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RESEARCH NOTE

THE PHOTOBIOLOGY OF *Paphiopedilum* STOMATA: OPENING UNDER BLUE BUT NOT RED LIGHT

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Abstract—The responses of stomata from *Paphiopedilum harrisianum*, Orchidaceae, to light and CO_2 were studied in epidermal peels. Stomatal opening under red light was indistinguishable from that in darkness, whereas blue light promoted opening above dark levels. The ineffectiveness of red light in causing stomatal opening was confirmed in the presence of 100 μM KCN; average apertures in both darkness and red light were 53% of those measured in the absence of the inhibitor, whereas under blue irradiation, the KCN inhibition was only 30%, with average apertures two-fold of those measured under red light or darkness. Fluence rate response curves under blue light were typical of a single photoreceptor; removal of CO_2 increased aperture values without a significant light–CO_2 interaction. The lack of a stomatal red light response contrasts with results obtained in species with chlorophyllous stomata in which red light consistently causes stomatal opening, and suggests that the previously reported red light responses in stomata from intact *Paphiopedilum* leaves resulted from indirect effects, such as depletion of intercellular CO_2 by mesophyll photosynthesis. In isolation, *Paphiopedilum* stomata appear to rely on a blue light photosystem for their responses to light and fail to open under red light because of their lack of guard cell chloroplasts.

INTRODUCTION

Stomatal responses to light can be convincingly demonstrated in epidermal peels or isolated guard cell protoplasts (Humble and Hsiao, 1970; Zeiger and Hepler, 1977; Zeiger *et al.*, 1977; Lurie, 1978; Travis and Mansfield, 1981; Jewer *et al.*, 1982; Pemadasa, 1982). The characterization of photoresponses of stomata in the intact leaf has been more difficult to achieve because of parallel responses of the mesophyll to light, and light–CO_2 interactions (Wong *et al.*, 1978; Zeiger, 1983). Several lines of evidence, however, point to direct stomatal responses to light in the intact leaf (Wong *et al.*, 1978; Sharkey and Raschke, 1981a,b; Zeiger and Field 1982; Morison and Jarvis, 1983) which appear closely related to those observed with guard cells in isolation.

The wavelength dependence of stomatal movements shows major peaks in the blue and in the red (Meidner, 1968; Hsiao *et al.*, 1973; Sharkey and Raschke, 1981b; Zeiger and Field, 1982) with the blue peak being higher under most experimental conditions. It is widely accepted that this wavelength dependence is the expression of the activity of two distinct photoreceptor systems: the guard cell chloroplasts, exhibiting the classical absorption spectrum of chlorophyll and a blue light photosystem which absorbs only in the blue. The combined responses of the two photoreceptor systems thus result in a wavelength dependence typical of PAR (photosynthetic active radiation) -dependent systems with an enhanced blue light response caused by the blue-light photosystem (Zeiger, 1983).

The reported wavelength dependence of stomatal responses in the genus *Paphiopedilum*, Orchidaceae (Nelson and Mayo, 1975), is at variance with this hypothesis. Stomata from *Paphiopedilum* lack guard cell chloroplasts (Nelson and Mayo, 1975; Rutter and Willmer, 1979; Thorpe, 1980; Zeiger, 1981) yet measurements of stomatal resistance in intact leaves show a red light response (Nelson and Mayo, 1975). These results are inconsistent with the postulated participation of guard cell chloroplasts in stomatal responses to red light. The photoresponses of *Paphiopedilum* stomata, however, were measured in intact leaves using a transient porometer (Nelson and Mayo, 1975), a method which cannot discriminate between direct light responses and those resulting from indirect effects, such as changes in intercellular CO_2 concentrations. We set out to characterize the photoresponses of *Paphiopedilum* stomata in epidermal peels, in an attempt to distinguish direct light responses from those mediated by CO_2 or by epidermis-mesophyll interactions. We report here that *Paphiopedilum* stomata in epidermal peels open in response to blue but not to red light, indicating that a direct response to red light requires the presence of guard cell chloroplasts.

MATERIALS AND METHODS

Plants of several *Paphiopedilum* species were purchased from a commercial nursery and kept in a greenhouse at 20°C under prevailing relative humidities of about 50%. The plants were shaded to reduce ambient light, with maximum irradiances not exceeding 0.75 mmol m^{-2} s^{-1}.

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Abaxial epidermal peels from *P. harrisianum* were taken from leaf segments *ca.* 4 mm wide. Peels were easily damaged when detached from the leaf; stomatal responses were most reliable when peels were made by pulling the epidermis at an acute angle with respect to the stripped mesophyll (Weyers and Travis, 1981).

Peels were incubated, cuticle up, in 60–80 m*M* KCl and 25 m*M* MES at pH 6.3. The temperature of the incubation medium was measured with a thermocouple thermometer (Wescor, Logan, UT) and controlled to 23 ± 0.3°C using a water bath (Forma Scientific, Marietta, OH). In the experiments investigating stomatal responses to CO_2, compressed air was continuously bubbled through the incubation solution. CO_2-free air was obtained by passing the air through Ascarite II (A. H. Thomas, Philadelphia, PA). Apertures were measured with an ocular micrometer at 400×, with peels mounted cuticle down, to avoid optical interference from the guard cell ledges forming the stomatal pore. The blue light source was a 150 W GE projection lamp (model DCL) in conjunction with a Roehm and Haas, Plexiglass No.2424 (Maximal transmittance at 470 nm, half band width, 100 nm) filter. Red light was obtained using a 500 W incandescent bulb (GE model 3200k) and a Kodak SAFE No.1A cutoff (50% cutoff at 645 nm) filter. Irradiances were measured with a Li-cor (Lincoln, NE, USA) quantum probe.

RESULTS AND DISCUSSION

None of the *Paphiopedilum* species studied, *P. insigne*, *P. philipinese*, *P. barbatum*, *P. boxalli* and their hybrid *P. harrisianum* had guard cell chloroplasts, as ascertained by fluorescence microscopy.

P. harrisianum was the only species yielding peels which were adequate for studies of stomatal movements. Initial experiments showed that, in contrast with stomata of other species usually used in work with epidermal peels (Weyers and Travis, 1981; Outlaw, 1983; Zeiger, 1983), those of *P. harrisianum* did not open when incubated in conventional KCl solutions. Opening was consistently observed upon incubation in 60–80 m*M* KCl and 25 m*M* MES at pH 6.3, with the stomata failing to open if either compound was omitted from the medium (Willmer *et al.*, 1983). Maximal pore opening varied between different sets of experiments and was usually higher at Stirling (Willmer *et al.*, 1983) than at Stanford (Table 1 and Figs. 1 and 2). The variability can be partially ascribed to different growing conditions and fluctuations in prevailing relative humidities (Williams *et al.*, 1983).

Aperture values after a 2 h incubation at 23°C ± 3 under 0.1 mmol m^{-2} s^{-1} of blue or red light and darkness are shown in Table 1. Stomata were closed at the beginning of treatments. In five out of the nine experiments, stomata had larger apertures under blue light; in the other four, opening under blue light was indistinguishable from that in darkness. We attribute the absence of a clearcut light effect to the variable, yet significant, opening in darkness, which appears to be a response to the incubation medium. The physiological implications of this response, if any, remain to be elucidated.

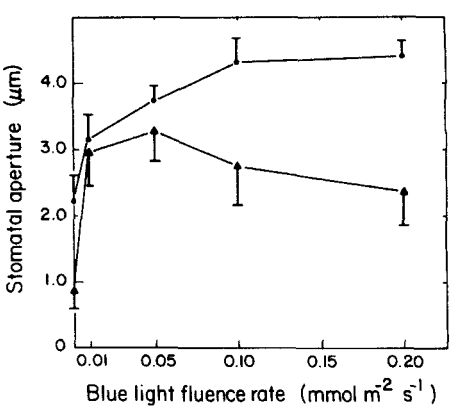

Figure 1. Fluence rate response curves of stomatal opening in peels of *P. harrisianum* under blue light, in normal air. Incubation medium had 70 m*M* KCl, 25 m*M* MES, pH 6.3 with (▲) or without (●) 100 μ*M* KCN. Each curve is the average of seven experiments, with 60 apertures measured at each fluence rate per experiment. Bars (depicted on only one side of each point, for clarity) represent one standard error of the mean.

Table 1. Stomatal apertures (μm) in peels of *P. harrisianum* illuminated for 2 h with blue or red light at 0.1 mmol m^{-2} s^{-1}

Experiment	Without KCN			With KCN		
No.	Dark	Red	Blue	Dark	Red	Blue
1	0.96	1.55	0.99	0.55	0.36	0.72
2	2.55	1.09	3.52	0.62	0.14	1.31
3	2.72	1.64	3.03	1.36	0.84	2.09
4	2.00	0.99	3.75	1.86	1.09	2.92
5	1.03	1.08	1.99	0.62	0.78	1.50
6	2.20	1.93	2.71	1.48	1.81	2.96
7	1.40	0.70	1.41	0.50	0.39	1.06
8	0.56	1.06	2.47	0.53	0.31	2.13
9	0.78	0.83	2.20	0.00	0.15	1.29
Mean	1.58	1.21	2.45	0.84	0.65	1.78
SEM	0.23	0.14	0.31	0.20	0.18	0.27

Incubation medium was 70 m*M* KCl and 25 m*M* MES at pH 6.3. KCN was added at 100 μ*M*. Each tabulated value represents the average of 60 aperture measurements.

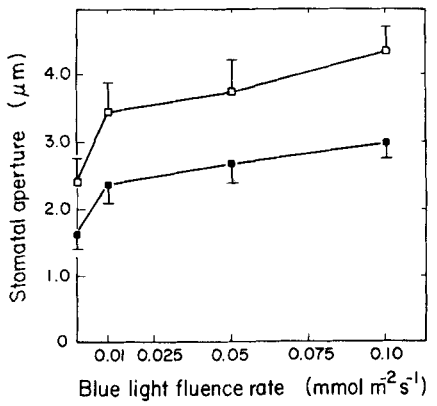

Figure 2. Fluence rate response curves of stomatal opening in peels of *P. harrisianum* under ambient (■) or CO_2-free (□) air. Incubation medium had 70 m*M* KCl and 25 m*M* MES, pH 6.3. Each curve is the average of seven experiments, with 60 aperture values measured at each fluence rate per experiment. Bars (depicted on only one side of each point, for clarity) represent one standard error of the mean.

Red light failed to enhance opening above dark levels in seven out of the nine experiments, with the two exceptional cases showing markedly low aperture values for all treatments. We conclude that the stomatal opening in response to red light observed in species with chlorophyllous guard cells (Hsiao *et al.*, 1973; Lurie, 1978; Ogawa, 1981; Travis and Mansfield, 1981; Pemadasa, 1982) is absent from *Paphiopedilum*.

Stomatal opening in darkness has been shown to be dependent on oxidative phosphorylation, and is largely inhibited by KCN (Zeiger, 1983). KCN at 100 μ*M* was therefore added to the incubation medium in a second set of experiments, in order to depress dark levels of stomatal opening and facilitate the characterization of specific light responses. As in the absence of KCN, average apertures values under red light were slightly lower than those seen in darkness and, in both treatments, opening in the presence of KCN was 0.53 of that seen in the absence of the inhibitor (Table 1). This identical KCN sensitivity further indicates that the opening measured under red light is actually a dark response and that red irradiation is ineffective in the promotion of stomatal opening in achlorophyllous stomata. Opening under blue light, on the other hand, was two-fold higher than that of the dark or red light treatments, clearly demonstrating a blue light effect. The blue light response was relatively insensitive to the presence of KCN, exhibiting average apertures which were 0.7 of those seen without KCN. This KCN sensitivity of the blue light response of *Paphiopedilum* stomata contrasts markedly with that of stomata in epidermal peels of *Commelina,* in which opening under blue light in the presence of KCN was only 0.2 of that seen in the absence of the inhibitor (Schwartz and Zeiger, 1982, and unpublished). These differences may reflect genus-specific metabolic properties connected with the functioning of the blue light photosystem.

The photoresponses from stomata of *P. harrisianum* shown here are at variance with the reported stomatal opening seen under red irradiance in intact leaves of *P. leeanum* (Nelson and Mayo, 1975). Since in species with chlorophyllous stomata, stomatal opening in red light can be observed in epidermal peels as well as in intact leaves (Lurie, 1978; Ogawa, 1981; Sharkey and Raschke, 1981b; Schwartz and Zeiger, 1982; Morison and Jarvis, 1983) we conclude that, in *P. leeanum*, the reported sensitivity to red light is an indirect effect, such as a response to decreasing intercellular CO_2 concentrations brought about by mesophyll photosynthesis, rather than a direct response to red light.

Fluence rate response curves of stomatal opening under blue light are shown in Fig. 1. In the absence of KCN, opening saturated at 0.1 mmol m^{-2} s^{-1}. As opposed to responses from chlorophyllous stomata (Travis and Mansfield, 1981; Pemadasa, 1982), where fluence rate response curves under blue light presumably reflect the activity of both the blue light photosystem and the chloroplasts of the guard cells, the data shown in Fig. 1 provide information on the response of the blue light photosystem without interference from guard cell chloroplasts.

Addition of KCN decreased both absolute aperture values and saturation levels (Fig. 1). However, KCN-treated stomata showed a proportionally larger response to a blue light step from darkness to 0.01 mmol m^{-2} s^{-1}. The inhibitory effect of KCN increased at higher fluence rates, presumably as a result of photoinhibition.

Nelson and Mayo (1975) reported that an increase in stomatal resistance occurred when *P. leeanum* leaves were exposed to CO_2-enriched air. Fluence rate response curves of stomatal opening in epidermal peels of *P. harrisianum* under normal and CO_2-free air (Fig. 2) demonstrate a direct response to CO_2 by these stomata; as in other species (Fischer, 1968; Travis and Mansfield, 1981; Donkin *et al.*, 1982) removal of CO_2 enhanced stomatal opening. However, unlike *Commelina* (Travis and Mansfield, 1981) or *Pisum* (Donkin *et al.*, 1982), the stomatal response of *Paphiopedilum* to CO_2 showed no significant light–CO_2 interaction. This differential behavior is consistent with the hypothesis that guard cell chloroplasts normally play a role in the CO_2 responses of stomata under illumination (Melis and Zeiger, 1982).

The wealth of information emerging from studies with *Paphiopedilum* stomata, renders its introduction as an experimental system (Nelson and Mayo, 1975, 1977) a valuable contribution to stomatal physiology. The isolation of the blue light response of stomata in *P. harrisianum* characterizes a useful means to study the properties of the blue light photosystem of guard cells. In addition, studies

of the physiology of *Paphiopedilum* stomata in comparison with orchids possessing chlorophyllous stomata are providing us with a useful method for the elucidation of the role of guard cell chloroplasts in stomatal function.

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