Effect of Colored Light on Stomatal Opening Rates of
Vicia faba L.¹

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ABSTRACT

The average opening rate of Vicia faba L. stomata was determined over an initial 20-minute light period following darkness. Nonsaturating intensities of broad band red and blue light had similar quantum effectiveness for the promotion of opening, whereas broad band green was about 40% and far red about 5% as effective. The opening rates under saturating red, green, and blue light were the same. Net photosynthesis was measured under various intensities of the same red, green, and blue light spectra. Red and blue light were equally efficient in causing photosynthesis, whereas green was 60% as effective. The light compensation points for the three colors were at higher intensities than those which saturated the opening rate response. These data suggest that only a single pigment system, probably the photosynthetic pigments, is responsible for initiating the light-induced opening response in V. faba stomata.

Most action spectra for stomatal opening have been determined for final apertures under constant light (4, 8, 9). Final apertures typically are measured after 2 to 3 hr in light. These action spectra are characterized by a greater response to blue light than to red light. The results have led to the proposal that two pigment systems, photosynthesis and a "blue-absorbing" pigment system, operate in stomatal opening (10).

It is possible that the pigments and mechanisms which are responsible for maintaining stomatal opening are different from those which initiate opening. Karvé (7) produced an action spectrum for the rate of opening of corn stomata. This is a reasonable measure of the relative efficiencies of light in commencing the opening process. Karvé's action spectrum was similar to those spectra produced for final opening, suggesting that the pigment systems that function for the maintenance of stomatal opening also function in the induction of opening in corn.

This paper reports evidence that the blue-absorbing pigment, which at least partially mediates the final aperture of Vicia faba stomata (4), does not operate in the induction of stomatal opening. The apparent photosynthetic nature of the response is discussed.

MATERIALS AND METHODS

Plant Material. V. faba L. var. Long Pod plants were grown in a temperature- and light-controlled growth chamber. Plants, grown singly in vermiculite in plastic pots (9 × 9 × 8 cm), were watered to field capacity daily and fertilized with a solution of Hyponex complete plant food (Hydroponic Chemical Co. Inc., Copley, Ohio) weekly. Light was supplied by white fluorescent tubes (Sylvania F72T12/CW/VHO) at an intensity of 1.5 to 2 mw cm⁻² (YSI-Kettering model 65 radiometer) and a photoperiod of 12 hr. Temperature was 25 to 26 C day and night. Plants were 3 to 5 weeks old when sampled.

Colored Light Sources. Fluorescent light (Sylvania F72T12/ CW/VHO for the opening rate experiments and GE 15T12 CW for the photosynthesis experiments) passing through cellulose acetate Roscolene filters supplied the red (two red no. 821 plus one yellow no. 807), green (one green no. 871 plus one yellow no. 807), and blue (one blue no. 857) light. Incandescent light (GE 60 w) passing through a sheet of "black" Plexiglas (FRF-700, Westlake Plastics Co., Lenni, Pa.) produced the far red light. Neutral density filters, made by spraying even coats of black acrylic paint (Mars Black, Liquitex) on glass sheets, were used to reduce the light intensities.

Spectral intensities were measured with an Isco model SR spectroradiometer. The energy units for each light spectrum were converted to quantum flux densities using the formula: Q = A/1987 × 1 × 10¹², where Q is the quantum flux density (quanta cm⁻² sec⁻¹), A is the wavelength in angstroms that corresponds to the peak intensity of the light spectrum, and I is the energy (µw cm⁻²) of the transmitted spectrum.

Opening Rate Determinations. The increase in the average stomatal aperture during the first 20 min in light was determined by microscopic measurement of epidermis (abaxial) impressions taken with a thin layer of Duco cement (DuPont Co.). Leaf discs (1 cm diameter) were cut from newly expanded leaves at the end of the daily light period. For a single opening rate, 12 discs were cut from two opposite leaflets and arranged in two groups, each containing discs from only one side of each leaflet. The discs were floated, abaxial side up, on distilled H₂O in darkness for 12 hr at 28 ± 0.5 C. (This length of dark incubation was found to yield the maximum opening rate under saturating light [6].)

Impressions were taken of discs from one group removed from darkness just prior to a light treatment, while impressions of the other discs were taken after a 20-min light treatment at 28 ± 0.5 C. The elapsed time from removal of discs from incubation or light treatments to when the cement had set was usually less than 4 min. Apertures were measured under a magnification of 1500 × to the nearest 0.33 µm. The difference in the average width of 150 apertures from each group was taken as the average opening rate. Each point in Figure 2 was computed from five separate rate determinations. Hence, 27,000 apertures were measured for the curves in Figure 2.

Photosynthesis Measurements. Net photosynthesis was determined using an open gas exchange apparatus coupled to a Beckman 315B IR gas analyzer. A single intact leaflet was measured early in the 12-hr photoperiod. High intensity white light was used to open the stomata. To assure minimum fluctuations in leaf resistance during the colored light measurements, steady-state photosynthesis was monitored under white light between each colored light determination.
RESULTS

The relative effectiveness of broad band blue, green, red, and far red light (Fig. 1) on the opening rate of V. faba stomata is shown in Figure 2. Under nonsaturating intensities, the quantum effectiveness of red and blue light for the promotion of opening were similar, as evidenced by the comparable slopes; green light was about 40% as effective, whereas far red light was about 5% as effective as either blue or red light. The maximum opening rate under saturating light was the same for red, green, and blue. Intensities of far red light sufficient to demonstrate a saturation response could not be obtained. However, it is evident that saturation of the response under red and blue light occurs near $6 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$, while saturation under green light seems to occur near $16 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$.

The relative responsiveness of the opening rate to the colored light suggested that the chloroplast pigments may be the photoreceptors. A determination of the relative responsiveness of photosynthesis to the broad band lights was made (Fig. 3). A similar photosynthetic response to the red and blue light was apparent. Green light was about 60% as effective (determined by slope) as blue or red light. The light compensation points were $12.2 \times 10^{14}$ for blue, $12.1 \times 10^{14}$ for red, and $18.8 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$ for green light.

DISCUSSION

Johnsson et al. (5) recently reported that the opening kinetics of grass-like stomata generally exhibited an initial enhanced opening under blue light compared to red light. Their survey of species with kidney-shaped stomata, including V. faba, generally showed no significant difference in the opening kinetics measured under red or blue light. Thus, a direct comparison of average opening rates under red and blue light, as measured here, is meaningful. The discrepancy between Karve's (7) report of blue light-enhanced opening rates for Zea mays stomata and this report of equal effectiveness of blue and red light for V. faba stomatal opening rates can be explained by the blue light component operating in corn stomata but not V. faba stomata.

Differences exist between the influence of colored light on the opening rate and the final opening of V. faba stomata. Using a 3-hr exposure time, Hsiao et al. (4) reported larger apertures under saturating 440 nm light than under 560 or 660 nm light for epidermal strips in CO$_2$-free air. A similar response seemed to occur when conductances of leaf discs were measured, although saturating 660 nm light doses were not attained. The difference in final aperture under different colors of saturating light indicates that at least two different pigment systems operate. The similar maximum opening rates reported here for red, blue, and green light are evidence for a single pigment system being responsible for "turning on" stomatal opening, if the maximum opening rate under saturating light is limited by metabolic generation of osmotica rather than water permeability.

The relative efficiencies of the colored light in producing the opening rate response, along with comparable relative efficiencies for causing photosynthesis, suggest that the photosynthetic pigments are involved in the early responsiveness of V. faba stomata to light. The generally held hypothesis that the stomatal aperture is at least partially regulated by photosynthetic CO$_2$ metabolism (2, 10) may also apply to the control of the opening rate. The mechanism must, however, be restricted solely to a chloroplastic event, since light saturation of the opening rate occurs below the light compensation point (cf. Figs. 2 and 3). That is, the maximum opening rate can be obtained when there is a net efflux of CO$_2$ from the leaf, and thus the only area of significant CO$_2$ depletion would be within the chloroplast.

It is interesting that low intensity (450 lux) green light will induce flattening and ion leakage from Elodea chloroplasts (1). CO$_2$-free air will also cause chloroplast shrinkage (3) and ion leakage (1). It may be that an important early event in light-induced stomatal opening is leakage of ions from chloroplasts in guard cells, which in turn regulate key enzymes in the osmotica (malate?)-generating pathway.

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LITERATURE CITED


