A LOSS OF PHOTOSYNTHETIC EFFICIENCY DOES NOT EXPLAIN STOMATAL CLOSURE IN FLOODED TOMATO PLANTS

Franciszek Janowiak [1], Mark A. Else [2], Michael B. Jackson [3]

[1] Department of Plant Physiology, Polish Academy of Sciences, Kraków
[2] Horticulture Research Institute, West Malling, Kent, UK
[3] Department of Agricultural Sciences, University of Bristol, Bristol, UK

Introduction

In tomato plants, stomata begin to close within 24 hours of soil flooding and remain closed for at least 72 h. The nature of the signal(s) generated by flooded roots that promotes stomatal closure in the shoots is not known. Our previous work has shown that this closure is neither prompted nor maintained by increased ABA export from flooded roots [ELSE et al. 1996]. Furthermore, a transient drop in shoot hydration, triggered by a flooding-induced suppression of the normal daily rise and fall in root hydraulic conductance, was not sufficiently intense to initiate stomatal closure [ELSE et al. 1995]. These findings suggest that signalling in the transpiration stream may not be involved; an alternative hypothesis is that stomata close in response to increased carbon dioxide (CO_2) concentrations in the sub-stomatal cavities, caused by a flooding-induced reduction in the efficiency of PS II. In this way, CO_2 could act as an 'accumulation message', linking oxygen-deficient roots with the induction of stomatal closure. We measured chlorophyll fluorescence parameters to assess whether soil flooding impaired the efficiency of the photosynthetic apparatus. Also, we attempted to determine whether any changes in photochemistry were a cause, rather than a consequence, of stomatal closure.

Material and methods

Plant material and growth conditions

Seeds of tomato (*Lycopersicon esculentum* MILL. cv. Ailsa Craig) were sown into a soil mix of peat, sand and clay (2 to 1 to 1, v/v/v). After two weeks, seedlings were replanted into pots (13 x 13 x 11 cm) filled with similar soil. Plants were maintained in growth chambers with a day/night temperature of 25/20°C

and a 16 h photoperiod (07^{00}–23^{00}) provided by Osram Vialox lamps, the light intensity at plant height was 200 μmol·m^{-2}s^{-1}. Relative humidity was uncontrolled. Plants were used for experiments at the 10–11 leaf stage.

Measurements of stomatal conductance and chlorophyll fluorescence

Plant root systems were flooded at 09^{00} by placing the pots of soil into larger pots filled with tap water. Well-drained plants were watered regularly throughout each photoperiod. There were 10 replicates per treatment.

The stomatal conductance of the 8th leaf was measured using a hand-held, AP4 porometer (Delta-T Devices, Cambridge, UK) on the day before flooding and after 2, 4, 8, 13, 26, 32, 37, 49 and 57 h of flooding. Chlorophyll fluorescence parameters were determined on three leaflets of the 8th leaf on the day before flooding and after 8, 32 and 57 h of flooding. Chlorophyll fluorescence was measured with a PAM 2000 fluorometer (Heinz Walz, Effeltrich, Germany). In light-adapted plants, effective quantum yield of photosystem II, $\Phi_{PS\ II}$ ($\Phi_{PS\ II}$ = (F_m – F_t/F_m) [GENTY et al. 1989] and photochemical fluorescence quenching qP (qP = (Fm' – Ft)/(Fm' – Fo); [WALZ 1998] were determined. In dark-adapted plants, maximum quantum yield of PS II F_v/F_m and non-photochemical fluorescence quenching qN (qN = (Fm – Fm')/(Fm – Fo'), [WALZ 1998] were measured.

Statistical evaluation

A fully randomised experimental design was used. The data are mean values of at least eight (stomatal conductance) or twenty four (fluorescence measurements) replicates. Least statistical differences (LSD) were calculated (p = 0.05) and are presented in the figures. Correlation coefficients presented in the figures are statistically significant at p = 0.05 (**).

Results and discussion

At the beginning of the photoperiod prior to flooding, stomatal conductances in both well-drained and soon to be flooded plants were similar. Conductances were also similar in the 16-h photoperiod following the start of soil flooding; stomatal conductances increased rapidly at the beginning of the photoperiod and reached a maximum in the early afternoon, before declining gradually throughout the evening (Fig. 1). However, stomatal conductances were reduced after 24 h of flooding, and stomata became more closed throughout the next two photoperiods.

$\Phi_{PS\ II}$, a measure of the efficiency of PS II photochemistry, decreased significantly after 32 h of flooding, as did photochemical quenching (Fig. 2A and 2C). This loss of photosynthetic efficiency was measured after stomata began to close. However, whether flooding-induced stomatal closure was triggered by a reduced efficiency of the photosynthetic apparatus is not clear from these data. Conversely, the lowered internal CO_2 concentration imposed by stomatal closure may have limited the efficiency of PS II during flooding stress. More detailed time-courses of changes in stomatal conductance and $\Phi_{PS\ II}$ following soil flooding are needed to resolve these possibilities.
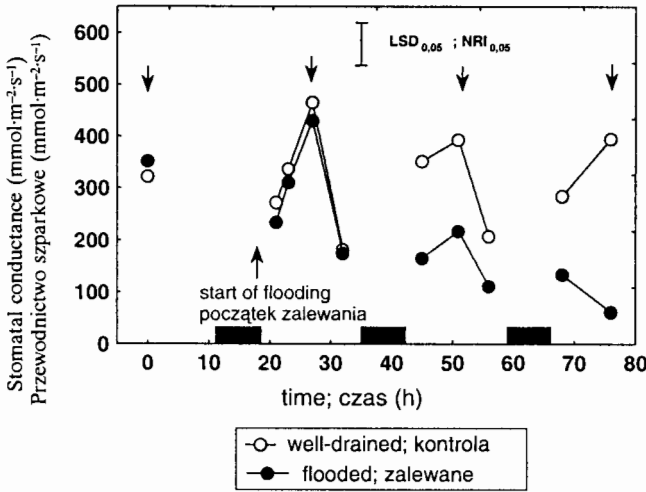
Fig. 1. Stomatal conductance in flooded and well-drained tomato plants. The arrows
         show the times at which measurements of chlorophyll fluorescence were made.

Rys. 1. Przewodnictwo szparkowe u zalewanych i kontrolowanych roślin pomidora.
         Strzałki pokazują czasy pomiarów fluorescencji chlorofilu.
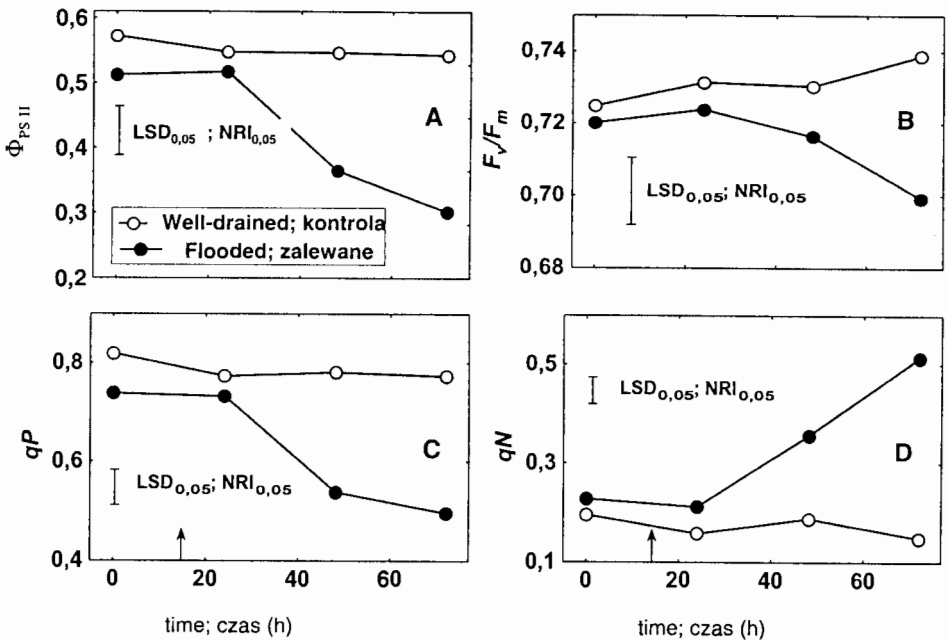

Fig. 2. Effective (A) and maximal (B) quantum yield of PS II, (C) photochemical and
         (D) non-photochemical fluorescence quenching in flooded and well-drained
         tomato plants. The arrows show the start of flooding.

Rys. 2. Efektywna (A) i maksymalna (B) wydajność kwantowa PS II, fotochemiczne
         (C) i niefotochemiczne (D) wygaszanie fluorescencji u kontrolnych i zalewa-
         nych roślin pomidora. Strzałki pokazują początek zalewania

In the longer term, the overall photosynthetic efficiency of dark-adapted plants, F_v/F_m, decreased after 57 h of flooding. Since F_v/F_m was measured in dark-adapted plants and was, therefore, independent of stomatal effects, this fall in F_v/F_m suggests that the integrity of the thylakoid membranes was reduced by prolonged flooding stress. QN, a measure of the proportion of energy dissipated as heat and, therefore, unused by the photosynthetic machinery, increased significantly after 32 h of flooding. This rise in qN occurred due to a reduction in both the photochemical efficiency of PSII and that of biochemical dark reactions.

There was no relationship between $\Phi_{PS\ II}$ and stomatal conductance in well-drained plants (Fig. 3A), indicating that the diurnal changes in stomatal conductance did not influence $\Phi_{PS\ II}$. However, in flooded plants, the correlation coefficient between stomatal conductance and $\Phi_{PS\ II}$ was high (0.70**), supporting the notion that the lowered CO_2 concentrations following stomatal closure limited $\Phi_{PS\ II}$ (Fig. 3B). Although the correlation between stomatal conductance and F_v/F_m was significant (Fig. 3C), the correlation coefficient was low (0.54**) since F_v/F_m is influenced mostly by the status of thylakoid membranes rather than by stomatal movements. QN correlated highly and negatively (–0.80**) with stomatal conductance (Fig. 3D) indicating that the amount of captured energy dissipated as heat and, therefore, not utilised in photosynthesis, was greatly influenced by patterns of stomatal closure.

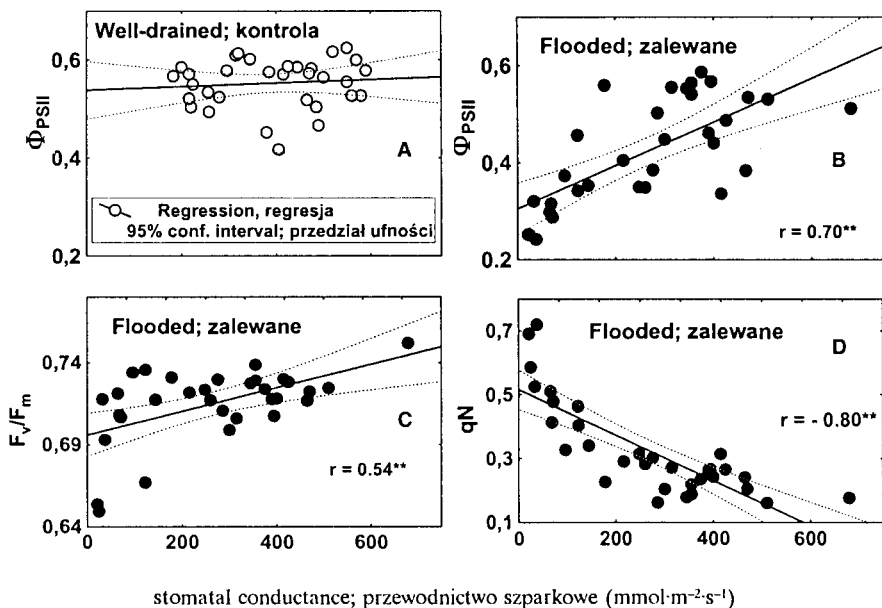stomatal conductance; przewodnictwo szparkowe $(mmol \cdot m^{-2} \cdot s^{-1})$

Fig. 3.   Relationships between stomatal conductance and (A) effective quantum yield of PS II in well-drained plants, (B) effective quantum yield of PS II in flooded plants, (C) maximal quantum yield of PS II and (D) non-photochemical quenching in flooded tomato plants

Rys. 3.   Zależność między przewodnością szparkową i efektywną wydajnością kwantową PS II u roślin kontrolnych (A) i u roślin zalewanych (B), maksymalną wydajnością kwantową PS II (C) i niefotochemicznym wygaszaniem fluorescencji u roślin zalewanych (D) pomidora. Współczynniki korelacji przedstawione na rysunku są statystycznie istotne przy p ≤ 0,05 (**)

In summary, stomata began to close after 24 h of flooding and reductions in the efficiency of PS II in flooded plants were detected after 32 h of flooding. Rather than triggering stomatal closure, the efficiency of PS II itself may be limited by reduced internal CO_2 concentrations imposed by stomatal closure. Further work is needed to separate cause from effect. The ways in which long-term flooding damage the thylakoid membranes are not known, but could involve altered root to shoot signalling in flooded plants. A consequence of this damage may be an accumulation of CO_2 in the sub-stomatal cavities that, in turn, helps to maintain stomatal closure in flooded plants.

References

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Abbreviations: ABA = abscisic acid; F_v/F_m = maximum quantum yield of PS II; LSD = least significant difference, qN = non-photochemical fluorescence quenching; qP = photochemical fluorescence quenching; PS II = photosystem II; $\Phi_{PS\ II}$ = effective quantum yield of PS II.

Key words: chlorophyll fluorescence, photosynthetic efficiency of PS II, tomato, flooding stress, stomata

Summary

Stomata begin to close within 24 h of imposing soil flooding. We investigated whether the stomatal response was triggered by reduced photosynthetic efficiency in young, fully expanded leaves of flooded plants. Chlorophyll fluorescence measurements indicated that $\Phi_{PS\ II}$, the effective quantum yield of PS II,

decreased after stomata began to close in flooded plants. Changes in qP mirrored those of $\Phi_{PS\ II}$. $\Phi_{PS\ II}$ was not affected by daytime patterns of stomatal conductance in well-drained plants but was reduced by stomatal closure in flooded plants. F_v/F_m, a measure of the overall photosynthetic efficiency of dark-adapted plants, decreased after 57 h of flooding. Therefore, prolonged soil flooding adversely affected the thylakoid membranes. QN, a measure of the amount of captured energy dissipated as heat and therefore, unused by the photosynthetic machinery, began to increase after 32 h of flooding and continued to rise thereafter. The interdependence of the changes in chlorophyll fluorescence parameters and the flooding-induced closure of stomata is discussed.

SPADEK FOTOSYNTETYCZNEJ WYDAJNOŚCI NIE WYJAŚNIA ZAMYKANIA SZPAREK U ROŚLIN POMIDORA W WARUNKACH STRESU ZALEWANIA

Franciszek Janowiak [1], Mark A. Else [2], Michael B. Jackson [3]
[1] Zakład Fizjologii Roślin im. F. Górskiego, Polskiej Akademii Nauk w Krakowie
[2] Instytut Badań Ogrodniczych, West, Malling Kent, Anglia
[3] Stacja Doświadczalna IACR-Long Ashton, Uniwersytet Bristol, Anglia

Streszczenie

W warunkach nadmiaru wody w glebie szparki u roślin pomidora zamykają się w czasie 24 h. W prezentowanych eksperymentach badano czy ta reakcja szparek była inicjowana przez spadek fotosyntetycznej wydajności młodych, rozwiniętych liści pomidora. Pomiary fluorescencji chlorofilu wykazały, że $\Phi_{PS\ II}$, efektywna wydajność transportu elektronów PSII, spadała dopiero po rozpoczęciu zamykania szparek u zalewanych roślin. Zmiany qP, fotochemicznego wygaszania fluorescencji, odzwierciedlały przebieg $\Phi_{PS\ II}$. Na $\Phi_{PS\ II}$ nie wpływał okołodobowy rytm szparek u roślin kontrolnych. Natomiast, u roślin zalewanych, Φ_{PSII} spadało wskutek zamykania szparek. Fv/Fm, miara całkowitej wydajności fotosyntetycznej roślin zaadaptowanych do ciemności, spadał po 57 h zalewania roślin. Wskazuje to na niekorzystny wpływ wydłużonego stresu zalewania na membrany tylakoidowe. Niefotochemiczne wygaszanie fluorescencji (NQ), która jest miarą rozpraszania niewykorzystanej energii w postaci ciepła, wzrastała po 32 h od rozpoczęcia stresu zalewania. W pracy dyskutowana jest niezależność zmian parametrów fluorescencji chlorofilu *a* i indukowanego stresem zalewania zamykania się szparek.

Dr Franciszek **Janowiak**
Zakład Fizjologii Roślin im. F. Górskiego
Polskiej Akademii Nauk
ul. Podłużna 3
30–239 KRAKÓW
email: fjanowiak@yahoo.com