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High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato

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Abstract

Potted tomato plants (*Lycopersicon esculentum* Mill. cv. Amalia) were submitted to three different treatments: control (C) plants were maintained at day/night temperature of 25/18 °C; preconditioned plants (PS) were submitted to two consecutive periods of 4 d each, of 30/23 and 35/28 °C before being exposed to a heat stress (40/33 °C lasting 4 d) and non-preconditioned (S) plants were maintained in the same conditions as the C plants and exposed to the heat stress. The inhibition of plant growth was observed only in PS plants. Heat stress decreased chlorophyll content, net photosynthetic rate and stomatal conductance in both PS and S plants. However, PS plants showed good osmotic adjustment, which enabled them to maintain leaf pressure potential higher than in S plants. Furthermore, at the end of the recovery period PS plants had higher pressure potential and stomatal conductance than in S plants.

Additional key words: leaf conductance, leaf osmotic potential, leaf water potential, photosynthesis, plant growth.

Introduction

Heat stress is a major factor limiting growth in many plant species and areas. Growth suppression involves changes in physiological processes, including plant water relations (Paulsen 1994, Jiang and Huang 2001, Mazorra *et al.* 2002).

Most crops present optimum growth within a range of temperatures of 10 - 30 °C, plant productivity decreasing substantially outside this range (Shabala 1996). Temperatures in the higher part of this range favour stomatal opening, while temperatures above it induce stomatal closing. Above 30 - 40 °C, respiratory processes are more favoured than photosynthetic ones, internal CO_2 concentration increases and stomata close (Barceló *et al.* 1987).

According to Zhuchenko (1988), the ability of plants to attenuate the negative effect of high temperatures through stress tolerance mechanisms is limited. Hence, plant heat resistance for most species mainly involves stress avoidance (Shabala 1996).

The aim of this work was to determine the response of preconditioned and non-preconditioned tomato plants (a new Cuban Amalia cultivar characterised by high yield and fruit quality) to heat stress. The ability of these plants to recover after heat stress was also examined. Growth, chlorophyll content, leaf water, osmotic and pressure potentials, root hydraulic conductivity, net photosynthesis rate and stomatal conductance were measured.

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Abbreviations: C - control plants; g_s - stomatal conductance; L_p - root hydraulic conductivity; P_n - net photosynthesis rate; PS - preconditioned stressed plants; RGR - relative growth rate; S - stressed plants; Ψ_{os} - osmotic potential in water saturated leaf; Ψ_p - leaf pressure potential; Ψ_s - leaf osmotic potential; Ψ_w - predawn leaf water potential.

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Materials and methods

Plant growth and treatments: Tomato plants (*Lycopersicon esculentum* Mill cv. Amalia) obtained from seeds were grown in 0.5 dm^3 pots containing washed silica sand in a growth chamber with a 13-h photoperiod, an irradiance of 380 $\mu mol\ m^{-2}\ s^{-1}$ (PAR), and day/night average temperature and relative humidity 25/18 °C and 60/70 %, respectively.

All pots were watered daily with a nutrient solution (Verdure 1981) beginning two weeks after sowing. When plants were at the four leaf stage (20-d-old), 192 plants of similar appearance were selected and submitted to three different treatments: control (C) and stressed (S) plants were maintained at day/night temperature of 25/18 °C for 8 d, and PS plants were submitted to two consecutive preconditioning periods of 4 d each, of day/night temperature 30/23 and 35/28 °C, respectively. After this, both S and PS plants were submitted to a heat stress period of day/night temperature 40/33 °C for 4 d. After the stress period, treated plants were maintained in the same conditions as the control plants for a further 12 d. In all treatments, at the beginning/end of the photoperiod the increase/decrease of temperature was imposed at a rate of around 1.3 °C h^{-1}.

The design of the experiment involved completely randomised blocks with four replications (four plants per replicate).

Measurements: Before the start of the preconditioning treatments, and at the end of the stress and recovery periods dry mass and allocation were measured in two plants per replicate. Leaf area was also measured using an *Image Analysis System* (*Δ-T Devices Ltd.*, Cambridge, UK).

To compare the effects of the treatments on plant growth, relative growth rate (RGR) was calculated using the formula:

$$RGR = (\ln DM_2 - \ln DM_1)/\Delta t$$

where DM_1 and DM_2 are the dry masses at the beginning and the end of a period, respectively, and Δt is the time between the beginning and the end of the period.

At the start and at the end of each experimental period the leaf water potential (Ψ_w) was estimated according to the method of Scholander *et al.* (1965), using a pressure chamber (*Soil Moisture Equipment Co*, Santa Barbara, USA). For this, the leaves were enclosed in a plastic bag and sealed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner 1988). The leaves used for the Ψ_w measurements were frozen in liquid nitrogen and, after thawing, the osmotic potential (Ψ_s) was measured in the expressed sap using a *Wescor 5500* vapour pressure osmometer (*Wescor*, Logan, USA) according to Gucci *et al.* (1991). Leaf pressure potential (Ψ_p) was estimates as the difference between Ψ_w and Ψ_s. For the measurement of Ψ_{os}, the leaf samples were harvested and subjected to a rehydration treatment involving dipping their petioles in distilled water overnight before proceeding as explained above for osmotic potential.

Midday stomatal conductance (g_s) and net photosynthetic rate (P_n) were measured at irradiance of 380 $\mu mol\ m^{-2}\ s^{-1}$ using a *LICOR LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, USA), respectively.

Leaf chlorophyll was extracted from discs with N,N-dimethylformamide using the Torrecillas *et al.* (1984) procedure and its content was determined according to Inskeep and Bloom (1985).

Predawn root hydraulic conductivity (L_p) was estimated according to Huxman *et al.* (1999). 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 obtaining a good seal, the pressure was raised from 0.2 to 0.8 MPa in 0.2 MPa increments. A small piece of plastic tubing was fitted to the stump, and every 3 min the exudate was collected and its volume measured. The exudate flux was normalised per root dry mass, to estimate water flux (J_v). Hydraulic conductivity was determined from the slope of the linear regression generated from a plot of applied pressure versus J_v.

Results

At the end of the stress period, plants from the preconditioning treatment (PS) showed a significant reduction in stem, leaf and root dry masses, total leaf area and RGR compared with the control, whereas the values in plants from the heat stress treatment (S) were similar to those of the control plants (C) (Table 1). At the end of the recovery period, these differences between C and PS plants were maintained (Table 1). It should be pointed out that in plants from the S and PS treatments bloomed later than C plants (data not shown).

A significant decrease in the chlorophyll content was observed during the preconditioning and stress periods in treated plants (Fig. 1). However, at the end of the recovery period, the chlorophyll contents of PS and S plants were similar to those of the C plants.

From the beginning of the first preconditioning period

Table 1. Root, stem and leaf dry mass [g plant^{-1}], total leaf area [cm^2 plant^{-1}] and relative growth rate (RGR) [g g^{-1} d^{-1}] in control (C), preconditioned and heat stressed (PS) and heat stressed (S) plants at the beginning of the experiment (0 d), at the end of the stress period (12 d) and at the end of the recovery period (24 d). Means within a column for each time followed by the same letter are not different at $P = 0.05$ (LSD test).

Time	Treatment	Root DM	Stem DM	Leaf DM	Leaf area	RGR
0 d	C	0.60 ± 0.08	0.36 ± 0.06	0.63 ± 0.07	293.46 ± 14.50	-
12 d	C	2.10 ± 0.03a	2.15 ± 0.03a	3.60 ± 0.18a	1046.41 ± 10.27a	441.52 ± 37.25a
	PS	1.19 ± 0.07b	1.11 ± 0.09b	2.13 ± 0.08b	633.46 ± 22.30b	279.81 ± 27.09b
	S	2.07 ± 0.06a	2.02 ± 0.11a	2.56 ± 0.21ab	914.01 ± 15.40a	402.81 ± 48.94a
24 d	C	2.51 ± 0.22a	3.57 ± 0.21a	6.61 ± 0.06a	1666.98 ± 12.18a	266.65 ± 17.41a
	PS	1.56 ± 0.19b	1.90 ± 0.17b	3.53 ± 0.21b	998.49 ± 13.02b	190.21 ± 19.98b
	S	2.48 ± 0.13a	3.23 ± 0.18a	6.45 ± 0.09a	1565.00 ± 18.62a	261.23 ± 16.72a

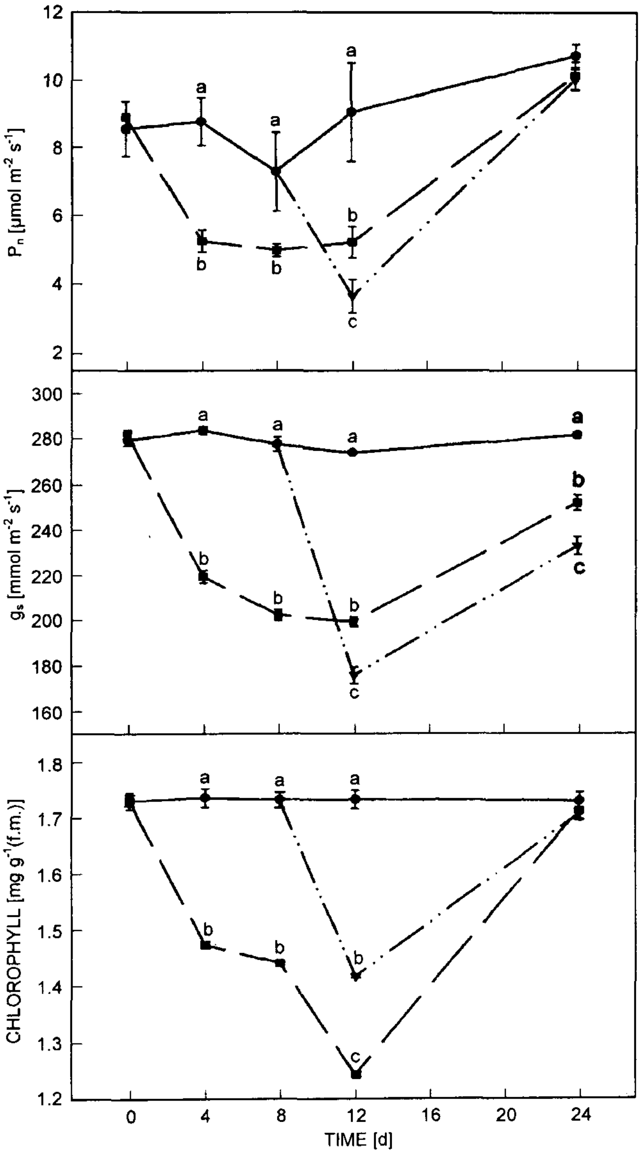

Fig. 1. Leaf chlorophyll content, midday stomatal conductance (g_s) and midday net photosynthesis (P_n) in control (*circles*), PS (*squares*) and S (*triangles*) plants throughout the experimental period. Values with the same letter are not different at $P = 0.05$. *Vertical bars* on data points present ± SE (not shown when smaller than the symbols).

(30/23 °C day/night temperature) P_n, g_s, Ψ_w, Ψ_p and L_p in plants from the PS treatment decreased (Figs. 1 and 2). Also, Ψ_{os} values in PS plants decreased during the second preconditioning period (35/28 °C day/night temperature) (Fig. 2).

During the heat stress, S plants showed a substantial decrease in P_n, g_s, Ψ_w, Ψ_{os}, Ψ_p, and L_p (Figs. 1 and 2). In PS plants Ψ_p values decreased to a lesser extent than in S plants, and P_n, g_s and L_p values remained nearly constant, whereas the Ψ_w and Ψ_{os} values decreased (Figs. 1 and 2).

During the recovery period, all the studied plant water relations parameters in PS and S plants recovered from minimum values. However, Ψ_{os} values in PS plants and Ψ_p and Ψ_w in S plants did not reach the levels of the control plants (Fig. 2). It is also important to point out that at the end of the experiment significant differences between treatments were found in g_s, with minimum values being achieved in S plants and maximum in C plants (Fig. 1).

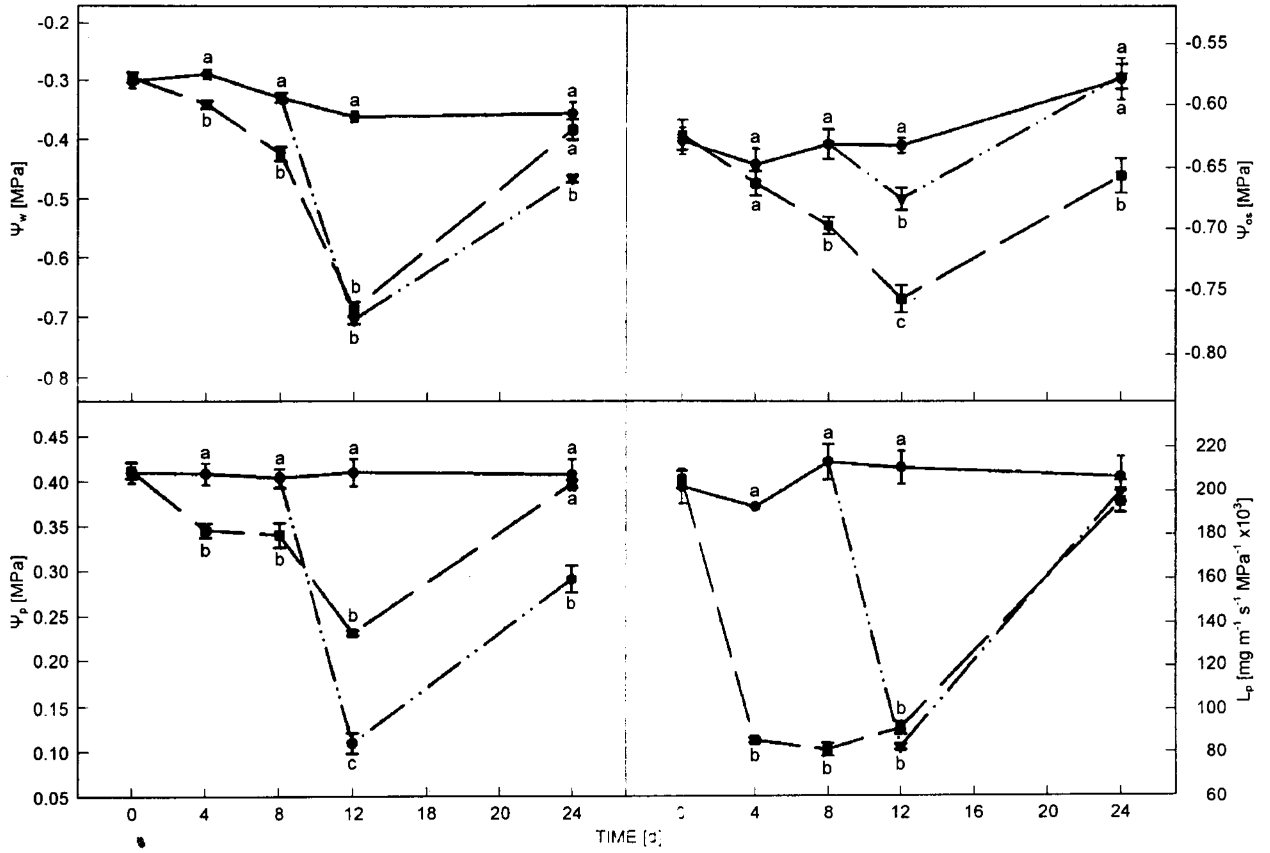

Fig. 2. Predawn leaf water potential (Ψ_w), leaf osmotic potential after saturation (Ψ_{os}), predawn leaf pressure potential (Ψ_p) and predawn root hydraulic conductivity (L_p) in control (*circles*), PS (*squares*) and S (*triangles*) treatments throughout the experimental period. Symbols as in Fig. 1.

Discussion

During the stress and recovery periods PS treatment plants showed a significant reduction in their biomass and relative growth rate (RGR), whereas plants from the S treatment were not affected (Table 1). Therefore, the effect of high temperature on tomato plant growth depends mainly on the duration of the stress. In this sense Georgieva (1999) indicated that prolonged periods under moderately high temperature can be more dangerous than a brief exposure to extreme temperatures.

The observed reduction in P_n in treated plants during the preconditioning and stress periods (Fig. 1) is in accordance with the results found by Starck *et al.* (1993). High temperature stress inhibits photosynthetic rate and increases respiration rate so that the amount of photosynthates is reduced (Hale and Orcutt 1987, Jiao and Grodzinski 1996). Moreover, it is important to point out that the osmotic adjustment (Ψ_{os} decrease) observed in treated plants is probably based on an active accumulation of amino acids (Chaitanya *et al.* 2001) at a high energy cost (Alarcón *et al* 1993, Torrecillas *et al.*

1994, 1995), and this expenditure of energy in PS plants could also reduce their biomass and RGR.

The observed chlorophyll decrease in treated plants (Fig. 1) might be related with damage to the reaction centres (Kyle 1987). According to Chaitanya *et al.* (2001), chlorophyll content and photosystem 2 activity are affected in plants under heat stress.

High temperature during the preconditioning and stress periods promoted differences in the plant water relations between treated and control plants.

At the end of the first preconditioning period (30/23 °C day/night temperature), the decreased root hydraulic conductivity (L_p) might explain why Ψ_w, Ψ_p, and gas exchange decreased in PS plants (Figs. 1 and 2). Rodríguez *et al.* (1997) indicated a direct effect of the root medium temperature on L_p. The question arises as to what changes may have caused the root to increase its hydraulic resistance in response to our experimental conditions.

During the second preconditioning period (35/28 °C day/night temperature), the observed decrease in Ψ_{os} (osmotic adjustment) in PS treatment plants would explain why Ψ_p remained constant in spite of low L_p (Fig. 2). Also, the fact that at the end of the stress period leaf Ψ_p was significantly lower in S plants than in PS plants could be ascribed to the higher osmotic adjustment achieved in PS plants (Fig. 2). Osmotic adjustment is considered an important mechanism of plant tolerance not only to drought but also to heat stress (Seeman *et al.* 1986, Smith *et al.* 1989, Ludlow *et al.* 1990).

At the end of the recovery period the fact that g_s values in PS and S plants did not recover completely, especially in PS plants, in which Ψ_w and Ψ_p had recovered, indicated that stomatal regulation was not a simple passive response to stress. The partial stomatal closure observed after rewatering can be also considered as a mechanism which allows the plant to regain full pressure potential more efficiently (Ruiz-Sánchez *et al.* 1997).

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