Differences in the effects of flooding the soil early and late in the photoperiod on the water relations of pot-grown tomato plants

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Received 16 March 2000; received in revised form 16 October 2000; accepted 17 October 2000

Abstract

The water relations of potted tomato plants (Lycopersicon esculentum Mill. cv INCA 9(1)) submitted to two flooding/recovery cycles imposed at the beginning and at the end of photoperiod were studied under controlled conditions. In both experiments, flooding induced stomatal closure due to leaf dehydration linked to a lowered root hydraulic permeability. Thus, during the early hours of flooding there was an effective negative hydraulic message from O2 deficient roots. However, when the soil was drained stomata did not re-open until some time after leaf water potential and leaf turgor potential returned to normal, supporting the view that chemical messages predominate. Flooding imposed at the end of photoperiod induced a delay in \(g_l, \Psi_l,\) and \(\Psi_p,\) recovery. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Flooding; Tomato plants; Root hydraulic conductivity; Water relations

1. Introduction

Stomata closure is one of the earliest plant responses to soil inundation. It can be as a consequence of a transient wilting [1], since one effect of soil is to limit the diffusion of oxygen to the root zone [2] and to reduce the permeability of the roots to water uptake [3]. Although, water relations and regulation of stomata in the flooded tomato plant has been intensively studied [4–7] there is a variety of experiments which have shown that a reduction in soil O2 can sometimes cause stomatal closure even when there is no change or increase in bulk leaf potential [8]. Therefore, it is unclear how the stomatal response is initiated and even whether stomatal control mechanisms vary according to the duration and time of flooding of the root system, Bradford and Hsiao [4], varied time at which flooding was started in the daily light–dark cycle. They found different stomatal responses and indirect evidence for an influence of roots in maintaining stomatal opening. An obvious consequence of conventional cohesion theory of water movement in plants is that the amount water supplied to the shoot by the roots will depend in the evaporative demand of the atmosphere. This means that the hydraulic resistance of roots decreased as rates of transpiration increase [9] if leaf water potential remains reasonably stable. However, there are contrasting reports as regards the extent and duration of the reduced hydraulic conductivity of flooded roots [10,11].

The purpose of the present work was to study the response of tomato plants to flooding stress imposed at the beginning and at the end of the photoperiod when the evaporative demand of the atmosphere is completely different. In these condi-
tions the amounts of solutes entering the shoots via the transpiration stream can be altered and the resulting effect on shoot physiology may be different [4,7]. Time courses of leaf and turgor water potential, leaf conductance and root hydraulic conductivity were generated during stress and recovery periods, to examine the mechanisms by which flooding affects the water relations of tomato plants.

2. Materials and methods

2.1. Plant material and treatments

Tomato plants (Lycopersicon esculentum Mill. cv INCA 9(1)) were grown in 0.5 l pots containing washed silica sand in a growth chamber with a 13 h photoperiod, a photosynthetically active radiation (PAR) of 350 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), a 25/20°C light/dark differential temperature and 60/75% light/dark relative humidities.

All pots were watered daily with a nutrient solution [12] beginning 2 weeks after sowing. Plants at the eight-leaf stage were submitted to two treatments in each of two different experiments. In both experiments, control plants were irrigated as indicated, and flooding stress was imposed by placing plant pots inside larger plastic pots filled with tap water at 25°C, with the water level maintained 10 mm above soil level for 36 h. In the first experiment, flooding stress was imposed 1 h after the beginning of the photoperiod, while in the second experiment, flooding stress was imposed 1 h before the end of the photoperiod. For recovery purposes, the pots containing the stressed plants were removed from the water, drained for 3 h and then placed in the same conditions as the control plants. Recovery was studied over a period of 108 and 120 h in the first and second experiment, respectively.

The design of the experiments involved completely randomised blocks with three replications (two plants per replicate).

2.2. Measurements

The oxygen content of the soil water surrounding the roots was measured with an Orion, model 810, dissolved oxygen meter (Orion Research Inc., Beverly, MA). One suction probe was installed in four pots per treatment. Measurements were made in extracted soil water immediately after collecting.

Abaxial leaf conductance \((g_l)\), leaf water potential \((\Psi_l)\), leaf turgor potential \((\Psi_p)\) and root hydraulic conductivity \((L_p)\) were measured at 2 h intervals during the light/dark period in the first/second experiment, respectively. During the recovery periods measurements were taken 6 h into the photoperiod, using six plants from each treatment. The abaxial conductance \((g_l)\) of attached leaf four of the control and flooded plants were determined using a LI-COR LI-1600 steady-state porometer (LI-COR Inc., Lincoln NE, USA). The water potential \((\Psi_l)\) of leaf four was estimated according to the method described by Scholander et al. [13], using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA). Leaves were enclosed in a plastic bag and sealed in the chamber within 20 s of collection [14] and pressurised at a rate of 0.02 MPa s\(^{-1}\).

Leaves from the \(\Psi_l\) measurements were frozen in liquid nitrogen. After thawing, the osmotic potential \((\Psi_s)\) was measured in the expressed sap using a Wescor 5500 vapour pressure osmometer (Wescor Inc. Logan, UT) according to Gucci et al. [15]. Estimates of leaf turgor potential \((\Psi_p)\) were based on the difference between \(\Psi_l\) and \(\Psi_s\).

Root hydraulic conductivity \((L_p)\) was measured according to Ramos and Kaufmann [16]. Plants were detopped with a razor blade and the sand was carefully washed away from the roots. Then the root system was immediately submerged in a container of water and placed in the pressure chamber with the cut stump exposed to the outside for 30 min. After a good seal was obtained, the air pressure was increased at an approximate rate of 0.4 MPa min\(^{-1}\) up to a final pressure of 0.8 MPa. A small piece of plastic tubing was fitted to the stump, and every 3 min the exudate was collected and its volume measured. After the exudation measurements, total root length was estimated using a Delta-T measurement system (Delta-T Devices Ltd., Cambridge, UK). Hydraulic root conductivity was calculated using the formula:

\[ L_p = J/(P \times L), \]

where \(L_p\) is expressed in mg m\(^{-1}\) s\(^{-1}\) MPa\(^{-1}\), \(P\) is the applied hydrostatic pressure (MPa), \(L\) is the root length (m) and \(J\) is the water flow rate through the entire root system (mg s\(^{-1}\)).
3. Results

3.1. Flooding at the beginning of photoperiod

A rapid decrease in the oxygen levels of the water surrounding the roots was noted from the beginning of the flooding period. The measured O₂ levels during the flooding period were 0.16 mmol l⁻¹ at 0 h, 0.08 mmol l⁻¹ at 1 h, 0.05 mmol l⁻¹ at 6 h, 0.023 mmol l⁻¹ at 24 h and 0.019 mmol l⁻¹ at 36 h.

Control plants showed a rapid increase in conductance (gᵢ) values from the beginning of the photoperiod, reaching maximum between 3 and 7 h before and then decreasing (Fig. 1A). Leaf conductance values began to fall within 3 h of the start of flooding, reaching a minimum of 92 mmol m⁻² s⁻¹ at 7 h and remaining at this level (Fig. 1A). During the second photoperiod, the stomata of flooded plants failed to open fully, their gᵢ values became even lower and more constant (around 65 mmol m⁻² s⁻¹) (data not shown). Following 36 h of waterlogging, recovery of leaf conductance after the plants were removed from the water occurred progressively. Flooded plants reached similar gᵢ levels as control plants 42 h after the end of the flooding period (Fig. 2A).

Leaf water potential (Ψᵢ) in control plants presented a typical daily rhythm with maximum values being reached at the beginning of the photoperiod before decreasing progressively and reaching minimum values about 5 h into the photoperiod (Fig. 1B). Flooding stress induced a clear reduction in Ψᵢ values during the first 6 h of the flooding period, after which a sudden and progressive increase to control levels was noted. This recovery of Ψᵢ was completed by 11 h into the photoperiod and maintained during the subsequent recovery period when the pots were drained (Fig. 2B).

Leaf turgor potential (Ψᵢ) in control plants ranged between 0.31 and 0.38 MPa (Fig. 1C). Flooding stress induced a progressive decrease in Ψᵢ during the first 4 h of the stress period, when it

Fig. 1. Leaf conductance (gᵢ), leaf water potential (Ψᵢ), leaf turgor potential (Ψᵢ) and root hydraulic conductivity (Lᵢ) in control (closed symbols) and flooded (open symbols) plants at the beginning of the photoperiod. Each point is the mean of six measurements. Vertical bars represent S.E. of the mean (not shown when smaller than the symbols). Asterisks indicate statistically significant differences by LSD₀.05.

Fig. 1.
reached values of 0.21 MPa. Subsequently, a progressive recovery of $\Psi_p$ was noted, reaching similar $\Psi_p$ values to the control plants after 11 h (Fig. 1C). Similarly to that observed for $\Psi_p$, the recovery of $\Psi_p$ was maintained when the soil was drained after 36 h flooding (Fig. 2C).

Different behaviour was observed in root hydraulic conductivity ($L_p$) of control and flooded plants. $L_p$ values decreased progressively in the flooded plants during the first photoperiod. This reduction was greater after 11 h of flooding, reaching a reduction of 60% with respect to the control plants (Fig. 1D). These values remained low and constant during the subsequent photoperiod (data not shown). Control plants, on the other hand, showed a progressive increase in $L_p$ from the beginning of the photoperiod. This parameter reached maximum values at 7 h, after which it decreased (Fig. 1D). Throughout the recovery period $L_p$ levels in flooded plants were lower than in control plants (Fig. 2D).

### 3.2. Flooding at the end of photoperiod

Oxygen levels in the water surrounding the roots decreased rapidly from the beginning of the flooding period, reaching 0.19, 0.10, 0.07, 0.025 and 0.022 mmol l$^{-1}$ at 0, 1, 6, 24 and 36 h, respectively.

After the first dark period, leaf conductance ($g_l$) in flooded plants presented very low and constant values (around 44 mmol m$^{-2}$ s$^{-1}$) throughout the photoperiod (data not shown). This is evidence of almost complete stomata closure. In control plants, $g_l$ showed a similar behaviour to that of plants depicted in Fig. 1A (data not shown). During the recovery period, a progressive increase in $g_l$ occurred in flooded plants, reaching similar values to those of the control plants at 96 h (Fig. 4A).

Differences between treatments in leaf water potential ($\Psi_l$) were found during the first dark period during flooding (Fig. 3A). In control plants, $\Psi_l$ increased from the beginning of the dark period, reaching maximum values after 3 h, after which they remained almost constant (around $-0.33$ MPa). On the contrary, the $\Psi_l$ of

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**Fig. 2.** Leaf conductance ($g_l$), leaf water potential ($\Psi_l$), leaf turgor potential ($\Psi_p$) and root hydraulic conductivity ($L_p$) in control and flooded plants at the beginning of the photoperiod, during a recovery period following 36 h of flooding. Black bars on abscissa indicate periods of darkness. Each point is the mean of six measurements. Symbols as in Fig. 1.
flooded plants decreased progressively from $-0.39$ to $-0.51$ MPa. The recovery of $\Psi_l$ after the plants were removed from the water after 36 h was progressive, reaching similar values to those of the control plants at 48 h (Fig. 4B).

Leaf turgor potential ($\Psi_p$) in control plants remained constant (at around 0.32 MPa) during the first dark period (Fig. 3B). Flooding stress induced a sudden decrease in $\Psi_p$ values at the beginning of the stress period, with values of 0.11 MPa being reached 2 h into flooding, increasing progressively to 0.18 MPa after that time (Fig. 3B). These $\Psi_p$ values in stressed plants were nearly constant during the remaining flooding period (data not shown). During the recovery period a progressive increase in $\Psi_p$ was noted, until similar levels to the control plants was reached at 48 h (Fig. 4C).

In the control plants root hydraulic conductivity ($L_p$) began to decrease gradually from the beginning of the dark period, reaching minimum values at 3 h and remaining constant thereafter (Fig. 3C). Flooding accentuated this decrease in $L_p$ values from the beginning of the stress period, and $L_p$ reached a minimum value of 442 $\mu$g s$^{-1}$ m$^{-1}$ MPa$^{-1}$ at 5 h (Fig. 3C). These values remained nearly constant during the rest of the flooding period (Fig. 3C). When the plants were removed from the water a partial increase in $L_p$ was observed after 100 h, although values were still lower than those in the control plants (Fig. 4D).

4. Discussion

The decrease in $L_p$ when tomato plants were flooded at the beginning of the photoperiod (Fig. 1D) could be attributed to the decrease in $O_2$ in the root zone and probably to increase in $CO_2$ concentration caused by import of gas from the soil [17]. Bradford and Yang [3] and Kozlowski and Pallardy [8] indicated that a shortage of $O_2$ at the roots interferes with water uptake of these organs, inducing a temporary depression in conductivity across the membranes, such an effect may induce a temporary loss of leaf hydration. In our conditions, the depression of $\Psi_l$ observed during the first 4 h of the flooding period (Fig. 1A) could have been the effect of a negative hydraulic message generated by $O_2$ deficient roots due to the fall in $L_p$. Consequently, the partial stomatal closure observed in these plants would appear to be the result of a leaf water deficit. This behaviour

![Fig. 3. Leaf water potential ($\Psi_l$), leaf turgor potential ($\Psi_p$) and root hydraulic conductivity ($L_p$) in control and flooded plants at the end of the photoperiod. Each point is the mean of six measurements. Symbols as in Fig. 1.](image-url)
has also been described by Else et al. [18] in tomato plants in similar conditions.

The fact that turgor potential ($\Psi_p$) also decreased during the first 4 h of flooding may be associated with stomatal closure (Fig. 1A and Fig. 1C), although it is also possible that osmotic changes resulting in lower guard-cell turgor could have occurred. Several authors have related osmotic changes with the migration of potassium ions from guard cells [19], the regulation of stomata by ABA may involve interference with this ionic transport between guard cells and adjacent cells [8].

During the recovery period there was a temporary partial closure of the stomata which was unrelated with $\Psi_l$ and $L_p$ showed minimum and constant values (Fig. 2). The fact that the complete recovery of $g_l$ values in flooded plants occurred later than the recovery of $\Psi_l$ and $\Psi_p$ values may mean that there was no simple passive response to water deficit as a result of high root resistance [4]. The pattern of stomatal behaviour seen in this period may be viewed as a more permanent effect of active chemical signals transmitted from roots, reflecting the effect of soil conditions on the activity and metabolism of the roots [17,18]. As a consequence, partial stomatal closure allowed the leaf water status to recover.

Root hydraulic conductivity ($L_p$) showed a different dynamic in the control plants of both experiments when the evaporative demand was different (Fig. 1D and Fig. 3C). Steudle and Peterson [9] pointed out that plants are designed to optimise water uptake according to demands from the shoot. In a transpiring plant, the hydrostatic pressure gradient generated between the soil solution and the xylem by transpiration will be high and the hydraulic resistance of the root low. This facilitates water uptake as demand increases. On the other hand, hydraulic conductivity will be lower during the night as can be observed from our results.

When plants were flooded at the end of the photoperiod the decrease of O$_2$ levels in the soil

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Fig. 4. Leaf conductance ($g_l$), leaf water potential ($\Psi_l$), leaf turgor potential ($\Psi_p$) and root hydraulic conductivity ($L_p$) in control and flooded plants at the end of the photoperiod, during a recovery period following 36 h of flooding. Black bars on abscissa indicate periods of darkness. Each point is the mean of six measurements. Symbols as in Fig. 1.
surrounding roots induced the progressive decrease in \( L_p \), and, as a consequence, a rapid decrease in \( \Psi_1 \) and \( \Psi_p \) (Fig. 3). The absence of a hydrostatic pressure gradient during the most of the dark period could explain the different behaviour in the recovery of \( \Psi_1 \) and \( \Psi_p \) in both experiments (Fig. 4B). Since, in the second experiment it seems that only the cell-to-cell component is left for water movement, which decreases the cross-sectional area available for apoplastic water flow, making it more difficult for the roots to take up water [20,21].

In this last experiment, the fact that \( g_l \) recovered later than \( \Psi_1 \) and \( \Psi_p \) after the flooding stress period had terminated confirms again that a chemical signal from roots was operating. The delay in \( g_l \) recovery could be due to the operation of factors exported by the roots and accumulated in the leaves, which have a direct effect on stomatal opening [21]. ABA, ethylene and ethylene precursor ACC are likely candidates, since they increase in shoots as a result of flooding [7,22].

Our results indicate that, in both experiments, flooding resulted in stomatal closure due to the leaf dehydration caused by increased resistance to water uptake as a result of the lowered permeability of the roots. The involvement of a hydraulic message from the roots during the early hours of flooding was evident. However, after that and during the recovery period, chemical messages are presumed to be responsible for the persistent partial stomatal closure, which is unrelated to the leaf water potential. Flooding imposed just prior to an 11 h dark period induced a greater delay in \( \Psi_1 \) and \( \Psi_p \) recovery than when flooding began early in a 13 h light period. The reasons for this are under investigation.

Acknowledgements

We thank M.D. Velasco for her assistance. This research was supported by the CSIC-CITMA (99CU0002) co-operation project and the CITMA (PNCT 013 05 008) project.

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