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Carbon dioxide assimilation, transpiration, and leaf conductance of bean (*Phaseolus vulgaris* L.) under blue and red radiation

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Abstract

In bean (*Phaseolus vulgaris* L.) seedlings well supplied with water, rates of transpiration (E) and CO_2 assimilation (P_N) of the primary leaves were measured under blue (BR) or red (RR) irradiance of 150 μmol(photon) m^{-2} s^{-1}. The leaf conductance to H_2O vapour transfer (g_{H2O}), as well as the intercellular concentrations of H_2O vapour (e_i) and of CO_2 (C_i) were calculated. Under BR, g_{H2O} was significantly greater, but P_N was lower, and E similar as compared with corresponding values found under RR. The increase of stomata aperture under BR was evident although C_i was higher and e_i was lower than under RR. Results agree with the suggestion that BR directly activates guard cell metabolism and in well watered plants determines mainly the stomata aperture.

Additional key words: intercellular CO_2 and H_2O vapour concentrations; leaf temperature; photosynthesis; stomata responses; water use efficiency.

Introduction

Photosynthetically active radiation (PAR, 400-700 nm), air humidity, soil moisture, and CO_2 concentration are the main environmental factors affecting stomata aperture. Stomata regulate transpiration and CO_2 diffusion into the leaf. It is not clear whether stomata response is a direct reaction to air humidity *per se*, water potential leaf-atmosphere difference, whole-leaf transpiration rate, or efficiency of water uptake and transport (El-Sharkawy and Cock 1986, Kappen and Haeger 1991, Mott and Parkhust 1991, Kearns and Assmann 1993, Monteith 1995, Bunce 1996, Meinzer *et al.* 1997, Mott *et al.* 1997, Yong *et al.* 1997, Jones 1998, Giorio *et al.* 1999).

In plants well irradiated and adequately supplied with water the stomata aperture is inversely correlated with leaf intercellular CO_2 concentration (Mott 1988). In spite of numerous investigations, the participation of CO_2 in mechanism of stomata control remains unknown (Morison 1998, Assmann 1999).

Opening of stomata is induced by two PAR components, blue (BR) and red (RR) radiation. However, the action spectrum of photosynthesis differs from that of stomata opening. The latter exhibits enhanced sensitivity to blue (425-475 nm, max. at 445 nm) radiation (Zeiger and Hepler 1977, Karlsson 1986, Vavasseur *et al.* 1990, Grantz *et al.* 1991, Karlsson and Assmann 1990, Briggs and Liscum 1997).

We found previously that bean-leaf conductance monitored by an air flow porometer (Strebeyko 1965) was several time higher under BR than under the RR of the same quantum irradiance (Sikorska *et al.* 1997, Maleszewski *et al.* 1999). The aim of the present work was to analyse the effects of BR and RR on bean-leaf conductance for water vapour (g_{H2O}), the rates of transpiration (E) and CO_2 assimilation (P_N), intracellular partial pressures of CO_2 (C_i), water use efficiency of photosynthesis (WUE_{Ph}), and the relations between these traits.

Materials and methods

The bean (*Phaseolus vulgaris* L. cv. Golden Saxa) seedlings were grown in an aerated Knop mineral liquid medium in a growth chamber for 3-4 weeks. The irradiance was 150 μmol(photon) m^{-2} s^{-1}. Radiation source were fluorescent tubes *Cool White* + *DayLight* in the ratio 1 : 1 (*Philips Lighting Poland*). Photoperiod was 16 h, day/night tem-

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Abbreviations: BR, blue radiation; C_a, ambient CO_2 concentration; C_i, intercellular CO_2 concentration; e_a, ambient H_2O vapour concentration; e_i, intercellular H_2O vapour concentration; E, transpiration rate; g_{H2O}, leaf conductance to H_2O vapour transfer; P_N, net photosynthetic rate; PAR, photosynthetically active radiation; RR, red radiation; WUE_{ph}, water use efficiency of photosynthesis.

perature 25/20±2 °C, day/night air humidity 70-85/85-90 %.

On the evening before measurements, at 20:00 of the local time, the bean shoot, with only two primary leaves left, of one side surface of 43-76 cm^2, was placed in a thermostated (25.0±0.5 °C) chamber (660 cm^3), provided with micro-fan (Fig. 1), and kept under darkness until the next morning. Humidity of the air entering (9.5 $cm^3\ s^{-1}$) the chamber was thermoelectrically controlled at the dew point of 5.0±0.5 °C. Leaf temperature was measured with a fine thermocouple probe (*BAT-12, Physitemp Instruments,* U.S.A.) inserted into leaf-blade. Root system, remaining outside the chamber, was immersed in aerated Knop medium. During measurements, made between 08:00 and 13:00, leaves were oriented perpendicularly to the incident radiation of 150 μmol(photon) $m^{-2}\ s^{-1}$, provided by a halogen lamp and the blue filter (transmission 360-500 nm, max. 477 nm, BR) or red filter (transmission 620-780 nm, max. 667 nm, RR). Water vapour and CO_2 concentrations in air entering and leaving the chamber were measured using infrared gas analyser (*LI-COR 6262, Lincoln,* USA), and E and P_N were calculated.

The partial pressure difference of water vapour between the intercellular spaces of the leaf and ambient air ($e_i - e_a$) was calculated using saturation vapour pressure at leaf temperature and the air humidity leaving the cuvette. Leaf conductance to water vapour transfer (g_{H2O}) and the partial pressure of CO_2 in the intercellular spaces of the leaf (C_i) were calculated according to Taiz and Zeiger (1991).

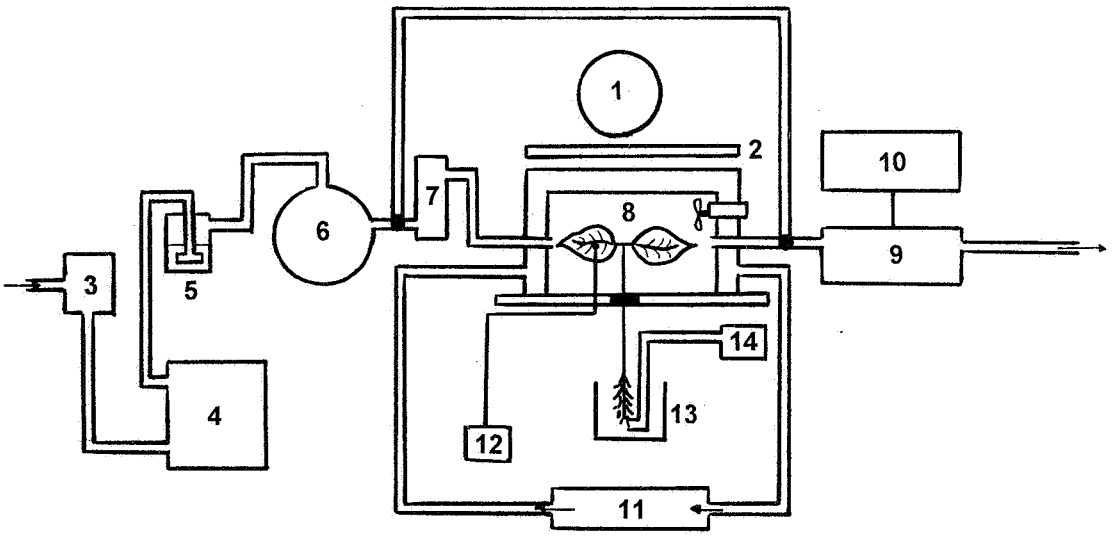

Fig. 1. The scheme of open system with CO_2/H_2O analyser (*LI-6262, LI-COR,* USA) and experimental arrangement used for determination of water vapour and CO_2 exchange parameters of bean (*Phaseolus vulgaris* L.) leaves. *1*, source of white radiation; *2*, radiation absorption filter blue (transmission in the range 360-500 nm, max. at 477 nm) or red (620-780 nm, max. at 667 nm); *3*, air supply pump; *4*, container equalising air composition; *5*, air humidifier; *6*, thermoelectric desiccator (dew point 5 °C) of air, *7*, air flow controller; *8*, thermostated (25 °C) leaf chamber with micro-fan; *9*, CO_2/H_2O analyser, *10*, computer; *11*, thermostat; *12*, thermocouple probe for determination of leaf temperature; *13*, container with growth solution; *14*, air pump.

Results

E, P_N, and g_{H2O} showed different kinetics of changes when bean seedling were irradiated with either BR or RR. A similar steady-state of E was attained more rapidly under RR than under BR (Fig. 2, Table 1). P_N also increased faster under RR, but achieved significantly higher value than under BR (Fig. 2, Table 1). The g_{H2O} also gained steady-state value earlier under RR, but it was always significantly lower than under BR (Fig. 2, Table 1).

Temperature of the leaf blades (T_l) closed in the thermostated (25.0±0.5 °C) chamber depended also on radiation spectral quality. Transient increase in T_l, registered instantly following onset of their irradiation, was under RR about twice as great as under BR. Also T_l settled during about 1 h of irradiation was over 1 °C higher under RR than under BR (Fig. 2, Table 1).

The average steady-state E of irradiated leaves was almost three-times higher than in darkness, but it did not significantly depend on radiation quality. Under steady-state E, g_{H2O} was smaller under RR than under BR, and this reduction was connected with lowered C_i. Probably, the lower C_i was due to P_N being significantly faster under RR than under BR.

During the period of stabilised E and P_N, $C_a - C_i$ was almost three times higher under RR than under BR. In experiments with RR an increase in $e_i - e_a$ was also found. The consequence of the above mentioned effects of RR on the leaf gas-exchange was an increase in WUE_{Ph} (Table 1).

Table 1. Mean values of steady-state exchange parameters for water vapour and CO_2 in *Phaseolus vulgaris* leaves in darkness (D) and under blue (BR) or red (RR) radiation. E, transpiration rate [mmol m^{-2} s^{-1}]; g_{H2O}, leaf conductance to water vapour [mmol m^{-2} s^{-1}]; P_N, CO_2 assimilation rate [μmol m^{-2} s^{-1}]; C_i, intercellular CO_2 concentration [μmol mol^{-1}]; $C_a - C_i$, the difference between ambient CO_2 concentration (C_a) and C_i; T_l, leaf temperature [°C]; $e_i - e_a$, the difference in the water vapor concentration between inside (e_i) and outside (e_a) of the leaf [mmol mol^{-1}]; WUE_{Ph}, the water use efficiency of photosynthesis [μmol(CO_2) $mmol^{-1}$(H_2O)]; (1),(2), the effect of irradiance quality not significant or significant, respectively.

	E	g_{H2O}	P_N	C_i	$C_a - C_i$	T_l	$e_i - e_a$	WUE_{ph}
D	0.37 ± 0.04	27.82 ± 4.43	-	-	-	-	-	-
BR	0.98 ± 0.04	165.13 ± 15.37	3.82 ± 0.29	235.4 ± 3.4	37.6	23.3 ± 0.1	6.06	3.9 ± 0.2
RR	0.95 ± 0.04	105.56 ± 17.64	6.26 ± 0.70	139.1 ± 10.8	101.9	24.7 ± 0.1	9.61	6.6 ± 0.3
BR/RR	1.03(1)	1.57(2)	0.61(2)	1.69(2)	0.37(2)	0.9	0.6	0.59

Discussion

The enhanced sensitivity of stomata opening to BR, since the first report of Zeiger and Hepler (1977), was found for several plant species (Farquhar and Sharkey 1982, Zeiger 1994). Radiation quality may directly affect radiation signal reception, intracellular signal transduction, or mechanisms of guard cell movement (Briggs and Liscum 1997). BR activated respiration (Kowallik 1967), activity of phospho-enolpyruvate carboxylase (Voskresenskaya 1972), proton pumps (H^+-ATPase) in the plasma membranes of guard cells (Assmann *et al.* 1985, Shimazaki *et al.* 1986), redox chain located in plasmalemma (Raghavendra 1990, Gautier *et al.* 1992), and increased ADP and ATP contents in leaves (Bukhow *et al.* 1995). BR influenced function of G-protein as well as another cellular components of signal transduction (Short and Briggs 1994). The carotenoid zeaxanthin is a specific receptor of BR in *Commelina communis* guard cells. This photoreceptor regulates the response of stomata to irradiation. It also may mediate irradiance-CO_2 interaction in guard cells (Zeiger and Zhu 1998, Zhu *et al.* 1998).

The influence of radiation quality on the stomata functions may be also indirect. This factor may affect T_l, E, water balance of plant, and P_N. In consequence it may change the other conditions affecting stomata aperture: the difference in $e_i - e_a$ as well as C_i. The sequence of events leading to the reaction of stomata to changes in the mentioned parameters, especially their co-operation, remains obscure.

In *C. communis* the BR response was greatest under low C_i and high irradiance. On the other hand, this response was diminished under conditions promoting water stress (Assmann 1988). In the present investigations we applied equal moderate irradiances of BR or RR to the upper surface of bean leaves. Leaving only two primary leaves on the experimental seedlings, we assured their efficient water supply. Introducing dry air of the dew point about 5 °C to the thermostated chamber, we constrained moderate transpiration of the leaves.

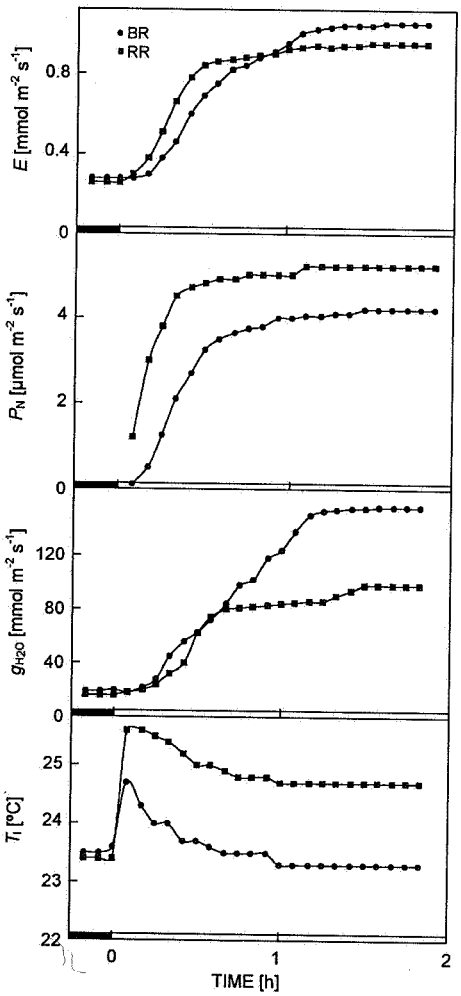

Fig. 2. Rates of transpiration (E) and net photosynthesis (P_N), leaf conductance to water vapour (g_{H2O}) and temperature (T_l) of *Phaseolus vulgaris* leaves in response to blue (BR) or red (RR) irradiance. The leaves were closed in the thermostated (25.0± 0.5 °C) chamber. *Black and white stripe* on the time scale mark the period of dark and light.

The g_{H2O}, calculated from the measured E, was under BR significantly greater than under RR (Table 1). The bean leaf conductance, monitored in the similar conditions with an air flow porometer, was under BR even to 6.8-12.8 times larger than under RR (Sikorska *et al.* 1997, Maleszewski *et al.* 1999). The demonstration of different g_{H2O} under BR and RR and simultaneously the similar E indicate that, at least under low irradiance, there may be not a direct relation between the values of these parameters. Thus BR might have affected directly the mechanism of guard cell movement, increased stomata aperture, however, E was limited by radiant energy inflow into the leaves.

BR irradiance of 150 μmol(photon) m^{-2} s^{-1} was equivalent of about 40.5 J m^{-2} s^{-1} supplied to the leaves. However, leaf E of 0.98 mmol m^{-2} s^{-1} (Table 1) consumed even more energy than that, considering the heat of water evaporation 43.9 kJ mol^{-1}, at 25 °C. For that reason the temperature of BR-exposed leaves was of about 2 °C lower than that of the thermostated chamber (Table 1).

Under RR, which is less effective in stomata opening, P_N was almost 40 % higher than under BR (Table 1). This could be caused by more efficient photosynthetic light reaction in mesophyll cells under RR of $\lambda > 620$ nm than under BR which was completely deprived of radiation of $\lambda > 500$ nm.

At low leaf conductance, P_N increased under RR, caused marked decrease in C_i, and so a respective increase of the CO_2 diffusion gradient ($C_a - C_i$) (Table 1). These factors may lead to the enlargement of the stomata aperture (Jarvis and Morison 1981). However, we found that under our experimental conditions their effects were incomparably weaker than those generated by the direct action of BR. No relation was found between C_i, ($C_a - C_i$), and E. Under RR we found only a marked increase in WUE_{Ph} (Table 1).

The present results confirm the suggestion that blue radiation (BR) affects directly the mechanism of stomata opening. In well-watered plants other factors do not play a significant role and the stomata aperture depends mostly on the irradiance parameters.

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