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Stomatal responses of bean (*Phaseolus vulgaris* L.) leaves to changing irradiance, air humidity, and water potential of root medium

S. MALESZEWSKI, E. BURCHACKA, M. RAKUS, and B. KOZŁOWSKA-SZERENOS

University of Białystok, Institute of Biology, ul. Swierkowa 20B, PL-15-950 Białystok, Poland

Abstract

Stomatal responses of attached bean (*Phaseolus vulgaris* L.) leaves to changing spectral composition ("white" - WL, blue - BL, or red - RL radiation), air humidity (100 % or about 4 % RH), and water potential of the root medium (close to 0 or -1.2 MPa) were determined by air flow porometer. Opening of stomata always increased under BL and decreased under RL. In response to decline in air humidity, leaf conductance showed transient increase before it reached lowered steady state. BL enhanced and RL diminished this response.

Additional key words: blue radiation; guard cells; leaf conductance; red radiation.

Introduction

The spectral quality of radiation affects the opening of stomata. Chlorophyll is the primary photoreceptor of PAR irradiance involved in stomatal opening. However, there is some evidence for the presence in guard cells of a second specific blue radiation (BL) absorbing receptor. It has been recently identified in *Arabidopsis* guard cell chloroplasts as zeaxanthin (Zeiger and Zhu 1998).

BL response may be a combined expression of activation of signal transduction and stomatal opening mechanisms. BL may affect activity of G proteins (Short and Briggs 1994), membrane proton pumps (Assmann *et al.* 1985, Shimazaki *et al.* 1986, 1997, Goh *et al.* 1996), redox chain participating in membrane polarization

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Fax: 048(085)457302; e-mail: stanmale@cksr.bialystok.ac.PL.
Abbreviations: BL, blue radiation; DA, dry air; g_l, leaf conductance; HA, humid air; LSCH, leaf steady conductance in HA; LPM, root medium of lowered water potential; LSCD, leaf steady conductance in DA; LTCD, leaf transient conductance after change of HA for DA; PAR, photosynthetically active radiation; RH, relative humidity; RL, red radiation; SM, simple liquid root medium; $Ts_{1/2}$, Ts, time required to reach in humid air half and steady leaf conductance, respectively; WL, "white light".

(Zeiger 1987, Raghavendra 1990, Gautier *et al.* 1992, Tallman 1992) as well as stomatal phosphoenolpyruvate carboxylase (Parvathi and Raghavendra 1997).

The stomata close in response to decrease in the humidity of ambient air. It is not clear whether this is a direct reaction to air humidity *per se*, water potential difference leaf–atmosphere, transpiration rate, or resistance connected with water uptake and transport (El-Sharkavy and Cock 1986, Kappen and Haeger 1991, Mott and Parkhurst 1991, Kearns and Assmann 1993, Monteith 1995, Meinzer *et al.* 1997, Mott *et al.* 1997, Yong *et al.* 1997).

In a previous work we found that under BL the stomata of bean leaves reached larger aperture than under the red radiation (RL) or "white light" (WL) of the same intensity. We also showed that the effects induced by BL or RL pre-irradiation led to increased or decreased steady state g_l during subsequent WL post-irradiation (Sikorska *et al.* 1997). In the present study the responses of attached bean leaf conductance to changes in the air relative humidity (RH) and in root medium water potential were analysed under WL, BL, and RL.

Materials and methods

Beans (*Phaseolus vulgaris* L. cv. Golden Saxa) were grown for 3-4 weeks in a growth chamber in aerated Knop mineral liquid medium. The PAR was 150 μmol m^{-2} s^{-1} (fluorescent tubes *Flora + Day Light* in the ratio 1 : 1), photoperiod 16 h, day/night temperature 25/20±2 °C, day/night air humidity 70-85/85-90 %.

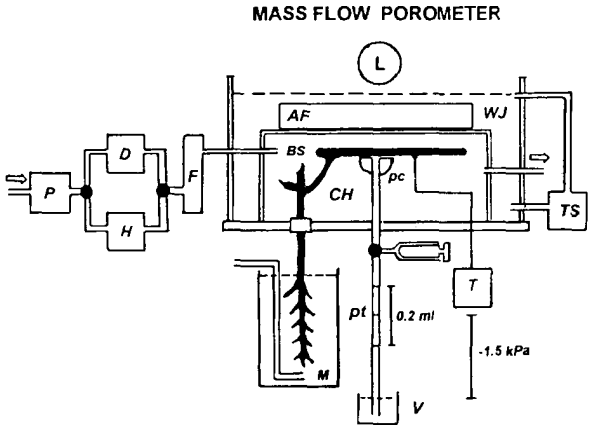

Fig. 1. The scheme of experimental arrangement with air flow porometer for determination of attached leaf conductance. *AF*, radiation absorption filter red (transmision in the range 620-780 nm, max. at 667 nm) or blue (360-500 nm, max. at 477 nm); *BS*, bean seedling with one primary leaf left; *CH*, thermostated leaf chamber; *D*, air desiccator; *F*, air flow meter; *H*, air humidifier; *L*, source of white radiation; *M*, container with areated growth medium (simple or lowered water potential to about -1.2 MPa); *P*, air pump; *pc*, porometric cuvette; *pt*, porometric vertical tube with syringe for uprising of manometric solution level; *T*, thermocouple probe; *TS*, thermostat (25 °C); *V*, vessel containing manometric solution; *WJ*, water jacket; *arrows* show the direction of air flow through leaf chamber.

Leaf conductance (g_l) was monitored by an air flow porometer (Fig. 1). On the evening prior to an experiment, at about 20:00 h local time, a bean shoot, with only one primary leaf left, was placed in a thermostated leaf chamber and kept under darkness until next morning. Small porometric cuvettes were fixed to the abaxial epidermis of the left basal parts of leaf. During measurements the mean underpressure of 1.5 kPa (1.7-1.3 kPa) was produced inside the cuvette by the column of manometric solution in vertical tube connected with the cuvette. The air flow was measured through an 0.2 cm^2 leaf area. Air introduced to the thermostated (25 °C) leaf chamber was humidified to RH of about 100 % or dried with a desiccant column to RH less than 4 %. Leaf was oriented perpendicularly to the incident radiation, 150 μmol m^{-2} s^{-1} of WL, RL, and BL, which was provided by a halogen lamp and red filter (transmision in the range 620-780 nm, max. at 667 nm) or blue filter (360-500 nm, max. at 477 nm). Leaf temperature (25-27 °C) was measured with a fine wire thermocouple probe pressed to the underside of the leaf. The measurements were made between 08:00 and 13:00 h.

Root system, remaining outside the chamber, was immersed in aerated simple Knop mineral liquid medium (SM) or in a medium of lowered to about -1.2 MPa water potential (LPM). To lower the medium water potential, 20 kg m^{-3} *PEG 6000* was used (Michel 1983).

The g_l as a rate of linear air flow through leaf blade (Strebeyko 1965) was expressed in μm s^{-1} Pa^{-1}. The presented values are means ± SD calculated from at least 10 measurements. Results were analysed using the Student's *t*-test.

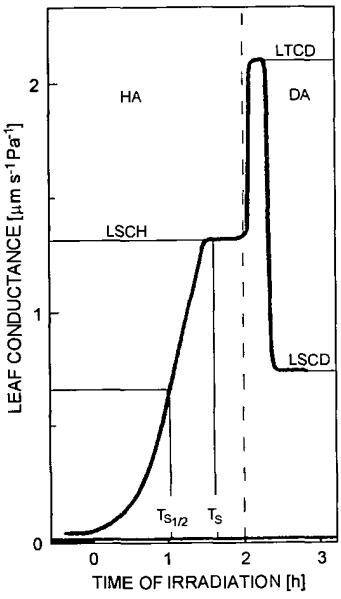

Fig. 2. Typical responses of *Phaseolus vulgaris* leaf conductance to the onset of irradiance in humid air and to the decrease of air humidity. *Black and white stripes* on the time scale mark the periods of dark and light. *Dashed line* indicates when humid air (HA) was changed for dry air (DA). The baseline leaf conductance (at the end of dark period) was close to zero. The measurements were done every 5 min at least. LSCH - leaf steady conductance in HA; LTCD - leaf transient conductance after change of HA for DA; LSCD - leaf steady conductance in DA; $Ts_{1/2}$, Ts - time required to reach in humid air half and steady leaf conductance, respectively.

Results

After onset of leaf irradiation in humid air (HA), g_l (Fig. 2) reached a steady value (LSCH) during 70-117 min (Ts). Half of the g_l steady value was reached during 53-80 min ($Ts_{1/2}$). Change of HA for dry air (DA) resulted in a two-phase response of stomata. In the first phase there was a rapid transient rise of g_l (LTCD), followed by conductance decrease and reaching of a new steady lower value (LSCD).

The leaf conductances (LSCH, LTCD, LSCD) were always significantly ($p<0.02$) higher under BL than those obtained under RL. Under WL intermediate values were found (Table 1). The mean values of steady g_l in dry air (LSCD) were always lower than those (LSCH) stated in humid air. However, under BL the effect of dry air was minimal (Table 1). The transient rise of g_l (LTCD), appearing always immediately after change of HA for DA, showed the same dependence on radiation quality as LSCH and LSCD. The stimulating effect of BL on LTCD was even the most pronounced (Table 1).

Table 1. Leaf conductances [$\mu m\ s^{-1}\ Pa^{-1}$] of *Phaseolus vulgaris* reached under "white" (WL), red (RL), and blue (BL) irradiation of 150 $\mu mol\ m^{-2}\ s^{-1}$ in humid air (LSCH), transient after the subsequent change to dry air (LTCD), and in dry air (LSCD). Results of plants with roots in simple medium (SM) and in medium of lowered water potential to about -1.2 MPa (LPM) are compared. Mean values ± SD.

Medium	Irradiation	LSCH	LTCD	LSCD	LSCD/LSCH
SM	WL	1.3 ± 0.8	2.1 ± 1.0	0.7 ± 0.3	0.5
	RL	0.5 ± 0.2	0.6 ± 0.5	0.2 ± 0.2	0.4
	BL	3.4 ± 1.2	7.7 ± 3.4	2.1 ± 1.3	0.6
	BL/RL	6.8	12.8	10.0	
LPM	WL	3.1 ± 1.5	5.3 ± 3.3	1.1 ± 0.7	0.4
	RL	1.1 ± 0.2	1.5 ± 0.3	0.5 ± 0.3	0.5
	BL	5.6 ± 2.5	13.9 ± 5.1	4.1 ± 1.5	0.7
	BL/RL	5.1	9.3	8.2	

Comparison of results obtained in experiments with root media of different water potentials reveals the unexpected enhancement of stomatal opening in plants with roots in medium of lowered water potential (LPM). It was also true for LTCD (Table 1). In plants with roots immersed in simple medium (SM), the time necessary to reach Ts and $Ts_{1/2}$ was not dependent on the radiation quality. However, when LPM was used, values of these parameters were significantly ($p<0.02$) decreased, but only under WL and RL. Under BL the lowered water potential of medium did not affect $Ts_{1/2}$ and Ts (Table 2).

Under BL, LSCD was not registered in about 20 % of experiments with plants the roots of which were in LPM. Following change of HA for DA, the leaves of these plants wilted and their g_l rapidly dropped to zero.

Table 2. Time [min] required to reach half ($Ts_{1/2}$) and steady (Ts) leaf conductance under "white" (WL), red (RL), and blue (BL) radiation of 150 μmol m^{-2} s^{-1} in *Phaseolus vulgaris* with shoot in humid air and roots in simple medium (SM) or in medium of lowered water potential to about -1.2 MPa (LPM). Mean value ± SD.

Irradiation	SM		LPM		LPM/SM	
	$Ts_{1/2}$	Ts	$Ts_{1/2}$	Ts	$Ts_{1/2}$	Ts
WL	62 ± 11	98 ± 16	43 ± 11	70 ± 11	0.69	0.71
RL	53 ± 7	78 ± 12	41 ± 12	63 ± 11	0.77	0.81
BL	61 ± 15	87 ± 17	63 ± 16	92 ± 17	1.03	1.06

Discussion

Under our experimental conditions the changes of g_l were dependent on the stomatal responses only. In a previous work we showed that spectral quality of irradiance did not affect the absorption of radiation, as well as the leaf temperature to a significant degree (Sikorska *et al.* 1997). Results of the present work indicate that the specific effects of BL and RL on stomatal opening are independent of water potential of atmosphere and of root environment.

Leaf conductance after decrease of air humidity transiently rose and reached a new steady level, lower than that in the humid air. This pattern of g_l changes was observed as a rule when the roots of the experimental plant were in the ordinary growth medium. When roots were immersed in medium of lowered water potential, a similar reaction of stomata was noted also in all measurements with WL and RL and in the majority of measurements with BL. However, in about 20 % of measurements with BL during the flow of dry air through leaf chamber the leaves wilted without reaching a steady lower conductance. It may be therefore suggested, that when the red PAR component is absent (under BL) the mechanisms of stomatal response to dry air or to increased resistance connected with water uptake, bringing a reduction of transpiration, may be less effective. The stomata may not ensure equilibrated water balance of plant. The water potential of about -1.2 MPa was close to the growth limiting one. At a more decreased water potential of medium the leaves of experimental plants wilted, even if they were in humid air.

The rate of stomatal opening was more rapid in stressed plants than in well-watered ones. Such unexpected phenomenon in *Phaseolus vulgaris* L. was already reported (Barradas *et al.* 1994). Our results suggest that water stress may cooperate with the red component of PAR. In humid air, g_l of plants with roots immersed in medium of lowered water potential reached the steady conductance faster than the plants in ordinary medium, but only under WL and RL. The decline in both $Ts_{1/2}$ and Ts appeared despite the fact that steady g_l in humid air in plants with roots in medium of lowered water potential was roughly twice than in those with roots in the ordinary medium.

In plants with roots in the medium of lowered water potential, steady g_l in humid air as well as transient leaf conductance in dry air were always significantly higher than in plants with roots in ordinary medium. This phenomenon appeared in plants with roots immersed in medium of lowered water potential through the previus dark period and during the conductance measurements. Tissues of these plants were, without doubt, in osmotic equilibrium with medium of lowered water potential. This equilibrium had to be related to decreased turgor of epidermal cells neighbouring guard cells and of mechanical forces affecting stomatal movements and aperture (Klein *et al.* 1996). Under these conditions the movement of guard cells which possess their own specific metabolic mechanism of turgor regulation may be facilitated and therefore the stomata not only do open more rapidly but also reach larger aperture than in plants of full turgor.

The phenomenon of transient phase in stomatal response in direction opposite to the final response is well-known (Raschke 1979). The transient increase of stomatal aperture due to elevation of vapour pressure deficit was observed in experiments with various plant species (Kappen *et al.* 1987, 1994, Kappen and Haeger 1991). Fast transient increase of g_l (LTCD) recorded by us as following the drop in humidity of ambient air may also be connected with the interaction of guard cells with neighbouring epidermal cells. The influence of "hydraulic" environment of epidermal cells on stomata was demonstrated already with other methods in *Vicia faba* (Klein *et al.* 1996). A rapid transient increase in aperture of stomata on small leaf area neighbouring another area which was submitted to dry air was also noted in experiments with *Xanthium strumarium* (Mott *et al.* 1997).

Our results may indicate that interaction between the stomata and the neighbouring epidermal cells depends also on radiation quality, and that it becomes more evident under BL. Probably, the effects found express the mechanisms which in the nature modulate the responses of stomata to environmental stimuli (Zeiger *et al.* 1981, 1987, Zeiger and Zhu 1998).

Under our experimental conditions an enhancement of stomatal opening under BL may be caused, at least partially, by excluding of red component of PAR. This suggestion is also supported by the restrictive effect of red pre-irradiation on the opening of stomata during subsequent "white" post-irradiation (Sikorska *et al.* 1997).

In laboratory experiments, BL that increases opening of stomata and water loss may be unfavourable for plants, especially under high evaporative demand and hindered water uptake. However, supplementing of WL with BL may in some cases, *e.g.*, in humid atmosphere or/and under increased CO_2 concentration, be advantageous to photosynthetic activity.

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