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PHOTOSYNTHETIC RESPONSES TO DEHYDRATION IN GREEN PEPPER (*Capsicum annuum* L.) LEAVES

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Abstract – Photosynthetic responses to dehydration were examined by the simultaneous measurement of O_2 evolution and chlorophyll (Chl) fluorescence in green pepper leaves. Dehydration was induced by immersing the plant roots directly in the Hoagland solution containing varying concentration (2-30%) of polyethylene glycol (PEG-6000). Water potential of the leaf was decreased time- and concentration-dependently by PEG-treatment. The decrease in water potential of leaf was correlated with the decrease in both the maximal photosynthesis (Pmax) and quantum yield of O_2 evolution, but Pmax dropped more rapidly than quantum yield at all water deficit conditions tested. However, Chl fluorescence parameters were not affected much. Dehydration did not change the initial fluorescence (Fo) and maximum photochemical efficiency (Fv/Fm) of photosystem (PS) II. Both the photochemical quenching (qP) and non-photochemical quenching (NPQ) were not changed by dehydration under low PFR (50 μmols m^{-2} s^{-1}). In contrast, under high PFR (270 μmols m^{-2} s^{-1}) qP was slightly decreased while NPQ was greatly increased. The fast induction kinetics of Chl fluorescence showed no change in Chl fluorescence pattern by dehydration at high PFR (640 μmols m^{-2} s^{-1}), but exhibited a significant drop in peak level (Fp) at low PFR (70 μmols m^{-2} s^{-1}). PS I oxidation and reduction kinetics revealed normal reduction but delayed oxidation to P-700$^+$, suggesting no lesion in electron flow from PSII to PSI, but impaired electron transport to NADP$^+$. These results suggest that water stress caused by PEG-treatment results in the reduction of photosynthesis, primarily due to the reduced electron transport from PSI to NADP$^+$ or hampered subsequent steps involving Calvin cycle.

INTRODUCTION

Plants in the nature are adversely affected by various environmental factors such as light, water and temperature beyond optimal range. Among these, water stress is probably the most common and of highest impact to plants.[1] Water stress may harmfully affect most physiological and biochemical processes in plants including photosynthesis which is pivotal to plant life. In consequence, effects of water stress on photosynthesis have been studied under various conditions in many plants to identify labile sites.

Decrease in photosynthesis due to water stress is attributed to both stomatal closure and unfavorable changes in the photosynthetic apparatus.[2-5] One of the initial responses of plants to the onset of water stress is closing of stomates.[3] Stomatal closing evokes decrease in photosynthesis by lowering the partial pressure of CO_2 within the leaf and elevating leaf temperature due to the reduced transpiration.[6-8] High temperatures reduce the electron transport capacity and stimulate photophospiration.[6] Measurement of O_2 evolution using leaf discs from dehydrated leaves at saturating CO_2 concentration demonstrated stomatal closure was the major cause for the observed decrease in leaf photosynthesis in plants subjected to a mild drought, but photosynthetic apparatus was little affected.[7,9] In *Phaseolus* and *Brassica*, photosynthesis was mainly inhibited due to the reduced transpiration and stomatal conductance after mild drought.[8,10] However, under severe water stress, photosynthetic activity of chloroplast *per se* was also reduced.[2,11,12] In sunflower, decrease in total leaf photosynthesis was well correlated with that in O_2 evolution by isolated chloroplasts.[2,11]

Substantial amount of other evidences suggest that direct impairment of chloroplastic function is involved.[13-18] The photochemical processes including water splitting, electron transport and photophosphorylation were shown to be inhibited.[13-16] Both PS II and PS I activities were also hampered with more damage to PS II.[15-17] Dark reactions of the stroma such as Rubisco and FBPase were altered after water stress or osmotic stress.[18-21] Similar studies were done taking advantage of Chl fluorescence measurements and photoacoustics method to avoid any artificiality during chloroplast isolation.[17,22,23] Specific changes in the fluorescence induction pattern in leaves suggested that

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† *Abbreviations:* Chl, chlorophyll; FBPase, fructose-1,6-bisphosphatase; Fm, maximal fluorescence after dark-adaptation; F(m, maximal fluorescence during illumination; Fo, initial fluorescence; Fp, maximal fluorescence of the induction kinetics; Fv, variable fluorescence; NPQ, non-photochemical quenching of fluorescence; PEG, polyethylene glycol; PFR, photon fluence rate; Pmax, maximal photosynthetic rate of O_2 evolution; PS, photosystem; qP, photochemical quenching of fluorescence; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

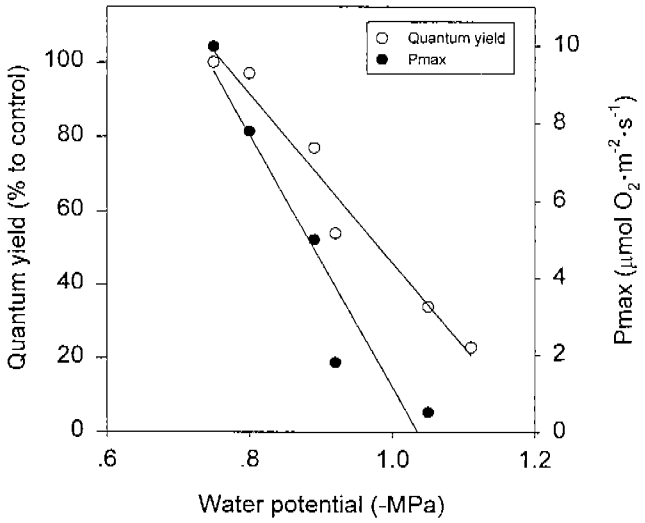

Figure 1. Changes in the quantum yield and maximal photosynthetic O_2 evolution (Pmax) in the dehydrated leaves.

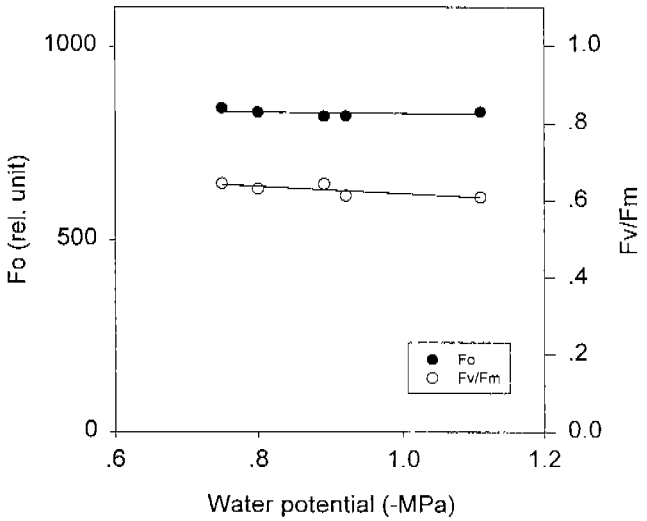

Figure 2. Changes in the maximal photochemical efficiency of PSII (Fv/Fm) and initial fluorescence (Fo) in the dehydrated leaves.

water stress hampered PS II activity.[16,17,22] Results are not conclusive, but all direct to the consensus that decrease in photosynthesis by water stress is largely due to the malfunctioning of chloroplasts.

The purpose of present study is to elucidate the underlying causes for the decline in photosynthesis due to water stress contributed by the non-stomatal factors using the indices of Chl fluorescence parameters and quantum yield of O_2 evolution. Identification of labile sites by non-intrusive method would eliminate the ambiguous results of *in vitro* system.

MATERIALS AND METHODS

Plant material and dehydration treatment. Green pepper plants (*Capsicum annuum L.*) were grown in pots filled with soil (Bioplug #2, Hungnong Seeds Co, Ltd., Korea) for 4 to 5 weeks in a growth chamber maintained at 25 ± 1°C with a diurnal cycle of 16 h light and 8 h dark. Light was provided by 4 banks of True-lite II fluorescent lamps (Durotest, U.S.A.) at the intensity of 100 μmols m^{-2} s^{-1}. For dehydration treatment, a few selected plants of the same age were carefully pulled out of the pots and washed in running water to avoid damage to the roots and were immersed in Hoagland nutrition medium containing appropriate concentration (2-30%) of PEG-6000 for the desired time. PEG was chosen for its chemical inertness other than dehydrating effect.[24,25]

Measurement of water potential. Water potential of leaf was measured psychrometrically using dewpoint microvoltmeter (HR-33T) with C-52 sample chamber (Wescor, Inc., U.S.A.) at room temperature.[26] A leaf disc of 0.7 cm-diameter was placed in the chamber and allowed to equilibrate for 15 min and was cooled for 45 s before reading voltage. Water potential was calculated from voltage value and corrected for temperature.

Measurement of Chl fluorescence and O_2 evolution. Chl fluorescence parameters (Fo, Fv, Fp and Fm) were measured with Plant Efficiency Analyzer (PEA, Hansatech, England) under the light intensity of 640 μmol m^{-2} s^{-1}. Pmax, quantum yield and quenching parameters were obtained by simultaneous measurement of O_2 evolution and Chl fluorescence using a leaf disc of 1.7 cm-diameter in Hansatech LD2 leaf disc chamber with Walz Pulse Amplitude Modulator (PAM) 101 Chl fluorometer and a Clark type electrode connected to Hansatech O_2 electrode control box at 25°C. Actinic light was provided by Schott illuminator at the intensity of 30, 50, and 270 μmol m^{-2} s^{-1}, respectively. NPQ (defined as Fm/F'm - 1) and qP were calculated as described by Schreiber *et al.*[27] Chl content was measured by conventional extraction method using acetone according to Porra *et al.*[28]

Measurement of fast induction kinetics of Chl fluorescence. Fast induction kinetics in Chl fluorescence was measured using PEA. Maximal fluorescence during induction was taken as Fp. Light was given at the intensity of 640 and 70 μmol m^{-2} s^{-1} for high and low PFR, respectively.

Measurement of kinetics in P-700 oxidation and reduction. PS I oxidation and reduction kinetics was measured using PAM 101 fluorometer connected to 830 nm LED (type ED 800T). PS I was first oxidized by illuminating with saturating far-red light (> 715 nm, 180 μmol m^{-2} s^{-1}). A single saturation light pulse was given for 1 s to reduce PS I and allowed re-oxidation for a while to reach plateau before turning off the far-red light for re-reduction of PS I.

RESULTS AND DISCUSSION

Changes in water potential by PEG treatment

Plants undergo water stress when there is not enough water available. Limiting water supply is the easiest and most common way to induce water deficit in plants, but conditions can vary depending on the water content in the soil, relative humidity in the atmosphere, and other factors.

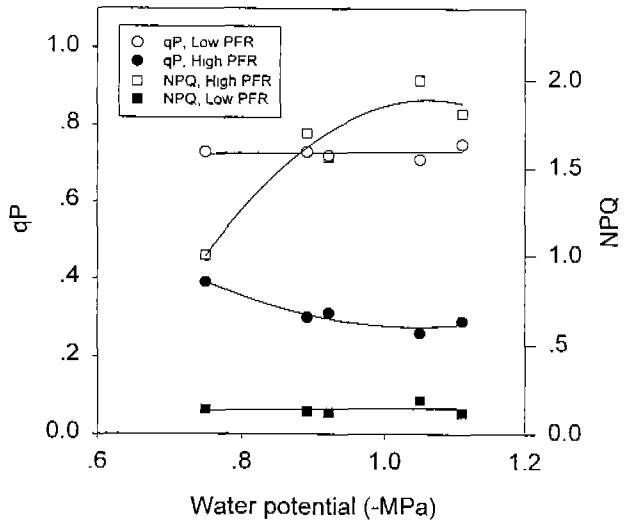

Figure 3. Changes in the photochemical quenching (qP) and nonphotochemical quenching (NPQ) under high (270 μmol m^{-2} s^{-1}) and low (50 μmol m^{-2} s^{-1}) PFR in the dehydrated leaves.

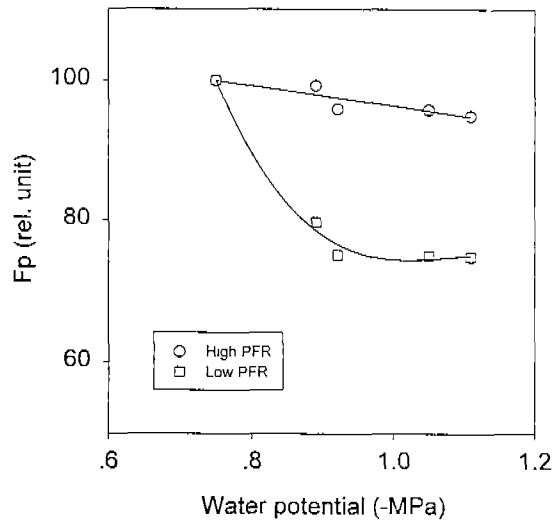

Figure 4. Changes in the maximal fluorescence level (Fp) during fast induction of chlorophyll fluorescence under high (640 μmol m^{-2} s^{-1}) and low (70 μmol m^{-2} s^{-1}) PFR in the dehydrated leaves.

In the present study, to provide more consistent and physiological condition, dehydration was induced by immersing plants in the nutrition medium containing varying concentrations (2-30%) of polyethylene glycol (PEG-6000) which is chemically inert. Green pepper plants responded to PEG-treatment with apparent wilting and concomitant decrease in water potential of the leaves. The decrease in water potential of leaf was time- and concentration-dependent by PEG-treatment (data not shown). PEG solution of 5% gave rise to a fast and sufficient dehydration, thus was chosen as the optimal condition for our experimental purpose. Water potential in the leaves prior to PEG-treatment was observed to be -0.75 $\pm$ 0.06 Mpa, and was linearly dropped to -1.11 $\pm$ 0.16 MPa as incubation time in 5% PEG solution was increased up to 8 h (data not shown). Longer treatment than 8 h did not further bring down water potential in the leaves. After 8 h-treatment, plants did not recover from wilting and die off even when they were returned to nutrient medium lacking PEG.

Changes in Pmax and quantum yield

The inhibition in photosynthesis due to water stress is attributed to both stomatal and non-stomatal factors.[2-5] Stomatal factors can be eliminated by measuring O_2 evolution of the leaf under saturating CO_2 concentration.[7] Therefore, changes in Pmax and quantum yield under this condition are solely incurred by non-stomatal factors. Pmax was determined under saturating PFR (270 μmol m^{-2} s^{-1}) while quantum yield was determined under low PFR (30 and 50 μmol m^{-2} s^{-1}). Both Pmax and quantum yield were linearly decreased in relation to the decreased water potential in the leaves by PEG-treatment (Fig. 1), demonstrating both maximal photosynthetic activity and efficiency in the chloroplasts were severely affected by water stress. Similar results were reported in sunflower and tobacco.[12,23] Faster decrease in Pmax implies that photosynthesis is mainly limited by the site most adversely affected by dehydration rather than the overall diminished efficiency.

Changes in Chl fluorescence parameters

Chl fluorescence at room temperature mostly originates from PS II, and initial fluorescence (Fo) and maximal photochemical efficiency (Fv/Fm) are generally accepted as indicators for PS II functionality.[29,30] Damages to PS II are often accompanied with rise in Fo level and drop in Fv/Fm. Changes in Fo reflect structural alterations at the PS II pigment level, while those in Fv/Fm indicate varied energy capturing efficiency in PS II.[30] As shown in Fig. 2, both Fo and Fv/Fm were not changed by the lowered water potential in the leaf. No change in Fo was previously observed in sorghum and *Nerium oleander* leaves.[3,31] PS II activity was reported to be hampered, but only under severe water deficit condition or when combined with photoinhibition.[3,31,32] In addition, direct measurement of PS II function using thylakoid membranes from wheat leaves after 24-h dehydration in PEG solution showed little reduction in PS II activity.[15] Our results showing no change in Fv/Fm even after maximal dehydration where no recovery is observed, support that PS II is indeed highly tolerable to water stress.

Changes in Chl fluorescence quenching parameters

After onset of illumination, the maximal Chl fluorescence declines as photosynthetic reaction proceeds. It is due to the photosynthetic electron flow represented as qP, and nonradiative energy dissipation and ΔpH formation manifested as NPQ.[27] At low PFR (50 μmol m^{-2} s^{-1}), both qP and NPQ did not change (Fig. 3). However, at high PFR (270 μmol m^{-2} s^{-1}) qP was decreased while NPQ was

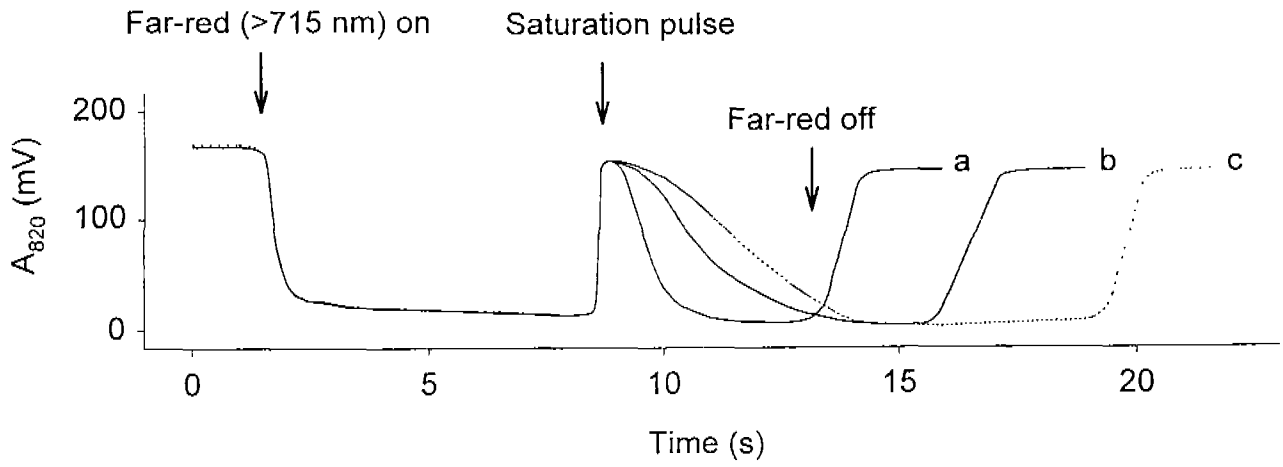

Figure 5. Changes in the reduction and oxidation kinetics of PSI after dehydration treatment in 5% of PEG solution: a, control (Ψ= -0.8 MPa); b, 4-h treatment (Ψ= -1.0 MPa); c, 8-h treatment (Ψ= -1.2 MPa).

significantly increased upon dehydration (Fig. 3). Decreased qP upon dehydration indicate that the acceptor side of PS II (Q_A) is overly reduced by hindered electron flow from donor side to the later chain.[27] Increased NPQ may reflect protection of PS II by the thermal dissipation and increased ΔpH formation across thylakoid membrane through cyclic electron transport.[27] Both may result from the increased back pressure as electron transfer to $NADP^+$ from PS I is impeded under water stress. At low PFR electron flow rate is not fast enough to evoke any back pressure to induce any change in NPQ and qP. It was reported that water stress brought in a decrease in qP and, combined with high temperature stress, caused a pronounced increase in NPQ and decrease in qP in *Arbutus unedo*.[30]

Fast induction kinetics of Chl fluorescence

Chl fluorescence at room temperature changes from initial level (Fo) to peak level (Fp) immediately after illumination and then declines to a steady-state level.[27,29] Fast induction kinetics allows to trace the fluorescence rise pattern to Fp. The overall pattern of fast induction kinetics was not changed after dehydration at high PFR (640 μmol m^{-2} s^{-1}), but was significantly altered at low PFR (70 μmol m^{-2} s^{-1}). Dehydration treatment did not change the induction pattern at high PFR other than a slight decrease in Fp level (Fig. 4), which was consistent with the result that no change in Fv/Fm was observed (see Fig. 2). However, the induction pattern at low PFR was much altered, leading to a considerable drop in Fp level. This may reflect the lowered energy transfer efficiency from antennae to reaction center after dehydration.[30] Under limited light availability it would lead to the decrease in quantum yield. At high PFR, the diminished efficiency would not be manifested with excessive light available.

PS I oxidation and reduction kinetics

Changes in absorbance at 820 nm reflects the oxidation and reduction state of P-700 that reflects electron flow from PS II to PS I and from PS I to $NADP^+$.[33,34] Oxidation of P-700 to $P\text{-}700^+$ is monitored by following the pattern of decrease in absorbance at 820 nm after a single saturation light pulse. As shown in Fig. 5, P-700 was oxidized more slowly in the dehydrated leaves, probably caused by hampered electron flow to $NADP^+$. Malfunctioning of Calvin cycle would also contribute to a slower formation of $P\text{-}700^+$. In contrast, re-reduction of $P\text{-}700^+$ after turning off far-red light was not altered (Fig. 5), suggesting that electron flow from PS II to PS I via Plastoquinone and cytochrome b_6-f complex was not affected. It is also possible that any damage to the electron flow chain from PS II to PS I is not monitored by the re-reduction of P-700 due to the fast turnover of PS I. Increased qP upon dehydration, as shown in Fig. 3, would be originated from the blocked electron flow from PS I to NADP rather than electron flow from PS II to PS I. Therefore, it is concluded that dehydration affects the electron flow from PS I to $NADP^+$ which is attributable to the lesion in electron transport chain *per se* or to the blocking of Fd-$NADP^+$ reductase or Calvin cycle reactions.

CONCLUSIONS

Dehydration of green pepper plants led to the reduction in photosynthesis manifested as rapid drop in Pmax and, to a lesser degree, in quantum yield. However, little changes in Fo, Fv/Fm and qP were observed after dehydration, indicating that decrease in photosynthesis was not caused by the reduced photochemical efficiency of PS II or damages to PS II. Electron flow to PS I from PS II seemed intact as indicated by normal reduction of $P\text{-}700^+$ after oxidation by far-red light. Increased NPQ and retarded P-700 oxidation point to the impaired electron flow to $NADP^+$ itself or malfunctioning of Calvin cycle. Improper operation in dark reaction would keep NADP pool in overreduced state and

lead to the inhibition of electron transfer from PS I.

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REFERENCES

1. McKersie, B. D. and Y. Y. Leshem (1994) Stress and stress coping in cultivated plants. Kluwer Academic Publishers, Dordrecht, p. 148.
2. Boyer, J. S. (1976) Water deficits and photosynthesis. In *Water Deficits and Plant Growth* (Edited by T. T. Kozlowski), Vol. IV. Soil Water Measurement, Plant Responses, and Breeding for Drought Resistance. Academic Press, New York, p. 153.
3. Björkman, O. and S. B. Powles (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. *Planta* **161**, 490-504.
4. Ögren, E. and G. Öquist (1985) Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. *Planta* **166**, 380-388.
5. Assmann, S. M. (1988) Stomatal and non-stomatal limitations of carbon metabolism: an evaluation of the path-dependent method. *Plant Cell Environ.* **11**, 577-582.
6. Farquhar, G. D. and T. D. Sharkey (1982) Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.* **33**, 317-345.
7. Kaiser, W. M. (1987) Effects of water deficit on photosynthetic capacity. *Physiol. Plant.* **71**, 142-149.
8. Cornic, G., J. L. Le gouallec, J. M. Briantais and M. Hodges (1989) Effect of dehydration and high light on photosynthesis of two C3 plants. *Phaseolus vulgaris* L. and *Elatostema repens*. *Planta* **177**, 84-90.
9. Chaves, M. M. (1991) Effects of water deficits on carbon assimilation. *J. Exp. Bot.* **42**, 1-16.
10. Shalhevet, J. (1993) Plants under salt and water stress. In *Plant Adaptation to Environmental Stress* (Edited by L. Fowden, T. Mansfield and J. Stoddart), Champman & Hall, Lndon, p. 133.
11. Boyer, J. S. and B. L. Bowen (1970) Inhibition of oxygen evolution in chloroplasts isolated from leaves with low water potentials. *Plant Physiol.* **45**, 612-615.
12. Mohanty, P. and J. S. Boyer (1976) Chloroplast response to low water potentials. IV. Quantum yield is reduced. *Plant Physiol.* **57**, 704-709.
13. Nir, I. and A. Poljakoff-Mayber (1967) Effect of water stress on the photochemical activity of chloroplasts. *Nature* **213**, 418-419.
14. Keck, R. W. and J. S. Boyer (1974) Chloroplast response to low water potentials. III. Differing inhibition of electron transport and photophosphorylation. *Plant Physiol.* **53**, 474-479.
15. Jun, X. H, J. Wang and H. G. Liang (1995) Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. *Physiol. Plant.* **93**, 771-777.
16. Wiltens, J., U. Schreiber and W. Vidaber (1978) Chlorophyll fluorescence induction: an indicator of photosynthetic activity in marine algae undergoing desiccation. *Can. J. Bot.* **56**, 2787-2794.
17. Havaux, M., O. Canani and S. Malkin (1986) Photosynthetic responses to water stress in leaves, expressed by photoacoustics and related methods. II. The Effect of rapid drought on the electron transport and the relative activities of the two photosystems. *Plant Physiol.* **82**, 834-839.
18. von Caemmerer, S. and G. D. Farquhar (1984) Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of laves of *Phaseolus vulgaris* L. *Planta* **160**, 320-329.
19. Kaiser, W. M. and U. Hever (1981) Photosynthesis under osmotic stress. Effect of high solute concentrations on the permeability properties of the chloroplast envelope and on the activity of stromal enzymes. *Planta* **153**, 423-429.
20. Berkowitz, G. A. and M. Gibbs (1982) Effect of osmotic stress on photosynthesis studied with isolated chloroplast. Site-specific inhibition of the photosynthetic carbon reduction cycle. *Plant Physiol.* **70**, 1535-1540.
21. Berkowitz, G. A. and M. Gibbs (1983) Reduced osmotic potential inhibition of photosynthesis. Site-specific effects of osmotically induced stromal acidification. *Plant Physiol.* **72**, 1100-1109.
22. Govindjee, W. S. Downton, D. C. Fox and P. A. Armond (1981) Chlorophyll a fluorescence transient as an indicator of water potential of leaves. *Plant Sci. Lett.* **20**, 191-194.
23. Havaux, M., O. Canani and S. Malkin (1986) Photosynthetic responses to water stress in leaves, expressed by photoacoustics and related methods. I. Probing the photacoustic method as an indicator for water stress *in vivo*. *Plant Physiol.* **82**, 827-833.
24. Michel, B. E. (1970) Carbowax 6000 compared with mannitol as a suppressant of cucumber hypocotyl elongation. *Plant Physiol.* **45**, 507-509.
25. Michel, B. E. (1971) Further comparisons between Carbowax 6000 and mannitol as suppressants of cucumber hypocotyl elongation. *Plant Physiol.* **48**, 513-516.
26. Boyer, J. S. and E. B. Knipling (1965) Isopiestic technique for measuring leaf water potentials with a thermocouple psychometer. *Proc. Nat. Acad. Sci. U.S.A.* **54**, 1044-1051.
27. Schreiber, U., W. Bilger and C. Neubauer (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In *Ecophysiology of Photosynthesis* (Edited by E.-D. Schulze and M. M. Caldwell), Springer-Verlag, Berlin, p. 49.
28. Porra, R. J., W. A. Thompson and P. E. Kriedemann (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* **975**, 384-394.
29. Walker, D. (1987) The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. pp. 19-57, University of Sheffield Print Unit.

30. Renger, G. and U. Schreiber (1986) Practical applications of fluometric methods to algae and higher plant research. In *Light Emission by Plants and Bacteria* (Edited by Govindjee, J. Amesz, D. C. Fork), p. 587.
31. Ludlow, M. M. and S. B. Powles (1988) Effects of photoinhibition induced by water stress on growth and yield of grain sorghum. *Aust. J. Plant Physiol.* **15**, 179-194.
32. Angelopoulos, K., B. Dichio and C. Xiloyannis (1996) Inhibition of photosynthesis in olive tree (*Olea europaea* L.) during water stress and rewatering. *J. Exp. Bot.* **47**, 1093-1100.
33. Harbinson, J. and F. I. Woodward (1987) The use of light-induced absorbance charges at 820 nm to monitor the oxidation state of P-700 in the leaves. *Plant Cell Environ.* **9**, 131-140.
34. Harbinson, J. and C. L. Hedley (1989) The kinetics of P-700$^+$ reduction in leaves: a novel in situ probe of thylakoid functioning. *Plant Cell Environ.* **12**, 357-369.