Enhancement of the Stomatal Response to Blue Light by Red Light, Reduced Intercellular Concentrations of CO₂, and Low Vapor Pressure Differences

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ABSTRACT

The effects of environmental parameters on the blue light response of stomata were studied by quantifying transient increases in stomatal conductance in Commelina communis following 15 seconds by 0.100 millimole per square meter per second pulses of blue light. Because conductance increases were not observed following red light pulses of the same or greater (30 seconds by 0.200 millimole per square meter per second) fluences, the responses observed could be reliably attributed to the specific blue light response of the guard cells, rather than to guard cell chlorophyll. In both Paphiopedilum harrisianum, which lacks guard cell chloroplasts, and Commelina, the blue light response was enhanced by 0.263 millimole per square meter per second continuous background red light. Thus, the blue light response and its enhancement do not require energy derived from red-light-driven photophosphorylation by the guard cell chloroplasts. In Commelina, reduction of the intercellular concentration of CO₂ by manipulation of ambient CO₂ concentrations resulted in an enhanced blue light response. In both Commelina and Paphiopedilum, the blue light response was decreased by an increased vapor pressure difference. The magnitude of blue-light-specific stomatal opening thus appears to be sensitive to environmental conditions that affect the carbon and water status of the plant.

Light is one of several environmental parameters which affect rates of water loss and CO₂ uptake in the leaves of higher plants (24). Following illumination, uptake of K⁺ and Cl⁻ and synthesis of malate by the guard cells results in osmotic swelling and an increase in the apertures of the stomatal pores through which gaseous diffusion occurs (15).

In the grasses, blue light elicits an initial rapid increase in transpiration followed by a second, slower increase. The initial increase requires blue light (4) and is presumably mediated by a specific blue light response that initiates ion uptake via a chemiosmotic mechanism (27). Blue light stimulates H⁺ extrusion (19) and ATP-dependent membrane hyperpolarization (1, 2) in guard cells, presumably creating an electrical gradient for the influx of potassium. The magnitude of the rapid blue light response is enhanced by illumination with continuous background red light (10, 21) or by red light pulses (4, 10). The slower increase in transpiration is evoked by both blue and red light and appears to be mediated by guard cell Chl in the so-called PAR² response of stomata (17).

Various hypotheses have been proposed to explain the enhancement of the blue light response by red light. In the present study, the plausibility of these hypotheses was investigated, using gas exchange techniques in which both stomatal conductance and photosynthetic assimilation could be measured. CO₂ conductance is a more reliable indicator of stomatal responses and guard cell tur-

MATERIALS AND METHODS

Plant Material. Plants of Paphiopedilum harrisianum G.S. Ball and Commelina communis L. were raised as described previously (1, 8) except for a different watering regime. Plants were either misted (P. harrisianum) or drip irrigated (C. communis) 6 d/week with an automatic irrigation system (Rain Master Irrigation Systems, Simi Valley, CA). Plants were hand-watered 1 d/week, at which time they were fertilized with Spoonit Orchid Food (Plantsmith, CA).

Gas Exchange. Leaves were sealed in the cuvette of a null balance gas exchange system (7). Leaf temperature was measured with a copper-constantan thermocouple pressed against the lower leaf surface and was maintained within ±0.2°C of the temperature chosen for a given experiment, typically between 23.5 and 24°C. Vapor pressure difference was regulated through adjustment of both air flow rate (Tylan mass flow controller, Carson, CA), and the dewpoint temperature of a custom built humidifier system. Except as noted, temperature and VPD were maintained constant throughout a given experiment.

Incoming relative humidity was calculated from the dewpoint temperature of the condensor and air temperature inside the cuvette. Chamber humidity was measured with a Vaisala probe (Vaisala US, Woburn, MA) located inside the chamber; thus, measurements of changes in conductance were essentially instantaneous.

Except as otherwise noted, air with a CO₂ content of 346 μl

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3 Abbreviations: PAR, photosynthetically active radiation; VPD, vapor pressure difference; cᵢ, intercellular concentration of CO₂; cₐ, ambient concentration of CO₂; g, stomatal conductance.
L⁻¹ flowed continuously through the chamber and was supplemented with 1% CO₂ in N₂ as necessary to avoid CO₂ depletion from photosynthesis. CO₂ content of air entering and leaving the chamber was measured using an ADC Mark 3 infrared gas analyzer in the differential mode. There was a flow-dependent delay in the measurement of assimilation that ranged from less than 1 min under the maximum background red light illumination to about 2 min in the absence of background illumination, when flow rates were kept low in order to maintain an adequate VPD. Because conclusions derived from the results presented here do not require precise kinetic analysis, the delay in measurement of assimilation was not deconvolved (cf. 14). Assimilation was calculated from flow rates and CO₂ measurements, and cᵣ was computed (11). Relevant parameters for the calculation of assimilation and conductance were measured at 60-s intervals through computer-assisted sampling.

**Photobiology.** The gas exchange system was located in a darkened room. The illumination protocol was a modification of the dual beam technique (8, 13). Background red light was obtained from a 300-W lamp (Sylvania PAR56/2MFL) filtered through a Kodak 1A filter (50% cutoff at 545 nm) and a layer of No. 5A Cinemoid. Background fluence rates were varied using neutral density screens. Pulsed light was obtained by passing light from a General Electric Gemini 500 projector lamp through either a Rohm and Haas 2424 blue Plexiglas filter (maximal transmittance at 470 nm) or a Kodak 2-61 red filter. Heat load on the chamber was reduced by filtering both background and pulsed light through wide-band hot mirrors (OCLI, Santa Rosa, CA) which transmit minimally in the IR. Unless otherwise noted, red and blue pulses were 15 s × 0.100 mmol m⁻² s⁻¹, with intensity adjusted as required using neutral density filters. Fluence rates were measured with a Licor quantum probe (Lincoln, NE) placed on top of the cuvette and were corrected for the transmission coefficient of the glass cuvette lid.

**RESULTS**

Red and Blue Light Pulses. In four separate experiments, a single attached leaf of *Commelina* was allowed to attain steady state levels of assimilation and conductance at each of four different background red light intensities. Responses to a red and to a blue light pulse were recorded at each background light intensity.

Responses are summarized in Figure 1a, and typical results are illustrated in Figure 2. At all background fluence rates of red light, stomata responded to a blue light pulse, resulting in a transient increase in conductance (Figs. 1a and 2a). In all four experiments, the magnitude of the response was enhanced at 0.263 mmol m⁻² s⁻¹ background red light (Fig. 1a). Figure 2b illustrates the absence of a conductance response following the administration of red pulses to the same leaf and in the same experiment as in Figure 2a. An occasional gradual increase in conductance to a new steady state level after application of a red light pulse at a background light intensity of 0.043 mmol m⁻² s⁻¹ was seen (not shown) but could not be consistently observed.

Additional Red Pulses. The absence of response to pulses of red light in the previous experiment was unexpected, given that stomata do respond to red light under continuous illumination (cf. baseline levels of conductance in Figs. 1a and 2). Additional experiments were performed in duplicate to confirm the absence of a response to pulses of red light. Data shown in Figure 3 typify results from these experiments, indicating a lack of response under a range of pulse lengths and intensities comprising three different total pulse fluences: 1.5 mmol m⁻² s⁻¹ (Fig. 3, a and b), 3.0 mmol m⁻² s⁻¹ (Fig. 3d), and 6.0 mmol m⁻² s⁻¹ (Fig. 3c). Results were obtained under 0.090 mmol m⁻² s⁻¹ background red light (Fig. 3, a, b, and c), where the PAR response is clearly not saturated (cf. baseline conductance in Figs. 1a and 2). An increased, but still subsaturating, level of background red light was not effective in activating the response to pulsed red light (Fig. 3d).

**Comparison of Commelina and Paphiopedilum.** Figure 4 shows characteristic responses of *Commelina* and *Paphiopedilum* from experiments performed in triplicate. Overall levels of conductance are low in *Paphiopedilum*, as has been observed previously (1, 22). In both species, stomatal conductances following a pulse of blue light were greater when background red light was present. The results of Figure 4 confirm the enhancement of the blue light response shown in Figures 1a and 2, and extend those results to the achlorophyllous guard cells of *Paphiopedilum*. Figure 4 shows a response of *Paphiopedilum* to a blue light pulse in the absence of red light; other replicates did show a small response (not shown).

**Carbon Dioxide.** Pressurized air tanks with different partial pressures of CO₂ were used to investigate the effect of manipulation of CO₂ concentrations on the magnitude of the blue light response in *Commelina*. Increasing cᵣ from 346 μL L⁻¹, a level typical of natural conditions, to 810 μL L⁻¹ resulted in an increase in cᵣ and an inhibition of the blue light response, while decreasing cᵣ to 120 μL L⁻¹ enhanced the response (Fig. 1b). Figure 5 exemplifies a typical experiment. The effect of such manipulations on the blue light response of *Paphiopedilum* was not tested.

** Vapor Pressure Difference.** The effect of VPD on the blue light response was determined using the same leaves as in the comparison of *Commelina* and *Paphiopedilum*. Following administration of the first and second blue light pulses in that experiment, the background red light intensity of 0.263 mmol m⁻² s⁻¹ was maintained, while the VPD was experimentally increased until steady state conductance was reduced to the level previously observed in the absence of background illumination.

In both *Commelina* and *Paphiopedilum*, the increased VPD diminished the magnitude of the blue light response (Fig. 6). This result was observed, even though the increased VPD lowered cᵣ (not shown), by decreasing baseline levels of stomatal conductance.

**Responses of Assimilation and cᵣ to Blue and Red Pulses.** Figures 7 and 8 show responses of assimilation and calculated values of cᵣ from the experiment providing the conductance responses shown in Figure 2. There was a short pulse of assimilation directly associated with both the blue (Fig. 7a) and the red pulses (Fig. 7b). At 0.263 mmol m⁻² s⁻¹ background red light, both assimilation (Fig. 7a) and cᵣ (Fig. 8a) increased after the blue light pulse. Otherwise, no significant increases in assimilation (Fig. 7) or cᵣ (Fig. 8) were observed following either blue or red light pulses.

**DISCUSSION**

The data reported in Figures 1 and 2 indicate that, in *Commelina*, pulses of blue light result in a transient increase in stomatal conductance (cf. 8) that is enhanced by background red light. This result is consistent with previous reports on red and blue light effects on transpiration in grasses (4, 10, 21). Comparable pulses of red light failed to elicit an increase in conductance (Fig. 2b) even though the background red light intensities used here were below the saturation levels for stomatal opening mediated by guard cell Chl (Figs. 1a and 2; 17). Altering the duration (Fig. 3a) or fluence rate (Fig. 3b) of the red pulses, increasing their total fluence (Fig. 3, c and d), or increasing the background fluence rate (Fig. 3d) were also ineffective. These results confirm that the conductance responses reported here are specific to blue light. The results also provide information on the PAR response of stomata. They suggest that, in addition to a fluence rate threshold (17), there may also be a threshold to the duration, or to the total fluence required to elicit a PAR response.
Results

Experiments were ambient CO₂, indicated by the numbers to the right of each response trace. Leaf temperature (Tₙ) and VPD are as indicated. The shaded region exemplifies the area of the conductance curves used in calculations of the magnitude of the blue light response for Figure 1 and Table I.

Fig. 2. Response of conductance in *C. communis* to 15 s × 0.100 mmol m⁻² s⁻¹ pulses of blue (a) or red (b) light. Pulses were administered under four different intensities of background red light, whose fluence rates, in mmol m⁻² s⁻¹, are indicated by the numbers to the right of each response trace. Leaf temperature (Tₙ) and VPD are as indicated. The shaded region exemplifies the area of the conductance curves used in calculations of the magnitude of the blue light response for Figure 1 and Table I.

Fig. 3. Absence of a response of conductance in *C. communis* to four different red light illumination protocols. In a, b, and c, the background red light was kept at the same fluence rate and the fluence × time combination of a red pulse (a, b) or the total fluence of the pulse (c) was altered. In d, the intensity of the background red light was increased. Leaf temperature (Tₙ) and VPD are indicated for each experiment.

Additional experiments addressed four hypotheses concerning the mechanistic basis of the enhanced blue light response. One possibility is that the enhancement reflects increased baseline levels of guard cell turgor under red illumination (4, 10). Upon stomatal opening from a completely closed state, there is an initial phase, the ‘Spannungphase,’ during which ion and water movement contribute to guard cell inflation and changes in guard cell shape, but there is little change in pore aperture (18) and consequently little change in stomatal conductance. The presence of background red light might increase turgor beyond this initial
state. Consequently, all subsequent solute uptake stimulated by a blue light pulse would be effective in driving aperture and conductance increases. The magnitude of response to a given blue light stimulus would then appear larger in the presence of red light than in its absence. However, if this explanation is correct, once the Spannungshe phase has been surpassed, as indicated by an increase in baseline levels of conductance, no effect on the blue light response of a further increase in red light fluence rate should be observed. In contrast, the data of Figures 1a and 2a show a clear enhancement of the blue light response between 0.043 mmol m⁻² s⁻¹ background red light, where the Spannungshe phase has already been exceeded (cf. Figs. 1 and 2, baseline conductance levels), and 0.263 mmol m⁻² s⁻¹ red light. These results, and previous results of Skaar and Johansson (21), do not support the hypothesis that the interaction with red light is an effect dependent on guard cell turgor. The relationship that is observed between baseline levels of conductance and the magnitude of the blue light response (Table I) may be a secondary correlation.

A second possible explanation for the enhanced blue light response is that red-light-driven photophosphorylation in the guard cell chloroplasts (20) provides energy for stomatal opening triggered by blue light (10, 21). However, chloroplasts must not be required for red light enhancement of the blue light response, since the response of the achlorophyllous guard cells of Paphiopedilum can also be enhanced (Fig. 4). The results cannot, of course, rule out an interaction between the blue light and PAR responses of chlorophyllous guard cells, but do suggest that another red-light absorbing pigment may be present in guard cells. An obvious candidate is phytochrome (16), and it is of interest

Fig. 4. Response of conductance in C. communis (a) and P. harrisianum (b) to a 15 s × 0.100 mmol m⁻² s⁻¹ blue pulse administered on a background of either darkness or 0.263 mmol m⁻² s⁻¹ red light.

Fig. 5. Effect of manipulation of cᵢ on the response of conductance in C. communis to a 15 s × 0.100 mmol m⁻² s⁻¹ blue pulse. The background fluence rate of red light was kept constant at 0.263 mmol m⁻² s⁻¹.

Fig. 6. Effect of VPD on the magnitude of the conductance response in C. communis (a) and P. harrisianum (b) to a 15 s × 0.100 mmol m⁻² s⁻¹ pulse of blue light. The background red light intensity was kept constant at 0.263 mmol m⁻² s⁻¹. Leaf temperature (Tₑ) and VPD are indicated for each experiment.

Fig. 7. Response of assimilation in C. communis to the blue (a) and red (b) pulses which resulted in the conductance responses shown in Figure 2. Background fluence rates of red light in mmol m⁻² s⁻¹ are indicated to the right of each response trace.
that red light, apparently acting via phytochrome, shifts the fluence response curve of blue light-stimulated phototrophic bending (3, 28).

A third possibility is that the magnitude of the blue light response is affected by a 'messenger' from the mesophyll whose concentration is proportional to the rate of carbon assimilation (23). In most of the experiments reported here (cf. Figs. 2a and 7a), the magnitude of the blue light response is positively correlated with the baseline level of mesophyll assimilation. But, in the experiments of Figures 1b and 5, increasing c_i resulted in a greater c_i and a greater assimilation rate (not shown), yet depressed the blue light response. These results speak against a control of the blue light response by mesophyll assimilation.

A fourth suggestion is that the 'red light' enhancement may be explicable as an indirect effect, mediated by the intercellular concentration of CO2. Both in experiments where c_i is altered using light intensity (Figs. 1a, 2, 4) and in experiments where c_i is manipulated by changing c_v (Figs. 1b and 5), there is a consistent negative correlation between the magnitude of the blue light response and c_i (Table I). Previous experiments with blue light also support such a correlation (4, 10). Although the causal basis for an interaction remains unknown, recent experiments suggest that both blue light (2) and CO2 (6) may directly affect ion transport at the plasmalemma of guard cells.

The intercellular concentration of CO2 is not the sole modulator of the blue light response, because combining the data from red light and c_i manipulations does not increase the total correlation coefficient with respect to blue light (Table I). In addition, increasing the VPD results in a decrease in c_i (not shown) yet decreases the blue light response (Fig. 6). One important aspect of future experiments will be to determine the relative magnitudes of the effects of red light, c_i, and VPD on the blue light response.

The data reported here have several ecological implications. The observation of conductance changes in response to blue but not red light pulses (Figs. 2 and 3) supports the suggestion (25) that the blue light response plays a significant role in conductance increases during sun flecks. At 0.263 mmol m^-2 s^-1 red light, there is an increase in assimilation (Fig. 7a) that is correlated with blue-light-stimulated increases in conductance and c_i (Figs. 7a and 8a). However, at all other background light levels, assimilation is limited by light rather than by CO2, as indicated by the short pulse of assimilation associated directly with the light pulse (Fig. 7). Thus, the level of background or 'shade' illumination in the natural environment may determine whether transient, blue light-stimulated increases in conductance have a significant effect on carbon gain.

The results shown here also illustrate a property of the blue light response of stomata that has not yet been demonstrated for the red light, CO2, or VPD responses of guard cells. That property is that the blue light response is directly sensitive both to factors which affect carbon gain, such as overall illumination levels and CO2 concentrations, and to factors affecting rates of water loss, such as VPD. The blue light response is greatest precisely under those conditions where CO2 is most likely to limit carbon gain, namely conditions of high background irradiance (Figs. 1, 2, and 4) or low c_i (Fig. 5). Conversely, under conditions promoting water stress, such as an increased VPD, the magnitude of the blue light response is diminished (Fig. 6). These results suggest that the blue light response may be the modulator responsible for 'fine-tuning' the ratio of carbon gain to water loss such that for a given steady state fluence rate, c_v, and VPD, a precise compromise is achieved between conflicting requirements for CO2 uptake and water conservation.

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* These experiments were performed twice.
STOMATAL RESPONSES TO BLUE LIGHT

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