增加速度의 低溫處理前後의 對比指數는 負의 有意相關을 나타내어 이들 螢光特性들은 稻體營養器官에 나타나는 低溫障害의 程度를 判斷하는데 有用하게 利用될 수 있을 것으로 판단되었다.

葉綠素螢光을 利用한 耐冷性檢定方法은 多量의 育種材料 檢定에 精密性, 經濟性, 迅速性과 함께 檢定에 供試된 個體를 계속 育種에 利用할 수 있어 이의 活用이 期待된다.

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