

Effects of radiation quality on the opening of stomata in intact *Phaseolus vulgaris* L. leaves

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Abstract

In intact French bean (*Phaseolus vulgaris* L.) leaves blue radiation enhanced opening of stomata both when it was used individually and when it was used as preirradiation before "white light" irradiation. Effects of red radiation were just the contrary.

Additional key words: blue and red radiation; porometry; stomatal conductance; "white light".

Introduction

The radiant energy is required for opening of stomata in the majority of plants. It is a signal initiating the stomatal opening and also a source of energy for the action of ion pumps at guard cell membranes, as well as for the photosynthetic CO₂ assimilation. Stomatal response to the "white light" (WL) is probably a joined expression of two distinct photoreceptor systems: one stimulated by blue radiation (BR) and the other stimulated by photosynthetically active radiation, PAR (Zeiger *et al.* 1987, Zeiger 1990). The BR activates the plasma membrane H⁺ pump in *Phaseolus* guard cells (Hedrich and Schroeder 1989). Stomata in adaxial and abaxial leaf epidermis may have different sensitivity to irradiance and spectral composition, and their response can be modified by the conditions in which the leaf develops and functions (Lu *et al.* 1993). Also mesophyll participates in stomatal control (Lee and Bowling 1992, 1993, 1995). The aim of this study was to compare the effectivity of a weak irradiance of different spectral ranges (BR, RR, WL) in the stomatal opening in intact bean leaves. The effects of BR and RR, applied directly or as preirradiation before WL, were measured.

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Abbreviations: BR, RR, WL, blue radiation, red radiation, "white light"; PAR, photosynthetically active radiation.

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Materials and methods

Plants: Bean (*Phaseolus vulgaris* L. cv. Golden Saxa) seedlings were grown for 3-4 weeks in an aerated nutrient solution inside a growth chamber. The PAR value was $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (fluorescent tubes *Flora+Day Light* in the ratio 1:1), photoperiod 16 h, day/night temperature 25/20 °C, day/night relative air humidity 70-85/85-90 %.

Porometric measurements: A shoot of bean seedling with only one primary leaf left was placed into a thermostated plexiglass chamber with a radiation source above it. Roots remained outside the chamber and were immersed into an aerated nutrient solution. For measuring the stomatal conductance a modified air flow porometer was used (Mikulska and Maleszewski 1990). A small porometric cuvette was fixed, using lanoline, to the abaxial epidermis of the primary leaf mounted perpendicularly to the incident radiation. The air flow through a 0.2 cm^2 epidermis fragment was measured under a mean underpressure in the porometric cuvette (1.5 kPa). The PAR value was $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (in experiments presented in Fig. 1C, it was also $600 \mu\text{mol m}^{-2} \text{s}^{-1}$), temperature in the thermostated chamber was 25 ± 0.5 °C, air relative humidity was about 100 % inside the chamber. Before measurements the plants had been left in darkness for 18 h. The leaf conductance values were expressed in absolute units ($g_1 = \mu\text{m s}^{-1} \text{Pa}^{-1}$) or as % of g_1 maximal for the given experiment (the relative conductance) presented on Fig. 1. The porometric measurements were carried out between 09:00 and 13:00 h local time. The control experiments indicated that the response of stomata on radiation and darkness did not significantly change even during three consecutive days (Mikulska and Maleszewski 1990). The temperature of leaf was measured using a thermocouple probe (*BAT-12, Physitemp Instruments, U.S.A.*).

Radiation quality: The source of WL was a halogen lamp (*LH 31 Dethal Polam, Poland*). The RR and BR were obtained with absorption filters, the characteristics of which were determined by a spectrophotometer (*Specord, Carl Zeiss, Jena, Germany*). The RR contained radiation in the range of 360-500 nm (maximum at 477 nm), BR in the range of 620-780 nm (maximum at 667 nm). None of the filters transmitted wavelengths of 500-620 nm. The irradiance as well as relative reflection and transmission of radiation by the leaf were estimated with a phytophotometer (*SONOPAN, FF-01, Poland*).

Results

Spectral properties of leaves: The reflection, transmission, and absorption of radiation by the leaf, as well as the leaf temperature during an irradiance of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ did not differ in dependence on the irradiance quality, which could affect the state of stomata (Table 1).

Stomatal opening: At a WL irradiance of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (WL150) the stomatal opening lasted about 75 min (Fig. 1A). Yet when the WL rose to $600 \mu\text{mol m}^{-2} \text{s}^{-1}$

(WL600), the relative g_1 increased twice (Fig. 1B). When the sequences of changes in the irradiance applied were RR-WL and BR-WL, $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ each time, the relative g_1 at RR attained 42 (Fig. 1C) or 63 % (Fig. 1D), respectively, of the maximal value.

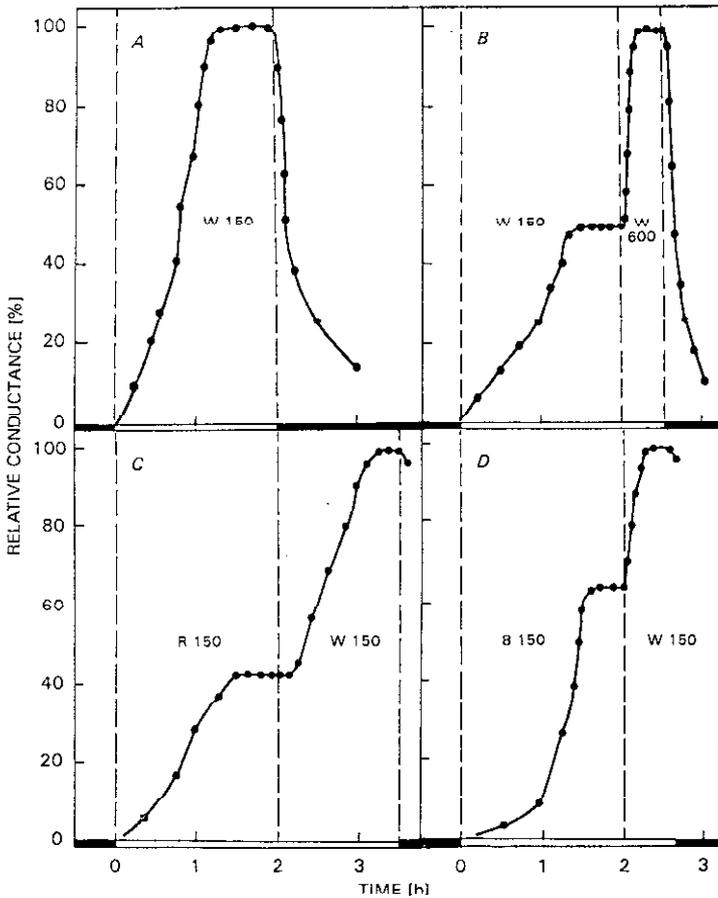


Fig. 1. Typical kinetics of stomatal movements during irradiation of a bean leaf with the "white light" (different irradiances: WL150 = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, WL600 = $600 \mu\text{mol m}^{-2} \text{s}^{-1}$), and sequences of red radiation (RR) or blue radiation (BR) of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ and WL150. $n > 6$. Black stripes on the time scale mark the dark periods.

Maximum g_1 , at an irradiance of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ was markedly dependent on the radiation quality. At BR, the maximum value of absolute g_1 was the largest, over two times larger than at RR (Table 1).

The curves of the g_1 changes in leaves irradiated only with $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ WL, RR or BR were used to estimate the time required to reach half ($T_{50\%}$) and maximal ($T_{100\%}$) opening of stomata for the given experimental conditions. Both these parameters were independent on the radiation quality (Table 1).

While changing the radiation quality during an experiment it was observed that the leaves under the WL postirradiation attained several times bigger g_1 if preirradiated with BR than if preirradiated with RR. Furthermore, the preirradiation with a weak RR reduced markedly the maximal g_1 achieved during the subsequent postirradiation with the WL (Table 2).

Table 1. Spectral properties of bean leaf, maximal values of leaf conductance (g_1) and duration of opening of stomata ($T_{100\%}$, $T_{50\%}$ - times required for maximal or 50 % opening, respectively) at "white light" (WL) or blue radiation (BR) or red radiation (RR) treatments of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Means \pm SD.

Parameter	WL	RR	BR
Reflection [%]	21 \pm 2	28 \pm 6	22 \pm 3
Transmission [%]	8 \pm 1	10 \pm 1	3 \pm 1
Absorption [%]	71	62	75
Leaf temperature [$^{\circ}\text{C}$]	25.9 \pm 0.3	26.1 \pm 0.4	25.7 \pm 0.3
g_1 [$\mu\text{m s}^{-1} \text{Pa}^{-1}$]	2.7 \pm 1.2	0.4 \pm 0.2	5.2 \pm 2.7
$T_{100\%}$ [h]	1.4 \pm 0.3	1.4 \pm 0.1	1.4 \pm 0.1
$T_{50\%}$ [h]	0.9 \pm 0.2	0.8 \pm 0.1	1.1 \pm 0.1

Table 2. Comparison of maximal values of bean leaf absolute conductance (g_1) on blue (BR) or red (RR) radiation and on "white light" (WL) after preirradiation with BR or RR. All irradiances were $150 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Results compared	Maximal values of $g_1 \pm$ SD and their ratio
BR/RR	5.2 \pm 2.7/0.42 \pm 0.2 = 12.4
WL after BR/WL after RR	6.7 \pm 4.3/1.0 \pm 0.7 = 6.7
WL after BR/WL	6.7 \pm 4.3/2.7 \pm 1.2 = 2.5
WL after RR/WL	1.0 \pm 0.7/2.7 \pm 1.2 = 0.4

Discussion

Our findings confirmed the greater BR effectivity in the process of stomata opening, stated also in other experimental conditions (Raschke 1975, Farquhar and Sharkey 1982, Zeiger 1983, Karlsson and Assmann 1990, Vavasseur *et al.* 1990, Lee and Bowling 1992, 1993, 1995, Liu *et al.* 1993). The g_1 of leaves irradiated with the BR of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ was more than 12 times higher than that achieved with RR, and almost twice as much than at the same WL irradiance (Tables 1 and 2). The time necessary for changing the state of stomata from the full closing (after 15-18 h of darkness) to the maximal opening in experimental conditions used did not depend on the radiation quality, its mean value was always about 86 min (Table 1). It indicates that the BR not only increases the aperture of stomata, but also enhances the rate of stomatal movement.

The effect of WL on the process of stomata opening is enhanced by preirradiation with the BR. We found that the RR was not only less effective in the process of stomatal opening in bean leaves, but if applied as preirradiation it also decreased the subsequent effect of WL (Table 2). The BR/RR ratio depends on the contribution of the direct solar radiation and that of the dispersed one, coming from the blue sky or clouds of different densities. A relatively weak irradiance during a low sun position, with cloudy sky, or shadowed stands is characterised by an increased contribution of the dispersed BR. The direct strong solar irradiance at open stands contains relatively more RR (Czarnowski 1993). In the latter case some other environmental factors increasing transpiration are usually involved: high temperature, low air humidity.

The high sensitivity of stomata to the BR controls the stomatal opening at dawn and enhances it early in the morning (Zeiger *et al.* 1981, Zeiger 1983). Our findings propose that the differential sensitivity of stomata to BR and RR reflects also the mechanism which optimizes the photosynthetic carbon fixation against the water loss during the transpiration. The BR may enhance the sensitivity of stomata to even a weak radiation stimulus. On the other hand, the RR component in the strong solar radiation, which reaches the leaf tissue, may be a signal for the decrease of stomatal aperture even before the negative balance and water deficit appears. This may cause, as noted in the studies of a beech canopy (Herbst 1995), that the stomatal conductance is considerably higher on an overcast day than on a sunny one. Our results do not permit excluding that the observed high effectivity of the BR in the stomatal opening is caused by the absence of the red component in the WL.

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