Leaf Age Dependence of Chlorophill Fluorescence Parameters in Water-stressed Leaves of *Phaseolus vulgaris* L.

Kazuo Tobe and Kenji Omasa*

(The National Institute for Environmental Studies,
16-2, Onogawa, Tsukuba, Ibaraki, 305-0053 Japan)

Abstract

To investigate leaf age dependence in the responses of photosynthetic functions to water stress, the changes in fluorescence parameters of bean (*Phaseolus vulgaris* L.) leaves were examined while the leaf water potential (Ψ_{LW}) was gradually decreased by withholding water supply to the soil; leaves of three different ages were used from each plant. No significant leaf age dependence was detected in any fluorescence parameters when the Ψ_{LW} was higher than -0.9 MPa, the point at which the net CO_2 uptake rate approached zero. However, at Ψ_{LW} lower than -0.9 MPa, both the maximum PS II photochemical efficiency (F_V/F_M) and the quantum yield of non-cyclic electron transport in PS II (ϕ_e) decreased with decreasing Ψ_{LW}. The decreases in both F_V/F_M and ϕ_e were larger in younger leaves. More conspicuous leaf age dependence was detected in the response of the steady-state nonphotochemical quenching coefficient (q_N). Water stress-induced changes in all these fluorescence parameters recovered to pre-stress levels within a day after rewatering. The results showed that the changes in the fluorescence parameters did not result from damage to the photosynthetic systems, but from changes in physiological conditions caused by the cessation of CO_2 supply to the stroma or changes in stromal water status. It was concluded that leaf age is an important factor in determining the responses of fluorescence parameters to water stress.

Key words: Chlorophyll fluorescence, Leaf age, *Phaseolus vulgaris* L., Photosynthesis, Water stress.

1. Introduction

Water stress causes stomatal closure even before the leaf water potential begins to decrease (Davies and Zhang, 1991), thus increasing the resistance of CO_2 entering the leaves. Reduced CO_2 supply to the chloroplasts invokes light energy dissipation to avoid damage caused by generation of surplus reductivity (Walker, 1992; Smirnoff, 1993). In addition, water stress can bring about decreases in photosynthetic capacity by inhibiting electron transport, photophosphorylation and metabolic processes

in the chloroplasts (Kaiser, 1987; Lawlor, 1995).

Fluorescence analysis has been applied to evaluate the effects of water stress on photosynthetic functions in leaves (*e.g.* Stuhlfauth *et al.,* 1988; Stuhlfauth *et al.,* 1990; Scheuermann *et al.,* 1991; Jefferies, 1994; Biehler and Fock, 1996). Genty *et al.* (1987) and Scheuermann *et al.* (1991) showed from fluorescence measurements that responses of photosynthetic functions of leaves to water stress differ among species. However, differences in the responses of photosynthetic functions of leaves of different ages to water stress are not clearly understood.

An individual plant comprises leaves of a wide age range and these are arranged vertically at different positions on the stem. Leaves of different ages exhibit different photosynthetic functioning (Jenkins and Woolhouse, 1981; Somersalo and Aro, 1987; Croxdale and Omasa, 1990). Moreover, when the

plant is water-stressed, the leaves show different water relations depending on leaf age (Ackerson and Hebert, 1981; Morgan, 1984; Zhang and Davies, 1989). Therefore, water-stressed leaves in individual plants can show age-dependent responses of photosynthetic functions.

The aim of the research presented here was to compare changes in fluorescence parameters of leaves of different ages within individual plants of bean (*Phaseolus vulgaris* L.) when the leaf water potential (Ψ_{LW}) was gradually decreased by withholding water supply to the soil. The study included an examination of the responses of fluorescence during the recovery from water stress.

2. Materials and Methods

2.1 Plant materials

Bean (*Phaseolus vulgaris* L. cv. Shin-edogawa) plants were used for the experiments. The seeds were sown in pots containing 1.6 l of artificial soil (mixture of vermiculite, peat, perlite and fine gravel, 2 : 2 : 1 : 1 v/v). After emergence, the seedlings were thinned to one plant per pot to increase their uniformity. These plants were grown in an environmentally controlled greenhouse (temperature: 25°C/20°C in day/night, relative humidity: 60%). The plants were watered every day and supplied with nutrients twice a week. The plants were used for the experiments 4–5 weeks after sowing when they had 6–7 fully expanded leaves.

2.2 Measurements

Five to ten plants were transferred to an environmentally controlled growth cabinet (temperature: 25°C; relative humidity: 70%) the day before the first measurements. The plants were illuminated from above with stannous halide vapor lamps (photosynthetic photon flux density (PPFD) on the upper portion of plants: 450 μ mol m^{-2}s^{-1}; light period: 6: 00–18: 00). These plants were water-stressed by withholding water supply to the soil, but two or three plants were watered every day as controls. After withholding water for 5–12 d, water supply to the soil was resumed. During the course of these treatments, gas-exchange rates and fluorescence parameters were measured every day using the same three leaves differing in age in each of two water-withheld plants or one water-withheld and one control plant. Leaf water potential (Ψ_{LW}) was measured every day in three leaves differing in age;

the leaf samples were selected from leaves of three to seven water-stressed plants. The measurements of Ψ_{LW}, gas-exchange rates and fluorescence were made during 9: 00–10: 00, 9: 00–12: 00 and 9: 00–17: 00, respectively; rewatering the water-stressed plants was done at about 17: 00. The above experiment was repeated six times. In addition to these experiments, in order to investigate the recovery from water stress precisely, plants were not watered for 5 d, then rewatered at about 9: 00. Subsequent changes in fluorescence in their young leaves were then measured.

Three leaf samples differing in age were selected as follows: young leaves were selected from the 6 th–7 th leaves having leaf areas of 20–30 cm^2; middle-aged leaves were selected from the 4 th–5 th leaves having the largest leaf area; old leaves were selected from the primary leaves. The terminal leaflets of the trifoliates were used for the young and middle-aged leaf samples.

Fluorescence measurements were made at about 25°C using the pulse amplitude modulation (PAM) chlorophyll fluorometer (PAM 101, 102, 103, Walz, Effeltrich, Germany) on the adaxial leaf surfaces of attached intact leaves following the method of Schreiber *et al.* (1986). Prior to the measurement, the leaf was kept in the dark for 20 min, and thereafter maximal (F_M) and variable (F_V) fluorescences were measured to determine maximum PS II photochemical efficiency (F_V/F_M) (Butler and Kitajima, 1975). Then the actinic light was illuminated. The saturation pulse of 700 ms was also radiated at 20-s intervals with the actinic light for the determination of induction and steady-state values of photochemical and nonphotochemical quenching coefficients (q_P and q_N, respectively). Further, quantum yield of non-cyclic electron transport in the PS II (ϕ_e) was calculated according to Genty *et al.* (1989). The incident PPFD on the leaf surface of the 100 kHz-pulsed measuring light, the actinic light and the saturation pulse were 1.5, 40, 2,200 μ mol m^{-2}s^{-1}, respectively.

Net CO_2 uptake and transpiration rates were measured in the cabinet under PPFD of 450 μ mol m^{-2}s^{-1} with a small open-flow gas-exchange chamber (Shimazu, SPB-H 2), which allows measurements of gas exchange rates of both sides of a leaf on a 6.25 cm^2 rectangular area. Ψ_{LW} was measured with an isopiestic psychrometer (Boyer and Knipl-

ing, 1965) using excised leaf samples.

3. Results

3.1 Changes in leaf water potential and gas-exchange rates

After water was withheld, the Ψ_{LW} decreased by a mean rate of about $0.1 \, \text{MPa d}^{-1}$, and Ψ_{LW} was almost the same among the three leaves differing in age in individual plants. Before the Ψ_{LW} began to decrease, Ψ_{LW} had been about 0.1 MPa lower in the young leaves than in the old and middle-aged leaves of the same plants. The lower Ψ_{LW} of non-stressed young leaves is an artefact caused by cutting growing tissue (Cosgrove *et al.*, 1984). Consequently, in this report, gas-exchange rates and fluorescence parameters of leaves differing in age were presented as functions of Ψ_{LW} of middle-aged leaves (Fig. 1-4).

Changes in net CO_2 uptake and transpiration rate with decreasing Ψ_{LW} are shown in Fig. 1 for the three leaves of different ages. Both the gas-exchange rates decreased with decreasing Ψ_{LW} from -0.3 to -0.9 MPa and were near zero when Ψ_{LW} was below -0.9 MPa. The gas-exchange rates of the young leaves approached zero at lower Ψ_{LW} than those of the older leaves. The old leaves showed lower gas-exchange rates than the younger leaves at higher Ψ_{LW}.

3.2 Changes in steady-state fluorescence

Figures 2-4 show changes in fluorescence parameters with changing Ψ_{LW}. When Ψ_{LW} was higher than -0.9 MPa, none of the changes was prominent, and no significant age dependence was detectable in these parameters, except that the old leaves showed slightly lower ϕ_e than the younger leaves. However, at Ψ_{LW} lower than -0.9 MPa, F_V/F_M, ϕ_e and q_N changed with decreasing Ψ_{LW}, and leaves differing in age showed differences in the fluorescence parameters. The decrease in F_V/F_M was less for the old leaves than for the younger leaves (Fig. 2). Also, the decrease in ϕ_e was most pronounced in the young leaves and smallest for the old ones (Fig. 3). For steady-state quenching coefficients (Fig. 4), q_N responded differently depending on the leaf age, while q_P was maintained near 1 over the entire range of Ψ_{LW}. In the young leaves, q_N was almost constant when the Ψ_{LW} was higher than -0.9 MPa, but with further decrease in Ψ_{LW}, it initially increased and subsequently decreased. Changes in q_N in the old leaves, however, were less conspicuous; these
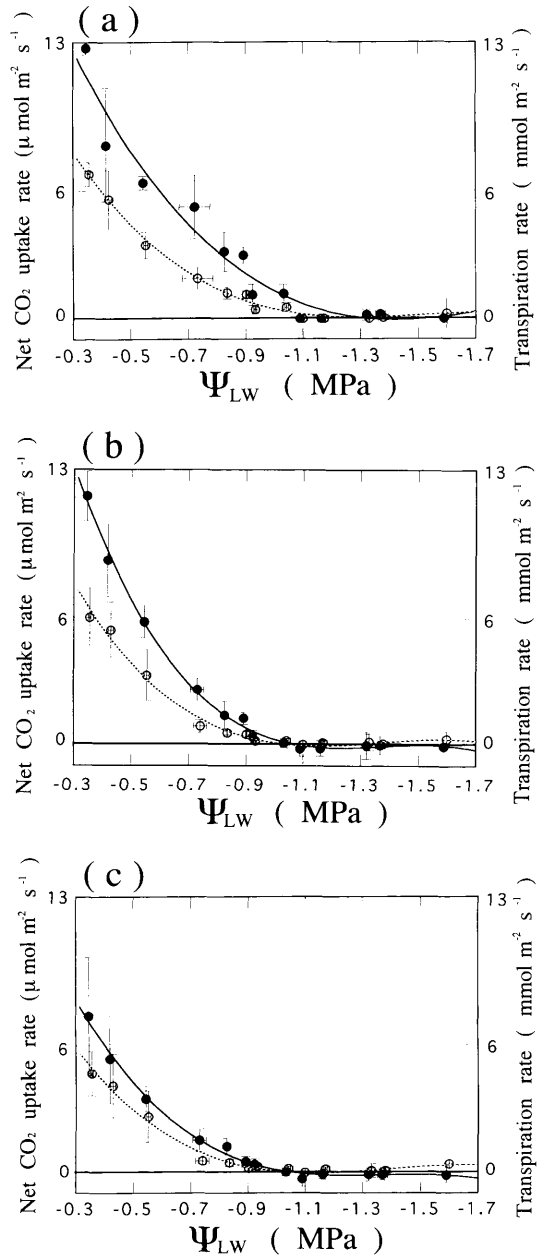

Fig. 1. Changes in net CO_2 uptake and transpiration rate with decreasing leaf water potential in young (a), middle-aged (b) and old (c) leaves. Symbols ● and ○ represent means of 3-11 replicates of net CO_2 uptake rate and transpiration rate, respectively; solid and dotted lines show changes in net CO_2 uptake and transpiration rates, respectively. Error bars indicate 90% confidence intervals. The gas-exchange rates were measured under a PPFD of $450 \, \mu \text{mol} \, \text{m}^{-2} \text{s}^{-1}$. The horizontal axis represents the leaf water potential of the middle-aged leaves.
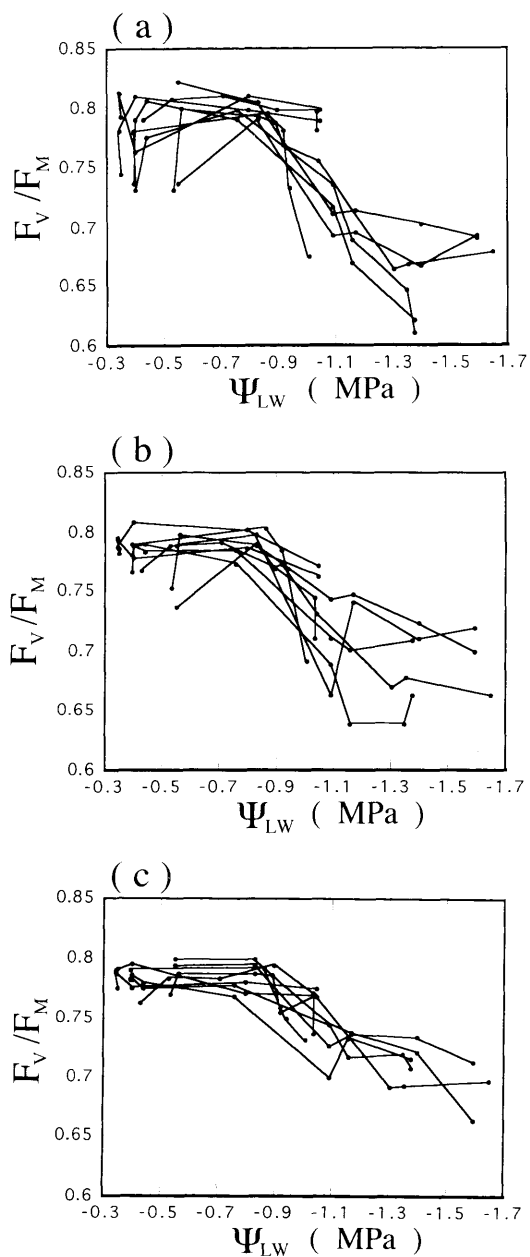
Fig. 2. Changes in F_V/F_M with decreasing leaf water potential. (a), (b) and (c) correspond to changes in the young, middle-aged and old leaves, respectively. Values of F_V/F_M of the same leaves are connected with solid lines. The horizontal axis represents the leaf water potential of the middle-aged leaves.
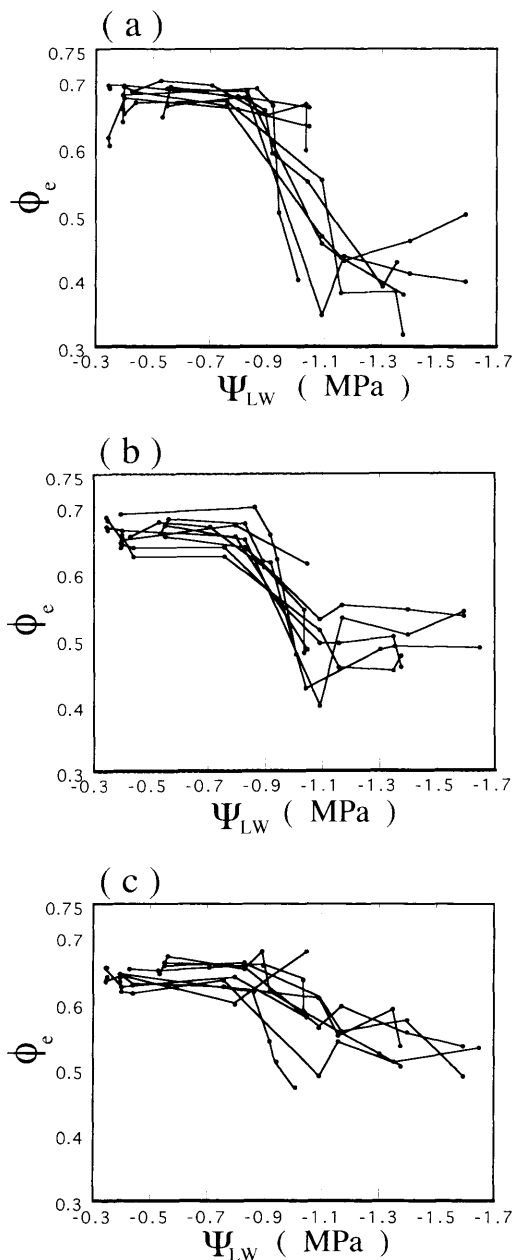
Fig. 3. Changes in the quantum yield of non-cyclic electron transport in the PS II (ϕ_e) with decreasing leaf water potential. (a), (b) and (c) correspond to changes in the young, middle-aged and old leaves, respectively. Values of ϕ_e of the same leaves are connected with solid lines. The horizontal axis represents the leaf water potential of the middle-aged leaves.
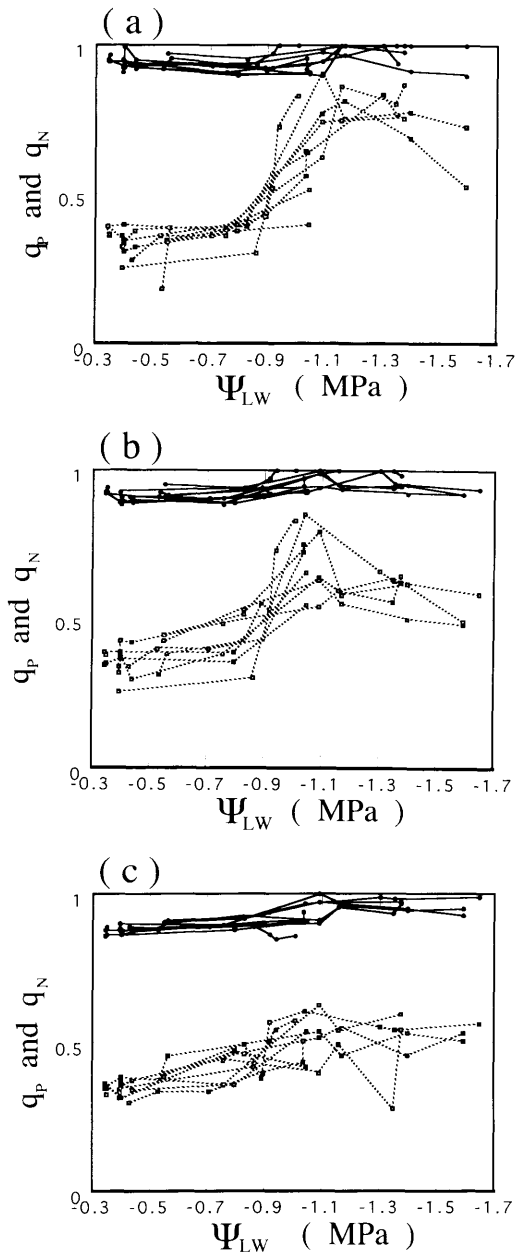
Fig. 4. Changes in steady-state quenching co-efficients with decreasing leaf water potential. (a), (b) and (c) correspond to changes in the young, middle-aged and old leaves, respectively. Solid and dotted lines represent, respectively, changes in q_P and q_N of the same leaves. The horizontal axis represents the leaf water potential of the middle-aged leaves.

showed a steady slow increase with decreasing Ψ_{LW}. For the middle-aged leaves, some showed the same changes as the young leaves and others exhibited the same tendency as the old leaves.

For all tested leaves, these water stress-induced changes in the fluorescence parameters returned to the pre-stress levels when fluorescence was measured 17–20 h after rewatering. For all the leaves, the steady-state q_P decreased by 2–5% after rewatering (see Fig. 5 c–e). In control plants, which were watered every day, no changes in these fluorescence parameters were detected in any tested leaves during the period of the experiments.

3. 3 Changes in induction of fluorescence

Changes in q_P and q_N after dark-light transition in all the tested leaves before and after water stress were similar to those shown in Fig. 5 a, b. Water stress caused a steeper initial q_P drop and greater overall rise in q_N (see Fig. 5 a, b). These changes in the patterns of transient quenching coefficients caused by water stress had recovered when fluorescence was measured 17–20 h after rewatering.

To investigate further the changes in the transient patterns of q_P and q_N during recovery from water stress, transient changes in q_P and q_N were examined in young bean leaves at various times during the periods when water was withheld for 5 d and during the subsequent rewatering (Fig. 5). The initial q_P drop was greater when the leaf was water-stressed (Fig. 5 b), but almost undetectable 4 h after rewatering (Fig. 5 c): it then gradually became deeper with time and approached the level before water stress (Fig. 5 d, e). The steady-state q_N recovered to the level before water stress within 4 h after resumption of the water supply (Fig. 5 c), but the recovery of the transient rises in q_N required more time.

4. Discussion

The close correlations in the rates of decreases of transpiration and net CO_2 uptake (Fig. 1) indicate that the decrease in CO_2 uptake rate caused by water stress was closely related to stomatal closure. The implication that stomata in the young leaves closed at lower Ψ_{LW} than those in the older leaves (Fig. 1) is in agreement with other reports (Turner, 1974; Jordan et al., 1975; Lawler and Milford, 1975).

In the fluorescence measurements, well-watered leaves did not show any significant age-dependent differences in fluorescence parameters except that the
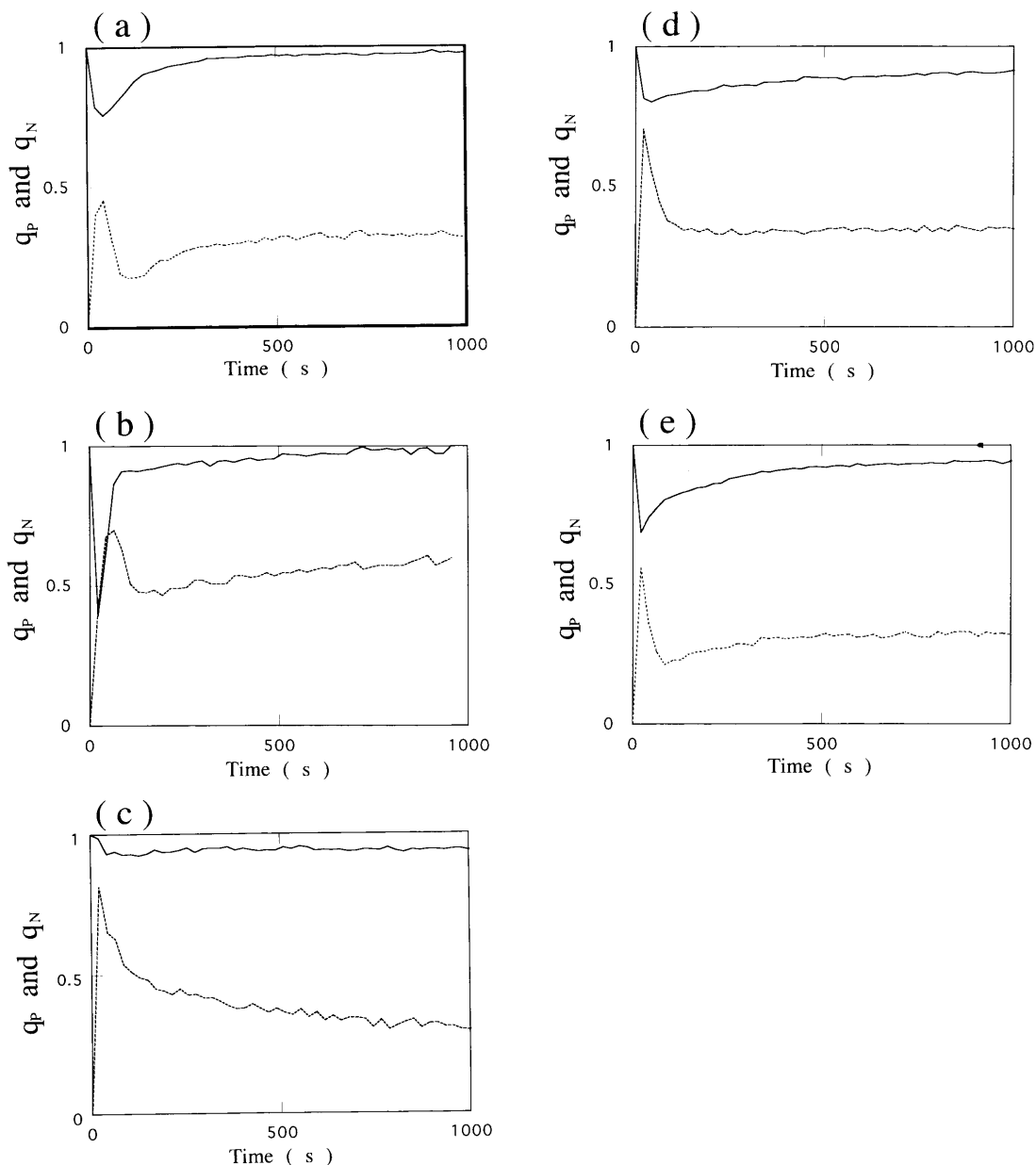
Fig. 5. Typical changes in induction of quenching coefficients of a young bean leaf: (a) before water-withholding, (b) 5 d after water-withholding and just before resuming water supply (Water supply to the plants was resumed at about 9 : 00), (c) 4 h after resuming water supply, (d) 7 h after resuming water supply, (e) 24 h after resuming water supply. Solid and dotted lines represent changes in q_P and q_N, respectively.

old leaves showed a slightly lower ϕ_e than younger leaves. This result is in contrast to other reports (Jenkins and Woolhouse, 1981; Somersalo and Aro, 1987; Croxdale and Omasa, 1990), which show different responses of fluorescence in leaves differing in age in non-stressed plants. This discrepancy may be because the range of leaf age was more limited in our measurements than in these previous reports. However, water stress brought about age-dependent differences in the responses of fluorescence parame-

ters. Because the fluorescence parameters of the control plants were unchanged during the experiments, the differences can be interpreted as resulting from water stress.

At Ψ_{LW} higher than -0.9 MPa, fluorescence parameters did not show significant changes, while the gas exchange rates decreased very markedly with decreasing Ψ_{LW}. Significant changes in fluorescence parameters occurred only after net CO_2 uptake rate became almost zero. Both F_V/F_M and ϕ_e suddenly decreased for all the tested leaves. This result agrees substantially with the results in the same species reported by Cornic *et al.* (1989), who measured changes in the quantum yield of O_2 evolution when leaf water deficit (LWD) of the leaves was gradually increased. They report that the quantum yield was constant when LWD was lower than 30%, but when LWD was higher than 30%, the point at which net CO_2 uptake rate approached zero, the quantum efficiency decreased with increasing LWD. It should be noted that the Ψ_{LW} at which F_V/F_M and ϕ_e began to decrease was almost the same among the leaves differing in age, but that the decreases in both F_V/F_M and ϕ_e were larger in younger leaves. This suggests that some water stress-induced changes in leaves, which are not leaf age-dependent, triggered the drops in both F_V/F_M and ϕ_e simultaneously, but to different degrees in leaves of different ages.

The decreases in F_V/F_M and ϕ_e contribute to reduced generation of residual reductivity and energy, but were not in parallel with changes in CO_2 uptake rate. Also, the decreases are rather modest compared with the drastic decreases in CO_2 uptake rate. Therefore, to compensate for the imbalance between the decreases in CO_2 uptake rate and the electron transport rate, some processes such as Mehler-ascorbate peroxidase reaction and/or photorespiration presumably must consume the residual energy under CO_2-limited conditions (Stuhlfauth *et al.*, 1988; Cornic *et al.*, 1989; Scheuermann *et al.*, 1991; Cornic, 1994; Biehler and Fock, 1996) to avoid damage caused by surplus reductivity generation (Walker, 1992; Smirnoff, 1993).

The steady-state q_P was near 1 for all the tested leaves regardless of the Ψ_{LW} (Fig. 4). This would be because the actinic light intensity was rather weak (PPFD: 40μ mol m^{-2}s^{-1}) in these measurements. However, the steady-state q_N showed different re-

sponses depending on the leaf age. For the old leaves and some of the middle-aged leaves, q_N showed a trend of constant slow increases with decreasing Ψ_{LW} (Fig. 4 b, c). Similar responses of steady-state q_N with changing Ψ_{LW} have also been reported in bean (*Phaseolus vulgaris* L.) and maize by Scheuermann *et al.* (1991). In the young leaves and some of the middle-aged leaves, however, steady-state q_N began to increase rapidly at Ψ_{LW} of -0.9 MPa (Fig. 4 a, b), while it remained constant at higher Ψ_{LW}. A higher q_N results from higher transthylakoidal pH gradient, which is caused by reduced photophosphorylation. Therefore, a q_N rise can be caused by restrictions of ADP-regenerating reactions (*e.g.* some reactions in Calvin cycle) and/ or ATP-synthesizing reactions. The different responses of steady-state q_N depending on leaf ages suggest different characteristics in the energization and deenergization of the membranes. These age-dependent changes in the responses of steady-state q_N to water stress suggest that the characteristics of the chloroplasts change at a developmental stage near the completion of leaf expansion.

The changes in the transitional quenching coefficients while the plant was water-stressed and subsequently rewatered in young leaves (Fig. 5) are similar in some aspects with those of *Digitalis lanata* leaves reported by Stuhlfauth *et al.* (1988). The most obvious differences in our results are 1) that the initial q_P drop was more conspicuously changed and 2) changes in steady-state q_N occured. These differences presumably resulted from differences in the species tested. The deepening of the initial q_P drop in the water-stressed leaves may result from dehydration-induced changes in the state of thylakoid membranes, and the changes in the initial q_P after rewatering may reflect dilution and subsequent readjustment of stromal solution. After resumption of the water supply to the water-stressed plants, the steady-state q_P was slightly decreased. The decrease in steady-state q_P after rewatering might indicate that the resumption of water supply to the chloroplasts caused some change in the thylakoid membranes.

In conclusion, water-stressed leaves within a plant can show leaf age dependence in the responses of fluorescence parameters. The rapid recovery of the fluorescence parameters after resumption of the water supply (*e.g.* Fig. 5 c–e) suggests that these

changes in the parameters did not result from damage to the photosynthetic systems, but from changes in physiological conditions caused by the cessation of CO_2 supply to the stroma or changes in stromal water status. The reasons for the responses of these parameters and for the age dependence of these responses remain unknown, but these results show that leaf age is an important factor in determining the responses of the photosynthetic system of leaves to water stress.

References

Ackerson, R. C. and Hebert, R. R., 1981: Osmoregulation in cotton in responses to water stress. I. Alterations in photosynthesis, leaf conductance, translocation, and ultrastructure. *Plant Physiol., 67*, 484–488.

Biehler, K. and Fock, H., 1996: Evidence for the contribution of the Mehler-peroxydase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiol., 112*, 265–272.

Boyer, J. S. and Knipling, E. B., 1965: Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proc. Natl. Acad. Sci. USA, 54*: 1044–1051.

Butler, W. L. and Kitajima, M., 1975: Fluorescence quenching photosystem of chloroplasts. *Biochim. Biophys. Acta, 376*, 116–125.

Cornic, G., 1994: Drought stress and high light effects on leaf photosynthesis. In Baker, N. R. and Bowyer, J. R., Editor: *Photoinhibition of Photosynthesis.* BIOS Scientific Publishers, Oxford, pp. 297–313.

Cornic, G., Le Gouallec, J.-L., Briantais, J. M. and Hodges, M., 1989: Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.). *Planta, 177*, 84–90.

Cosgrove, D., van Volkenburgh, E. and Cleland, R., 1984: Stress relaxation of cell walls and the yield threshold for growth. Determination and measurement by micropressure probe and psychrometer techniques. *Planta, 162*, 4–54.

Croxdale, J. G. and Omasa, K., 1990: Chlorophyll fluorescence and carbon assimilation in developing leaves of light-grown cucumber. *Plant Physiol., 93*, 1078–1082.

Davies, W. J. and Zhang, J., 1991: Root signal and the regulation of growth and development of plants in drying soil. *Ann. Rev. Plant Physiol. Plant Mol. Biol., 42*, 55–76.

Genty, B., Briantais, J.-M. and Baker, N. R., 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta, 990*, 87–92.

Genty, B., Briantais, J.-M. and Viera Da Silva, J. B., 1987: Effects of drought on primary photosynthetic processes of cotton leaves. *Plant Physiol., 83*, 360–364.

Jefferies, R. A., 1994: Drought and chlorophyll fluorescence in field-grown potato (*Solanum tuberosum*). *Physiol. Plant., 90*, 93–97.

Jenkins, J. I. and Woolhouse, H. W., 1981: Photosynthetic electron transport during senescence of primary leaves of *Phaseolus vulgaris* L. II. Noncyclic electron transport. *J. Exp. Bot., 32*, 467–478.

Jordan, W. R., Brown, K. W. and Thomas, J. C., 1975: Leaf age as a determinant in stomatal control of water loss from cotton during water stress. *Plant Physiol., 56*, 595–599.

Kaiser, W. M., 1987: Effects of water deficit on photosynthetic capacity. *Physiol. Plant., 71*, 142–149.

Lawlor, D. W., 1995: The effects of water deficit on photosynthesis. In Smirnoff, N., Editor: *Environment and Plant Metabolism.* BIOS Scientific Publishers, Oxford, pp. 129–160.

Lawler, D. W. and Milford, G. F. J., 1975: The control of water and carbon dioxide flux in water-stressed sugar beet. *J. Exp. Bot., 26*, 657–665.

Morgan, J. M., 1984: Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol., 35*, 299–319.

Scheuermann, K., Biehler, K., Stuhlfauth, T. and Fock, H. P., 1991: Simultaneous gas exchange and fluorescence measurements indicate differences in the response of sunflower, bean and maize to water stress. *Photosyn. Res., 27*, 187–197.

Schreiber, U., Schliwa, U. and Bilger, W., 1986: Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosyn. Res., 10*, 51–62.

Smirnoff, N., 1993: The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol., 125*, 27–58.

Somersalo, S. and Aro, E.-M., 1987: Fluorescence induction in pea leaves of different ages. *Photosynthetica, 21*, 29–35.

Stuhlfauth, T., Scheuermann, R. and Fock, H. P., 1990: Light energy dissipation under water stress conditions. *Plant Physiol., 92*, 1053–1061.

Stuhlfauth, T., Sueltemeyer, D. F., Weinz, S. and Fock, H. P., 1988: Fluorescence quenching and gas exchange in a water stressed C_3 plant, *Digitalis lanata. Plant Physiol., 86*, 246–250.

Turner, N. C., 1974: Stomatal behaviour and water status of maize, sorghum and tobacco under field

conditions. II. At low soil water potential. *Plant Physiol.*, **53**, 360-365.

Walker, D., 1992: Excited leaves. *New Phytol.,* **121**, 325-345.

Zhang, J. and Davies, W. J., 1989: Sequential re-

sponse of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. *New Phytol.,* **113**, 167-174.

水ストレスをあたえたインゲン (*Phaseolus vulgaris* L.) の葉におけるクロロフィル蛍光パラメータの葉齢依存性

戸部和夫・大政謙次*

国立環境研究所生物圏環境部
（* 現在　東京大学大学院農学生命科学研究科）

要　約

　水ストレスが植物個体中の葉齢の異なる葉の光合成機能におよぼす影響を調べるため，鉢植えのインゲン (*Phaseolus vulgaris* L.) への給水を停止して葉の水ポテンシャルを徐々に低下させ，その間のパルス振幅変調 (PAM) 式クロロフィル蛍光測定から得られるパラメータ (F_V/F_M, ϕ_e, q_P, q_N) の変化を調べた。測定は，同一個体中の葉齢を異にする 3 枚の葉のそれぞれに対して行った。葉の CO_2 吸収速度は，葉の水ポテンシャルの低下とともに急激に低下し，水ポテンシャルが $-0.9\,\mathrm{MPa}$ でほぼゼロとなった。これに対し，クロロフィル蛍光測定から得られるパラメータのいずれについても，水ポテンシャルが $-0.9\,\mathrm{MPa}$ 以上の場合には，葉齢の差による顕著な差異は認められず，また，水ポテンシャルの低下に伴う変化もみられなかった。一方，水ポテンシャル

が $-0.9\,\mathrm{MPa}$ 以下に低下すると，水ポテンシャルの低下とともに F_V/F_M および ϕ_e の低下がみられ，この低下の度合いは若い葉のほうが顕著であった。さらに，水ポテンシャルが $-0.9\,\mathrm{MPa}$ 以下のとき，水ポテンシャルの低下とともに q_N の定常値は変化したが，この変化の態様は葉の葉齢によって異なるものとなった。水ストレスを与えた植物に給水を開始すると，F_V/F_M, ϕ_e および q_N の値は，給水開始から 1 日以内に，水ストレスを与える前の値に戻った。以上の結果から，クロロフィル蛍光測定から得られるパラメーターにより水ストレスが光合成機能におよぼす影響を評価する際には，測定に用いる葉の葉齢を考慮する必要があることが明らかとなった。

キーワード：インゲン，クロロフィル蛍光，光合成，水ストレス，葉齢