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형광분석법에 의한 시설재배작물의 광합성 Stress 측정

유장걸* · 송성준** · 한센 우테***

Stress Effects on Photosynthesis of Greenhouse Plants as Measured by the Fluorescence Method

Zang-Kual U.*, Sung-Jun Song** and Ute Hansen***

Abstract

To assess the effects of plant stress by light, temperature, NaCl and soil moisture on photosynthetic activitity, the fluorescence method was used for Chinese cabbage(*Brassica perkinensis* Rupr.), strawberry(*Fragaria grandiflora* Ehrh.) and citrus tree(*Citrus unshiu* Marc.). With decreasing the light intensity, Fv/Fm ratios of intact leaves of Chinese cabbage and strawberry increased significantly, indicating lower photochemical efficiency in PSII system, resulting in an inverse relationship with the photosynthetic activity. Chinese cabbage and strawberry that were grown at higher temperature had higher Fv/Fm ratios and photosynthetic activities, while those given high concentration of NaCl and having low soil moisture had lower values. Chinese cabbage more resistant to salt stress and requiring more water had a greater Fv/Fm ratio than strawberry. In citrus tree, Fv/Fm ratio was lower in the non-irrigated group than the irrigated one. From these results, the fluorescence method was found to be a useful tool which can be used to assess the degree of *in vivo* stress induced by various environmental factors.

Introduction

Since Kautsky and Hirsch[1] reported that fluorescence intensity in green plant leaves displayed characteristic changes on illumination, the use of chlorophyll fluorescence has at first been limited

* Dept. of Agricultural Chemistry, Cheju National University, Cheju, Korea
** Cheju Applied Radioisotope Research Institute, Cheju National University, Cheju, Korea
*** Research Institute for Biophysics, Hannover University, Hannover, Germany

studies of the primary processes of photosynthesis in the laboratory[2]. In recent years, however, there has been remarkable progress in the understanding and practical use of chlorophyll fluorescence in plant science[3,4,5,7]. Furthermore, a new measuring system, the PAM chlorophyll fluorometer[8,9,10] monitors the chlorophyll fluorescence in sun light, thus making it possible to apply chlorophyll fluorescence to plant stress-physiology[11, 12] as well as ecophysiology work[13].

Light quanta absorbed by chlorophyll a are mainly utilized by photochemistry but partly dissipated as fluorescence[14]. If the photochemical reaction is inhibited, the fluorescence intensity increases greatly, giving a closely inverse correlation with photochemical utilisation of light energy. When the intact leaf of the plant is dark-adapted, PSII, reaction centers are open, producing fluoresence (Fo). When such a leaf is illuminated, PSII reaction centers become closed, while fluorescence intensity (Fv) increases gradually, reaching a maximum peak (Fm) when completely photoinhibited by the saturating light pulse[8,14,15,16]. The Fv/Fm ratio (Fv=Fm−Fo) indicates the quantum efficiency of open PSII reaction center [17], thus showing a characteristic change by environmental stress that affects the photochemical efficiency of PSII reaction center[14]. Therefore, the fluorescence signal represents a parameter of the physiological state of the photosynthetic apparatus in intact plant leaves.

The present study aimed to determine by the chlorophyll method the effects of stresses on crops which may develop by conditions in the greenhouse.

Materials and Methods

1. Plant materials

Three plant species were selected for this study, Chinese cabbage (*Brassica perkinensis* Rupr. var. Seoul Ŏskari), strawberry (*Fragaria grandiflora* Ehrh. var. Bokyo), citrus tree (*Citrus unshiu* Marc). Seeds of Chinese cabbage purchased from a commercial seed company were germinated in a petri dish after surface-sterilizing with 1% of NaOCl. The 10 day-old seedlings were transplanted in pots containing Cheju dark brown soil or acid-washed sea sand. Strawberry supplied from a farm in the outskirts of Cheju-shi was transplanted in pots and grown for 1 month in a polyethylene film-covered greenhouse under the daylight.

Also, two varieties (Miyagawa and Okitsu) of citrus grown in pots containing Cheju dark brown soil were used for the experiment.

2. Measurement of Chlorophyll Fluorescence

The Fv/Fm ratios were measured with a portable PAM chlorophyll fluorometer (Walz, Effeltrich, Germany). After dark-adaptation for 5 min, the intact leaf of each of the three plant species (Chinese cabbage, strawberry and citrus) was illuminated by continuous measuring light (650nm, 0.008−10u E m^{-2} S^{-1}) to measure Fo and followed by saturation pulse light (<710 nm, max. 12, 000u E m^{-2} S^{-1}) to measure Fm. Variable fluorescence, Fv was obtained by subtracting Fo from Fm and then, the Fv/Fm ratio was calculated.

3. Photosynthetic Activity

The photosynthetic activity of the intact leaf was measured using a CO_2 analyzer (LI6200, Li-cor, USA).

4. Stress Treatment

Light stress : Chinese cabbages and strawberries were transferred to polyethylene film-covered greenhouse and grown for 1 day at four levels of light intensity (control, 50%, 15%, 5% of sunlight) which were adjusted with the combination of light blocking net.

Temperature stress : Chinese cabbages and strawberries were grown in a growth chamber (10,000 lux) controlled at 10, 20 or 30°C for 1 day for temperature stress.

Salt stress : Sand-cultured Chinese cabbages and strawberries in the growth cabinet(20°C, 10, 000 lux) were supplied with 200ml of 0, 2.5, 50 mM NaCl containing one-fourth dilution of Hoagland solution[18] every day for 4 days.

Water stress : To give water stress, all pots with chinese cabbages and strawberries were irrigated sufficiently to wet the soil, divided into three groups to give three levels (12, 16 and 22%) of soil moisture, weighed every day to record the loss of water, and then supplemented water to maintain each level of soil moisture. In case of citrus trees after all pots were given the same amount of water, half of pots were irrigated 6 liters of water at day 12.

5. Statistical treatment

Five replica were used for each experiment and treatment means were compared with Duncan's multiple range test at 5% level.

Results and Discussion

Light stress : Fv/Fm ratio increased significa-

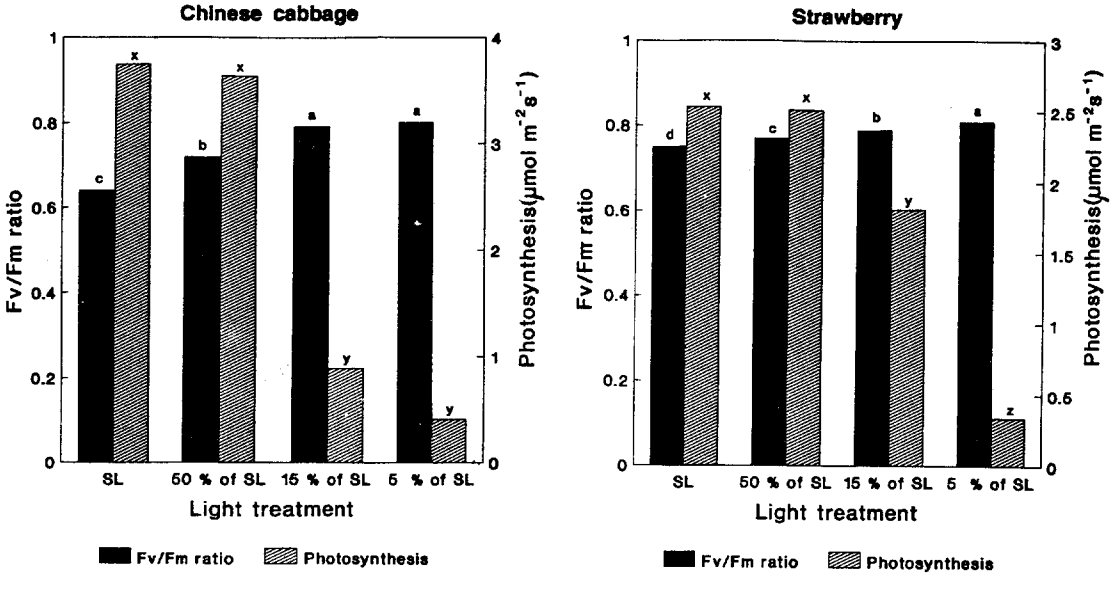

Fig. 1. Changes in Fv/Fm ratios and photosynthesis of Chinese cabbage and strawberry affected by light treatments. Different letters at the top of the bar indicate a significant difference at P=0.05.

ntly with decreasing the light intensity (Fig. 1). Changes in the Fv/Fm ratio were greater in Chinese cabbage than in strawberry, indicating that the efficiency of photochemical reaction was higher with lower light intensity. Also, Fv/Fm ratio was inversely proportional to the photosynthetic activity, suggesting that fluorescence method can be used as a tool for the measurement of photosynthetic activity change. Photosynthetic activity of Chinese cabbage, which has higher light saturation point[19,20], thus being less photoinhibited, was greater than that of strawberry.

Temperature stress : Low temperature reduces the efficiency of enzymes involved in the photochemical reaction. As shown in Fig. 2, Fv/Fm ratio of Chinese cabbage and strawberry were lower in 10°C than in 30°C. Also, Chinese cabbage used for this experiment was a cultivar which grows well in the summer season, while strawberry was resistant to lower temperature[19,20], thus showing lower Fv/Fm ratio of Chinese cabbage than strawberry. As Fv/Fm ratio shows a good correlation with the photosynthetic activity, the fluorescence method can be used in measuring temperature injury of plants.

Salt stress : Most of the salt stresses in nature are due to Na salts, particularly NaCl which affects the osmotic dehydration in plant cell as well as inhibits photosynthesis, respiration and protein metabolism[21]. It was known that chlorophyll content of tomato plant[22] and the rate of CO_2 fixation of *Phaseolus aconitrifolius*[23] decreased by salt stress. In this experiment, when the different concentrations of NaCl were treated to Chinese cabbages and strawberries, their Fv/Fm ratios decreased because of the inefficient photochemical reaction[15] caused by the increased NaCl concentration. Also, Chinese cabbage which was

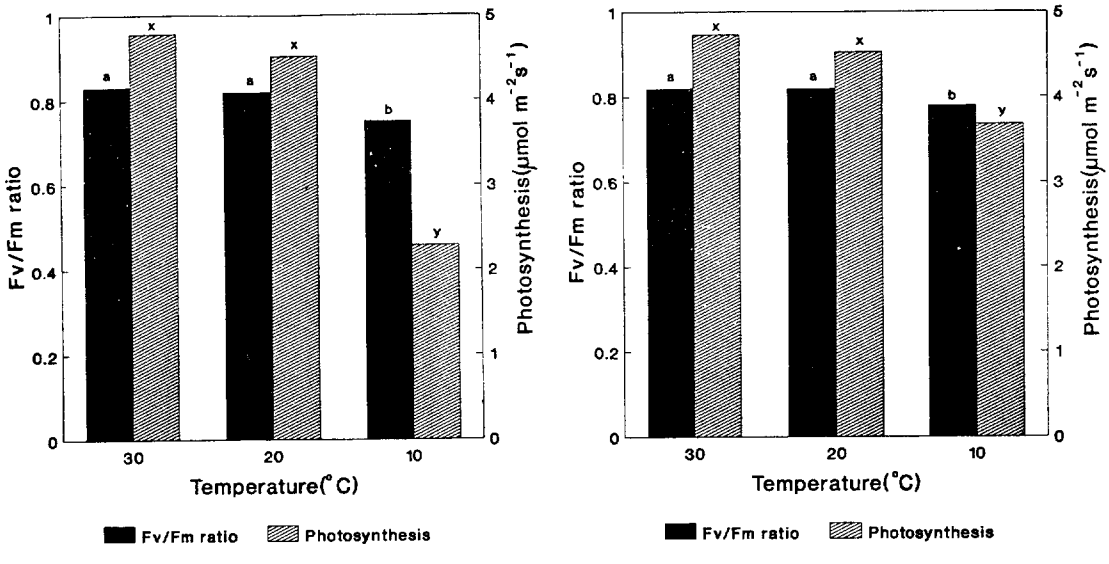

Fig. 2. Changes in Fv/Fm ratios and photosynthesis of Chinese cabbage and strawberry treated with different temperatures. Different letters at the top of the bar indicate a significant difference at P=0.05.

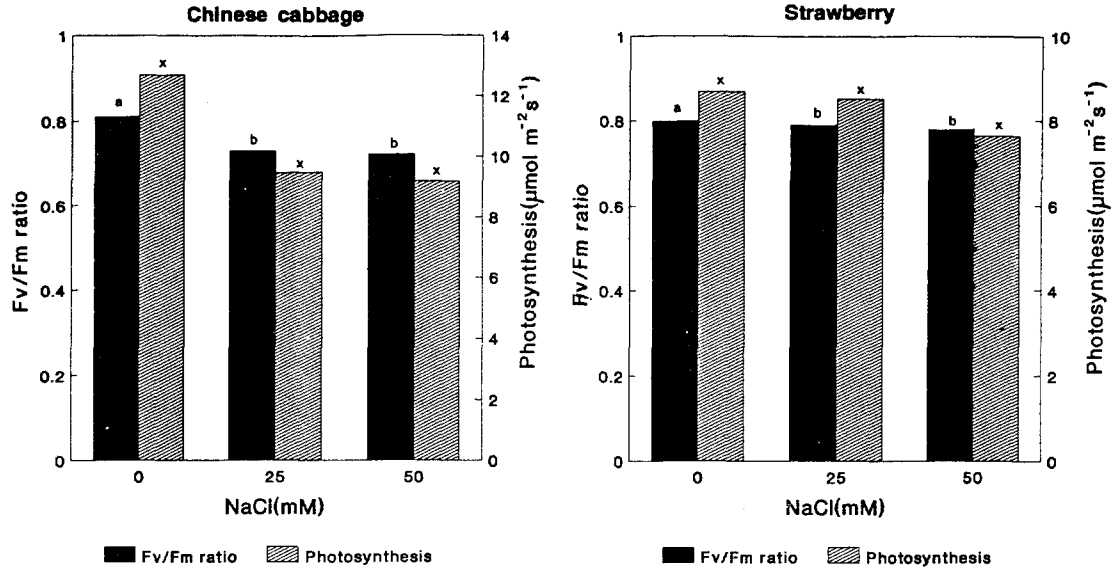

Fig. 3. Changes in Fv/Fm ratios and photosynthesis of Chinese cabbage and strawberry stressed by NaCl treatments. Different letters at the top of the bar indicate a significant difference at P=0.05.

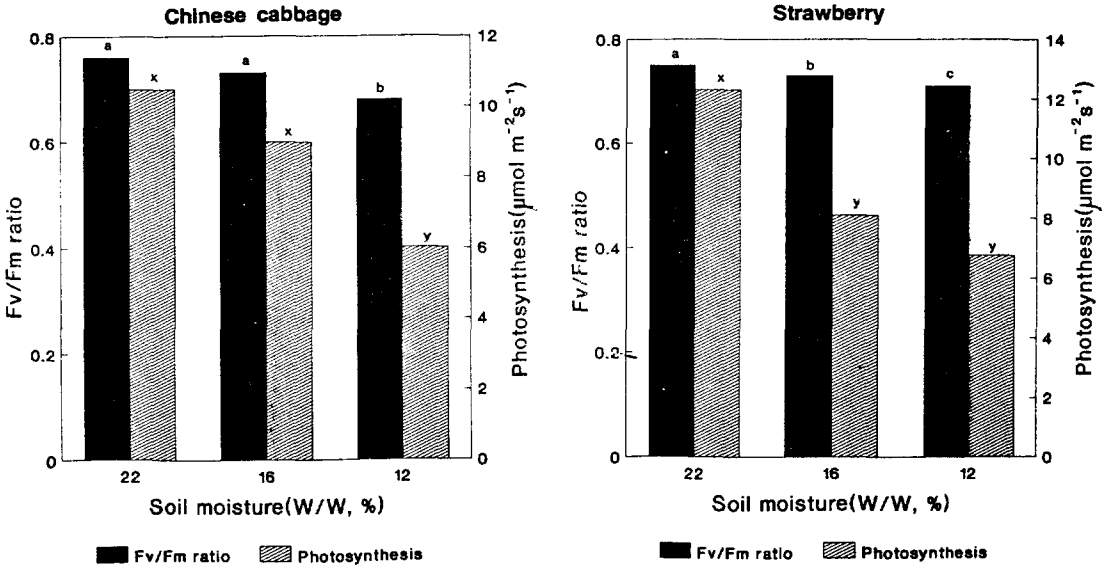

Fig. 4. Changes in Fv/Fm ratios and photosynthesis of Chinese cabbage and strawberry following water stress. Different letters at the top of the bar indicate a significant difference at P=0.05.

much more resistant to salt stress had a greater Fv/Fm than strawberry (Fig. 3).

Water stress : Water stress has negative effects on the development of the plant depending on the degrees of dehydration, the stage of development, the length of exposure, the kind of plant, and so forth[21]. And it was found that some physiological injury (e.g.osmotic dehydration in plant cell, the inhibition of photosynthesis, respiration and protein metabolism) of plant following water stress was similar to salt injury[21].

Like in salt stress, Fv/Fm ratios and photosynthetic activities of Chinese cabbage and strawberry were lower under water stress.

As Chinese cabbage requires much more water than strawberry[19,20], Fv/Fm ratios were lower in Chinese cabbage than in strawberry under the low soil moisture. Also, there was a negative correlation between the Fv/Fm ratio and photosynthetic activity in both plants (Fig. 4).

In citrus trees, changes in Fv/Fm ratio following water stress were also observed. Fv/Fm ratios of both cultivars, Miyagawa and Okitsu were of the same value between watered-/non-watered group at day 5 after irrigation, but differed at day 16, and its difference was bigger at day 23. Therefore, we suggest that Fv/Fm ratio is a good indicator for the detection of water stress in the greenhouse citrus. And the effect of water stress was greater in Miyagawa than Okitsu, indicating that Miyagawa was weaker to water stress than Okisu (Tab. 1).

Table 1. Changes in Fv/Fm value of citrus tree following water stress.

Cultivar	Treatment	Fv/Fm Days after water treatment		
		5	16	23
Miyagawa	Non-watered	0.72	0.67	0.59
	Watered	0.72	0.78	0.77
Okitsu	Non-watered	0.75	0.73	0.69
	Watered	0.75	0.74	0.74

In conclusion, Fv/Fm ratio had a very close correlation with stress factors (light intensity, temperature, salt concentration, soil moisture) affecting the cultivation in greenhouse and photosynthetic activity measured under such stress. Therefore, the fluoresence method can be used as a tool for assessing plant stress as well as photosynthetic activity in intact leaves.

요 약

작물의 광합성 상태를 신속, 정확하게 알아내는 것은 농업생산력 향상은 물론 각종 환경요인에 의해서 작물이 받게 되는 stress 평가를 하기 위해 중요한 일이므로, 형광분석법을 시설재배작물의 광합성 상태 측정에 이용하고자 배추와 딸기를 식물재료로 하여 비닐하우스의 재배 환경 요인으로 작용하는 인자(광도, 온도, NaCl 농도, 토양수분)들이 엽록소형광에 미치는 영향을 조사하였다. 광도가 낮아짐에 따라 배추와 딸기의 Fv/Fm 비가 증가되었는데, 이는 광도가 낮아짐에 따라 PSII 반응센터의 광에너지 전환효율이 낮았기 때문이라고 생각된다. 특히, Fv/Fm 비가 CO_2 analyzer에서 측정된 광합성능과 역비례 관계를 보인 것은 Fv/Fm 비가 식물의 광합성상태와 매우 밀접한 관계가 있음을 나타낸 것이다. 온도처리의 경우 처리온도가 높아짐에 따라 Fv/Fm 값이 높아졌다. NaCl 처리에 의해서 Fv/Fm 비와 광합성능이 낮아졌으며 딸기보다 배추에서 더 컸다. 또한 토양수분함량이 적어짐에 따라 Fv/Fm 비가 감소하였는데 이는 Fv/Fm 비가 광합성과 수분 stress 생리 관계를 연구하는데 좋은 자료로 활

용될 수 있음을 보인 결과로써, 감귤의 경우도 토양 수분 함량이 낮을 때 Fv/Fm 비가 낮은 값을 보여 감귤나무의 수분 stress 정도를 *In vivo* 상태에서 경시적으로 관찰할 수 있는 예민한 방법으로 형광분석법이 활용될 수 있으리라 기대된다.

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