

CHLOROPHYLL A FLUORESCENCE TRANSIENT AS AN INDICATOR OF WATER POTENTIAL OF LEAVES*

GOVINDJEE**, W.J.S. DOWNTON***, D.C. FORK and P.A. ARMOND

Carnegie Institution of Washington, 290 Panama Street, Stanford, CA 94305 (U.S.A.)

(Received July 1st, 1980)

(Revision received September 26th, 1980)

(Accepted October 2nd, 1980)

SUMMARY

The ratio of the maximum (P level) to the minimum (O level) chlorophyll *a* fluorescence, measured at 685 nm, decreased from a value of about 4–1 as *Nerium oleander* plants were water stressed (water potential of leaves decreasing from –8 bars to –9 bars). Furthermore, this change was reversed to a large degree when water-stressed plants were re-watered. No measurable effect was observed on the O level. A similar relationship between the P/O ratio and the water potential was also observed in the leaves of *Atriplex triangularis* and *Tolmiea menziesii*. These data indicate that water stress inhibits the electron donation (or the water oxidation) side of photo-system II (PSII).

INTRODUCTION

Water stress leads to several changes in the photosynthetic apparatus of green plants [1]. Low water potential has been observed to cause a decrease in the quantum yield of O₂ evolution in chloroplasts and leaves from sunflower plants [2], a decrease in the ability of the coupling factor, isolated from spinach leaves, to bind fluorescent nucleotides (ϵ -ATP, ϵ -ADP) [3], and a decrease in the ratio of the maximum (P level) to the minimum (O level) fluorescence in the red algae *Porphyra sanjuanensis* [4]. The P/O ratio is a good indicator of the activity of photochemical system II. In this communication, we present data on the relationship between the P/O ratios

*CIW Publication Number 720.

**To whom correspondence should be sent to: Department of Botany, 289 Morrill Hall, University of Illinois, Urbana, IL 61801 (U.S.A.).

***Present address: CSIRO Division of Horticultural Research, Box 350, G.P.O., Adelaide, 5001, Australia.

and water potential of the leaves of *Nerium oleander*, *Atriplex triangularis* and *Tolmiea menziesii*. These data suggest that water stress blocks electron flow from the water side to the reaction center chlorophyll *a* of photo-system II.

MATERIAL AND METHODS

N. oleander plants were grown in 4 or 18-l pots outdoors for 7 months and were about 1 m in the height when used. Mean daily temperatures during the period of water stress studies were 27° max./5° min. Water potential of *N. oleander* leaves was decreased by withholding water over various periods preceding experiments. In *A. triangularis* and *T. menziesii* different water potentials were obtained by either picking leaves at different times of the day or by dehydration of fresh leaves. Low water potential leaves were rehydrated by rewatering intact plant or by simply placing the leaf in water. Paired leaves were detached, one leaf was used for chlorophyll *a* fluorescence transient measurements after 10-min dark treatment and the other leaf, used for water potential measurements also after a 10-min period. Water potential was measured in Wescor C52 psychrometers (Wescor

TABLE I

RATIO OF P/O CHLOROPHYLL A FLUORESCENCE INTENSITY AS A FUNCTION OF WATER POTENTIAL OF *N. OLEANDER* LEAVES

Conditions	Water potential, ψ_w , bars	P/O
(1) Grown in a 4-l pot; plant watered daily; sample time: 12:30.	-8.3	4.3
(2) Grown in a 18-l pot; watered daily; 15:00.	-8.7	3.1
(3) Same as No. 2; a different plant.	-9.9	2.8
(4) Grown in a 4-l pot; water stressed by the addition of only small quantities of water (1/3 of control plant requirement) over the preceding 4 days; 14:30.	-14.6	2.6
(5) Same as No. 4; a different plant, 11:30.	-16.2	2.7
(6) Grown in 18-l pot; plant received no water for the preceding 4 days; 16:00.	-17.6	2.8
(7) Grown in 4-l pot; water restricted (to ¼ of the control plant requirement) for the preceding 3 days; 13:30.	-20.5	2.5
(8) Leaf of No. 7 left for an hour for dehydration in room light.	more negative than -20.5 (est. -25)	1.8
(9) Grown in 4-l pot; plant received no water for the preceding 5 days; 13:30 p.m.	-39.2	1.1
(10) No. 9 watered, allowed to recover for 18 h; 11:00.	-9.9	2.5

Inc., Logan, UT) and chlorophyll *a* fluorescence with the instrument described elsewhere [5]. Front surface photosystem II chlorophyll *a* fluorescence was monitored from the lower surface of the leaves at 685 nm and was excited by saturating ($\sim 5 \text{ nE} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) green light (550 nm).

RESULTS AND DISCUSSION

Table I and Fig. 1 show the relationship between water potentials and the P/O ratios of *N. oleander* and other leaves under different conditions. The inset in Fig. 1 shows the fluorescence transient at several water potentials in *N. oleander* leaves. No significant effect was observed on the O level. It is clear that P/O decreases from a high value of 4.0 in well watered *N. oleander* plants ($\psi_w = -8$ bars) to a low value of 1.1 in a severely stressed plant ($\psi_w = -39$ bars). Thus, the maximum fluorescence intensity

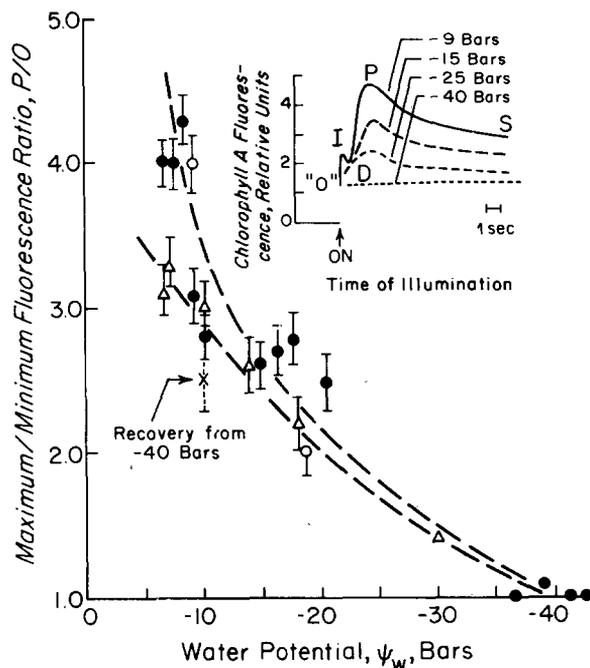


Fig. 1. A plot of the ratio of P/O chlorophyll *a* fluorescence as a function of the water potential of the leaves from *N. oleander* (\bullet), *A. triangularis* (\blacktriangle) and *T. menziesii* (\circ). *Inset*: chlorophyll *a* fluorescence transient in *N. oleander* leaves at different water potentials. The meaning of the various points on the chlorophyll *a* fluorescence transient, referred to as O, I, D, P and S, are described elsewhere [6]. The lower curve in the main figure is a representative curve for *A. triangularis*, and the upper curve for *N. oleander*. The cross (\times) indicates measurements for a *N. oleander* leaf recovered to about -10 bars from about -39 bars (rounded off to -40 bars in the figure). The vertical bars indicate the range of values obtained in different measurements.

in severely water-stressed plants approached the O level. *N. oleander* plants having a water potential of -39 bars recovered to a large extent on rewatering (cf. No. 10 with No. 9, Table I). The recovery of the P/O ratio did not occur if the leaves were crisp dry (water potentials substantially lower than -40 bars). A similar recovery was observed when *A. triangularis* leaf was soaked in water. No recovery measurements were made with *T. menziesii* leaves. In all cases examined, the P/O decreases as the water potential is decreased. Thus, the P/O ratio serves as a qualitative indicator of the leaf water potential.

A decrease in P/O ratio is equivalent to a decrease in the variable (P minus O) fluorescence since O level did not change. If the electron donation from the donor (water) side of PSII is inhibited, Q (the stable electron acceptor of PSII) cannot be reduced. Since chlorophyll *a* fluorescence is low when Q is in the oxidized state and is high when Q is in the reduced state [7], chlorophyll *a* fluorescence remains low, i.e., P/O is low, when the block is on the water side of PSII. If the water stress had blocked electron flow beyond Q, we would have expected a higher P/O ratio. Our results suggest that water stress blocks electron flow on the water side of PSII in the three species examined. P. Mohanty and J. Boyer (pers. comm.) have also observed that the P/O ratio is diminished in chloroplasts isolated from dehydrated sunflower leaves supporting the present conclusions.

ACKNOWLEDGEMENTS

One of the authors (G) thanks the Carnegie Institution of Washington for a summer fellowship.

REFERENCES

- 1 J.S. Boyer, in: T.T. Kozlowski (Ed.), *Water Deficits and Plant Growth*, Vol. 4, Academic Press, New York, 1976, p. 153.
- 2 P. Mohanty and J.S. Boyer, *Plant Physiol.*, 57 (1976) 704.
- 3 J.M. Younis, J.S. Boyer and Govindjee, *Biochim. Biophys. Acta*, 548 (1979) 328.
- 4 J. Wiltens, U. Schreiber and W. Vidaver, *Can. J. Bot.*, 56 (1978) 2787.
- 5 S. Markin, D.C. Fork and P. Armond, *Carnegie Inst. Wash. Yearbook*, 77 (1978) 237.
- 6 Papageorgiou, in: Govindjee (Ed.), *Bioenergetics of Photosynthesis*, Academic Press, New York, 1975, p. 319.
- 7 L.M.N. Duysens and H.E. Sweers, in: *Japanese Society of Plant Physiologists (Eds.), Studies of Microalgae and Photosynthetic Bacteria*, University of Tokyo Press, Tokyo, p. 353.