

Stomatal Movement in Response to Root Zone Temperature in Purple Heart (*Tradescantia pallida*)

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Received: 09 February 2016

Accepted: 24 May 2016

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The effects of root temperatures (25, 35 and 45°C) and temperature duration (30, 60 and 90 min) on net photosynthesis rate, stomatal conductance and transpiration rate in *Tradescantia pallida* were investigated. The experiment was conducted under controlled conditions with factorial arrangement based on a completely randomized design (CRD) and four replications. Result showed that, net photosynthesis rate was not significantly different between plants treated with 25 and 35°C. However, aperture area and width increased at 35°C and declined sharply at 45 °C as compared with that at 25 °C. Net photosynthesis rate and stomatal conductance of plants treated with 45°C decreased to 76 and 68%, respectively, as compared with those at 35°C. Stomatal aperture area of plants treated with 35°C was 27% and 320% higher than those treated with 25 and 45°C, respectively. Stomatal resistance of plants treated with high temperature (45°C) were higher (174%) than those treated with 35°C. In 35 °C, aperture area of plants after 30 min was 61% and 45% higher than those after 60 and 90 min exposure, respectively. The results revealed that, a heat shock of roots at 45°C could lead to a significant decrease in stomatal conductance (by 81%) and transpiration rate (by 60%) as compared with those at 35°C. Overall, the results suggest that the root temperature affects leaf gas exchange and stomatal behavior and has to be taken into account in plant production system, in particular, hydroponics.

Abstract

Keywords: Heat shock, Photosynthesis, Root zone temperature, Stomatal conductance.

Abbreviations: C_i , Intercellular CO₂ concentration; P_N , Net photosynthetic rate; g_s , Stomatal conductance; r_s , Stomatal resistance.

INTRODUCTION

Tradescantia pallida is an evergreen perennial plant of scrambling stature. It is distinguished by elongated, pointed leaves that have large stomata and used as a model plant for research on stomata behavior. Leaves of plants are equipped with stomata made of pores surrounded by pairs of adjacent guard cells that tightly regulate the pore aperture. Stomata govern leaf diffusive conductance, and thereby influence the photosynthesis and transpiration processes in plants. Plants are exposed to environmental stresses such as temperature and drought in both natural and agricultural environments. Initial responses to stress such as changes in CO₂ fixation, stomatal limitations, and decrease in transpiration occur at the leaf level (Haldimann and Feller 2004; Iriti *et al.*, 2009). Stomata play a dominant role in the control of plant water relations and photosynthesis.

To achieve the optimal response to multifactorial environmental changes, stomata perceive many environmental factors i.e. temperature and drought, and have the ability to integrate environmental and endogenous signals (Schroeder *et al.*, 2001). Temperature is one of the most important environmental factor limiting growth and survival of plant. High temperature may affect plant metabolism indirectly by promoting evaporative water loss from the soil or directly by injuring the photosynthetic apparatus (Haldimann and Feller, 2004). In warm and moist environments, stomata have been shown to open wide (Feller, 2006), while at low temperatures they tend to close (Wilkinson *et al.*, 2001; Veselova *et al.*, 2006). Understanding stomatal regulatory strategies is important in determining a plant's overall stress response. When plants are exposed to drying soil, stomatal conductance (gs) and leaf expansion can be regulated by long distance chemical signals travelling from root to shoots (Davies and Zhang, 1991). However, knowledge about the effects of root zone temperature on stomata remains limited. In previous studies attention has been directed to the measurements of stomatal responses to temperature around aerial parts of plants. In this study we focus on gas exchange parameters and stomatal behavior in response to changes in root zone temperature.

MATERIALS AND METHODS

Plant materials and growth conditions

The experiment was carried out in a greenhouse at research field of Faculty of Agriculture, Lorestan University, Khorramabad, Iran (latitude 33° 29' N and longitude 48° 22' E and an altitude of 1125 m) during April - July 2015. The greenhouse conditions were 25 ± 2°C / 18 ± 2°C (day night⁻¹), 70 ± 5% RH and light intensity of about 400 μmol m⁻² s⁻¹. Terminal stem cuttings of *Tradescantia pallida* were obtained from mother plants in the same greenhouse and placed in a sand substrate for rooting. Uniformly rooted cuttings were then transplanted into plastic pots (15 cm diameter and height) filled with a mixture of equal proportions of clay soil, sand and decayed cow manure. Plants were kept well-watered and given a soluble fertilizer once two weeks throughout the cultivation. Physical and chemical properties of soil used in this study were loam texture, pH of 7.45, EC of 2.1 dS m⁻¹, K content of 380 mg kg⁻¹, P content of 18.76 mg kg⁻¹, total N content of 0.15%, and organic carbon content of 1.79%. Young fully expanded leaves were used in the experiments.

Experimental design and salinity treatments

To investigate the response of *Tradescantia pallida* under root zone temperatures treatments, a greenhouse experiment was conducted with factorial arrangement based on a completely randomized design (CRD) with four replications in 2015.

Roots were treated at 25, 35 or 45°C for 30, 60 or 90 min. A water bath was adapted to induce root zone temperature treatment. Then, gas exchange parameters were measured using a portable gas exchange system.

Gas exchange measurements

An LCA4 portable gas exchange system (*ADC Bioscientific, Ltd.*, Hoddesdon, England) was used to measure the rate of net photosynthesis (P_N), transpiration rate (E), stomatal resistance

Table 1. Analysis of variance of the effects of root zone temperature and duration on stomatal behavior and gas exchange parameters in *Tradescantia pallida*

S.o.V	Mean square							
	df	Photosynthesis rate	Internal CO ₂	Transpiration rate	Stomatal conductance	Stomata resistance	Aperture width	Aperture area
Temperature	2	48.2 ^{***}	10136 [*]	6.01 ^{***}	0.13 ^{***}	214.6 ^{***}	456.5 ^{**}	313894 ^{***}
Duration	2	2.21 ^{ns}	986.6 ^{ns}	0.333 ^{ns}	0.007 ^{ns}	2.53 ^{ns}	17.95 [*]	65350 ^{***}
Duration × Temperature	4	3.8 ^{ns}	756.4 ^{ns}	0.264 ^{ns}	0.011 ^{ns}	16.49 ^{ns}	10.68 [*]	3712 ^{ns}
Error	24	1.92	2234.7	0.424	0.014	11.2	4.03	4604
CV (%)		32	15.6	29.2	31.4	31.3	18.6	24.4

^{ns}: not significant, ^{*} significant at 5% level probability, ^{**} significant at 1% level probability, ^{***} significant at 0.1% level probability.

(r_s), and stomatal conductance (g_s) on four fully expanded leaves placed at mid-canopy height on each plant. For gas exchange measurements, four upper fully expanded leaves in every individual plant were selected for measurements.

Stomatal aperture was determined using a silicon rubber impression technique (Smith *et al.*, 1989). Stomatal aperture was determined from young fully expanded leaves at approximately two-thirds of the distance from the base to the tip. One leaf per plant and one impression (5 mm × 10 mm) per location on the leaf were used. Then stomatal aperture was determined from 10 randomly selected stomata per impression. Aperture area and width were measured from digitized video images of stomata using a microscope connected to a Nikon digital imaging camera. Image processing was done using the free UTHSCSA Image Tool Program (University of Texas Health Science Centre at San Antonio, TX, USA).

Statistical analysis

Data were subjected to analysis of variance using SAS statistical software (*Version 9.1; SAS Institute*, Cary, NC, USA) package. Duncan's multiple range test was used for mean separation ($P < 0.05$).

RESULTS

Analysis of variance (ANOVA) showed that root zone temperature had a significant effect on gas exchange parameters and stomatal characteristics. The effect of temperature duration and interaction of temperature and duration was significant for stomatal characteristics (Table 1).

Stomatal aperture area increased significantly when root zone temperature changed from

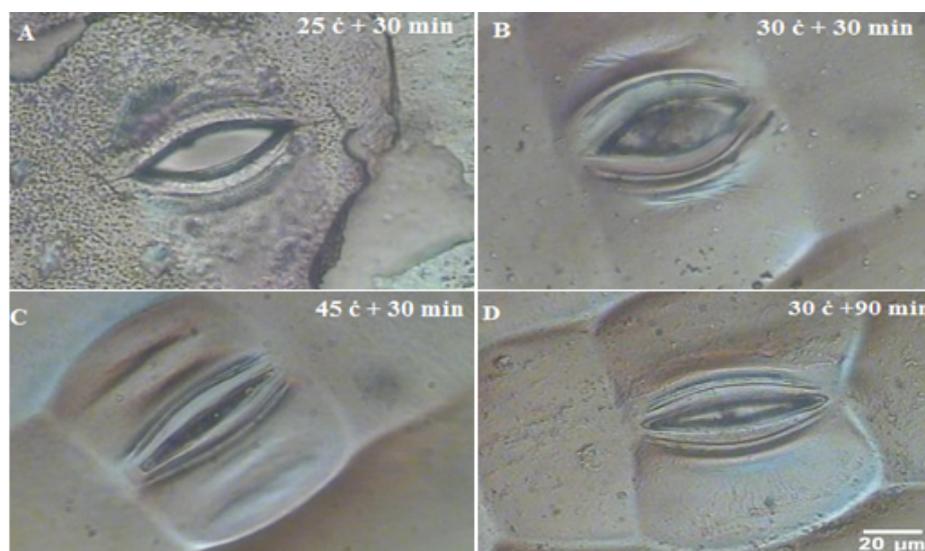


Fig. 1. Stomata of *Tradescantia* in different root zone temperature and duration

Table 2. Effects of root zone temperature on stomatal behavior and gas exchange parameters of *Tradescantia pallida*

Temperature (°C)	Photosynthesis rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Internal CO_2 (vpm)	Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)	Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	Stomatal resistance ($\text{m}^2 \text{s}^{-1} \text{mol}^{-1}$)	Aperture width (μm)	Aperture area (μm^2)
25	4.52 ^a	294.4 ^b	2.56 ^a	0.25 ^a	4.35 ^b	12.69 ^b	323.5 ^b
35	4.9 ^a	278.4 ^b	2.7 ^a	0.29 ^a	4.93 ^b	15.76 ^a	411.5 ^a
45	1.25 ^b	334.8 ^a	1.41 ^b	0.09 ^b	11.95 ^a	3.88 ^c	97.9 ^c

In each column, values with same letter are not significantly different at 5% probability level, according to Duncan Multiple Range Test

25 to 35°C. Fig. 1 presents pictures of the stomata located on the lower leaf surface of *Tradescantia pallida*. The stomata are kidney shaped and there were few stomata on upper surface. Results showed that temperature caused changes in stomatal aperture width and area.

No significant differences were found between temperature duration for P_N , E , g_s , and r_s , but aperture width and area were remarkably affected (Table 1). Interaction between temperature and duration was significant for stomatal aperture width ($P < 0.05$) (Table 1).

Significant difference among root zone temperature was observed for all the measured parameters. Root zone temperature at 25°C significantly increased photosynthesis rate (261%) and stomatal aperture width (227%) as compared with 45°C (Table 2). Although P_N and E were higher at 35°C as compared with 25°C but no statistical difference was observed between them. Area of stomatal aperture at 35°C was higher than that 25 and 45°C by 27% and 320%, respectively. Stomatal conductance decreased (177%) as temperature increases. Results show that stomatal resistance (r_s) increased with increasing temperature. R_s in plants treated with high temperature (45°C) were higher (by 174%) than that in plants treated with 35°C (Table 2).

No significant differences were found between temperature duration in measured parameters except stomatal aperture width and area. Aperture area in duration of 30 min after treatment was higher (61%) than that in 60 min and 90 min (45%). Although r_s was higher in 60 min than 90 and 30 min, their differences were not significant (data not shown).

Plants with roots exposed to 30°C for 30 and 60 min showed the greatest photosynthesis rate. Application of 35°C for 30 minute had 491% and 38% greater photosynthesis rate than 45 and 25°C, respectively. Regardless of temperature duration, transpiration at 35°C was the highest and no significant difference was observed between temperature durations. Moreover, transpiration rate was the lowest in plants treated with 45°C for 90 min. Stomatal aperture area of plants treated with 35°C was higher than that treated with 25 and 45°C with identical duration. Stomatal aperture area of plants treated with 35°C for 30 min showed the greatest value. Stomatal aperture at 35°C was higher (24% and 254%) than that at 25 and 45°C, respectively. Also, aperture width was higher at 35°C as compared with that in other treatments. Plants treated with 45°C for 90 min showed 114% and 153% less stomatal aperture that those treated with 25 and 35°C, respectively (Fig. 2).

Significant correlation was found between photosynthesis rate with stomatal aperture area ($r = 0.6$, $P < 0.001$) and stomatal conductance ($r = 0.43$, $P < 0.001$) (Table 3). Stomatal resistance showed negative significant correlation with photosynthesis rate ($r = -0.61$, $P < 0.001$) and stomatal aperture area ($r = -0.55$, $P < 0.001$), transpiration rate ($r = -0.72$, $P < 0.001$), and stomatal conductance ($r = -0.65$, $P < 0.001$). Furthermore, aperture showed negative correlation with C_i ($r = -0.48$, $P < 0.001$) and r_s ($r = -0.63$, $P < 0.001$). The highest r_s was found in plants treated with 45°C for 90 min (Fig. 2).

Stomatal resistance (r_s) of plants treated with 45°C for 60 min was 297% higher than that treated with 25°C for the same duration. In addition, 60-minute application of 35°C had 69% higher r_s than that in 25°C. However, plants treated with 35°C for 30 min showed 36% lower stomatal resistance than those treated with 25°C (Fig. 2). Stomatal conductance (g_s) of plants treated with 35°C for 60 min was 20 and 285% higher than that at 25 and 45°C, respectively (Fig. 2).

Results showed that as root zone temperature increased to 45°C, stomatal aperture width

Table 3. Correlation coefficient of measured parameters

Correlation	Photosynthesis rate	Aperture area	Internal CO ₂	Transpiration rate	Stomatal conductance	Stomatal resistance
Aperture area	0.60***	1				
Internal CO ₂	0.4*	0.45**	1			
Respiration	0.44**	0.52**	0.52**	1		
Stomatal conductance	0.43**	0.45**	0.37*	0.76***	1	
Stomatal resistance	0.61***	0.55***	0.41*	0.75***	0.65***	1
Aperture width	0.72***	0.88***	0.48**	0.54***	0.48**	0.63***

ns: not significant, * significant at 5% level probability, **significant at 1% level probability, *** significant at 0.1% level probability

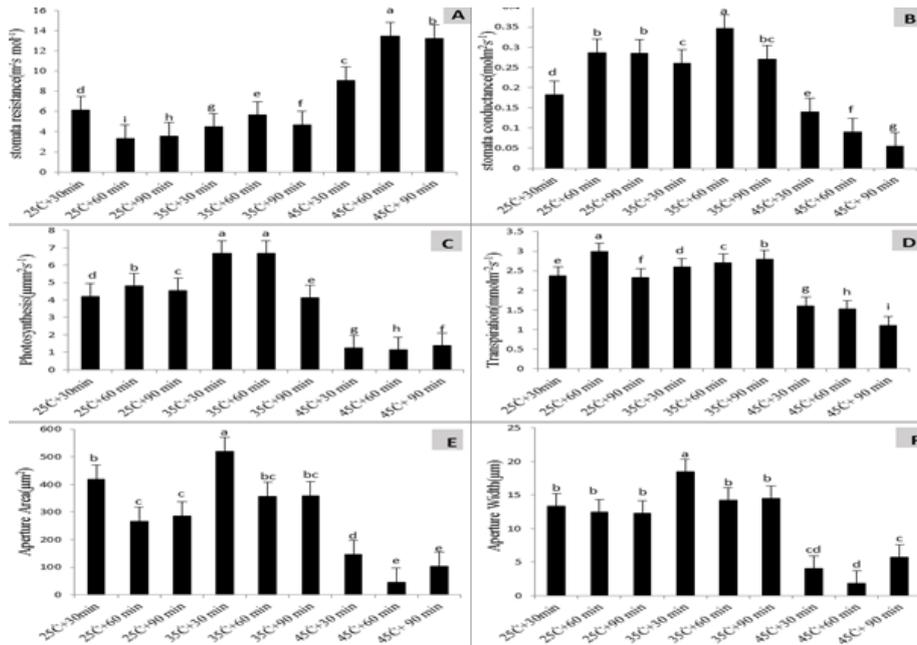


Fig. 2. Interaction of root zone temperature and duration for stomatal behavior and gas exchange parameters of *Tradescantia pallida*. (Values with same letter are not significantly different at 5% probability level, according to Duncan Multiple Range Test).

and photosynthesis rate decreased. There was positive significant correlation between aperture width and photosynthesis rate ($r = 0.72$, $P < 0.001$). But stomatal resistance increased with temperature. Also, negative correlation was found between r_s and aperture width and photosynthesis rate ($r = -0.62$, $P < 0.001$). Furthermore, a negative correlation was found between photosynthesis rate and stomatal resistance (Table 3). The highest stomatal resistance and the lowest photosynthesis rate were found in plants treated with 45°C for 60 min (Fig. 2).

DISCUSSION

Plant leaves are the most adaptable organs in reaction to environmental adverse conditions (Marchi *et al.*, 2008). For example, photosynthesis and leaf conductance are partially inhibited by abscisic acid or pH signal under drought conditions (Davies and Zhang, 1991; Liang and Zhang, 1999). It is well known that *Tradescantia* sp. is a model plant for investigation of stomatal movement because of having big stomata (Fig. 1). It has been illustrated that some environmental conditions, like water stress, may affect the stomata behavior and aperture (Rouhi *et al.*, 2007). Stomatal apertures movement is important to alter environmental conditions and has an important role in transpiration and photosynthesis processes that are closely associated with adaptability of the plants (Brownlee, 2001). Water stress results in stomatal closure and decreases transpiration rates, thus reducing photosynthesis and growth restraint (Yordanov *et al.*, 2000). It has been pre-

viously reported that gs and photosynthesis decreased with the increase in soil water deficit.

Reynolds-Henne *et al.* (2010) reported that heat stress on leaf in darkness led to the increased stomatal opening in *Phaseolus vulgaris*. Our results showed that root zone temperature of 30°C decreased stomatal resistance, thus stomatal aperture increased. But when temperature rises to 45°C, stomata closed and photosynthesis rate decreased. The results of this study could not describe the reasons of stomatal closing but showed the effects of heat shock on stomatal closure.

It has been proved that heat shock could trigger physical signals such as electronic wave transmission (Roblin and Bonnemain, 1985), and could change ions concentration in xylem sap (Yang *et al.*, 2006). ABA, H₂O₂, pH, and ions are well-known factors playing important roles in stomatal movement. Root zone temperature treatment is likely to lead to changes in concentration and composition of these factors in guard cells. It is reported that heat shock in root zone caused stomatal closure (Yang *et al.*, 2006) which is in line with our results. It has been shown that partial root treatment of *Commelina communis* caused changes in ABA and H₂O₂ content, therefore decreased stomatal conductance and increased stomatal resistance (Yang *et al.*, 2006). Long distance signal conduction activated by heat treatment in various plant species such as sunflower and maize has been reported (Yang *et al.*, 2006). Thus, some physical signals or the changes in concentration of chemical factors in xylem sap might involve in the signal transmission, merit to attract more investigation. The present study showed for the first time that root zone temperature can activate some long-distance signal transmission in *Tradescantia pallida* resulting in changes in stomatal behavior. Therefore, it is important to determine the temperature that causes stomatal opening or closing in greenhouse and hydroponic culture and thus managing the plant productivity.

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How to cite this article:

Ashrafi, N., and Rezaei Nejad, A. 2016. Stomatal movement in response to root zone temperature in purple heart (*Tradescantia pallida*). *Journal of Ornamental Plants*, 6(3): 133-139.

URL: http://jornamental.iaurasht.ac.ir/article_524564_228993ee6ff337737ae96d10da12a3f2.pdf

