Plant Physiol. (1990) 92, 495–499
0032-0889/90/92/0495/05/$01.00/0

Received for publication June 5, 1989
and in revised form October 7, 1989

Two Distinct Blue-Light Responses Regulate Epicotyl Elongation in Pea[1]

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ABSTRACT

Blue light induces a long-term suppression of epicotyl elongation in red-light-grown pea (*Pisum sativum* L.) seedlings. The fluence-response characteristics are bell-shaped, indicating the possibility of two different blue-light responses: a lower fluence response causing suppression and a higher fluence response alleviating the suppression. To determine if two responses are in effect, we have grown pea seedlings under dark conditions hoping to eliminate one or the other response. Under these growth conditions, only the lower fluence portion of the response (suppression of elongation) is apparent. The kinetics of suppression are similar to those observed for the lower fluence response of red-light-grown seedlings. The response to blue light in the dark-grown seedlings is not due to the excitation of phytochrome because a pulse of far-red light large enough to negate phytochrome-induced suppression has no effect on the blue-light-induced suppression. Furthermore, treatment of the dark-grown seedlings with red light immediately prior to treatment with high fluence blue light does not elicit the higher fluence response, indicating that the role of red light in the blue high fluence response is to allow the plant to achieve a specific developmental state in which it is competent to respond to the higher fluences of blue light.

Blue light plays a pivotal role in the development of many eukaryotic organisms including the higher plants. Several of the events regulated by blue light have biphasic fluence-response characteristics, occurring primarily as bell-shaped curves. Thus, a response elicited by lower fluences of blue light can be negated or compensated by higher fluences of blue light. These responses to blue light are apparent when plants are grown in continuous red light, indicating that the responses are probably not due to the excitation of phytochrome (2, 3, 10–12). Those responses showing bell-shaped fluence-response characteristics under continuous red-light conditions include phototropic curvature for several monocot and dicot species, including pea (curvature toward a unilateral light source at low fluences, less curvature at higher fluences) (2, 3), long-term suppression of epicotyl elongation in pea (suppression at low fluences, alleviation at higher fluences) (16), and control of the steady-state level of *Cab*[2] RNA and pEA215 RNA in pea (accumulation at low fluences, return to control levels at high fluences) (17).

In the case of phototropic curvature, the bell-shaped curve is amply explained by the attenuation of a unilateral beam of light as it passes through the thickness of a stem (14). However, the biphasic nature of epicotyl elongation and steady-state levels of RNA are elicited by bilateral illumination and are not explained by a spatial model. It is, therefore, possible that two distinct blue-light responses exist, one with a threshold to lower fluences of blue light (a blue LF response), affecting the suppression of stem elongation and an increase in *Cab* and pEA215 RNA levels, and a second with a threshold to higher fluences of blue light (a blue high fluence response), alleviating the suppression of epicotyl elongation and causing a return to control levels for *Cab* and pEA215 RNA.

One way to confirm that two distinct blue-light responses are in effect is to demonstrate that either one or both responses can exist independent of the other. It is possible that growth in red light, through either the achievement of a particular developmental state or the presence of the Pfr, allows for one of the responses. To test this hypothesis we have used plants grown in absolute darkness to examine the effects of blue light on long-term growth suppression. Under these conditions, we find that only the blue low fluence response is present. Controls indicate that neither LF nor VLF phytochrome responses are responsible for the effects observed to blue light and that the blue high fluence response is not dependent upon the presence of Pfr at the time of blue light irradiation.

MATERIALS AND METHODS

Plant Growth Conditions

Seeds of *Pisum sativum* L. var Alaska (J Mollema, Sons, Grand Rapids, MI) were imbibed and grown in absolute darkness for 7 d in 80% RH at 21°C. Blue light sources and irradiation protocols have been described previously (13, 16). Red and far-red irradiations were done with the same light source used for blue light with the following filters: red light, two sheets of Rohm and Haas (Philadelphia, PA) No. 2423 red plexiglass and two sheets of Roscolux (Rosco, Port Chester, NY) fire No. 19; far-red light, one sheet of North Carolina Biological (North Carolina) far-red filter No. 68–6800 CBS. Red light treatments had a total fluence of 10^4 μmol m^{-2} delivered as a 10 s pulse. Far-red treatments had a total fluence of 10^5 μmol m^{-2} and were delivered over a 15 min period.

[1] Supported by U.S. Department of Agriculture grant No. 86CRCR12228 to L. S. K.

[2] Abbreviations: *Cab*, Chl *a/b* binding protein; VLF, very low fluence; LF, flow fluence.

Fluence Response Experiments

Six days after planting, separate trays of seedlings were irradiated with either 10^{-1}, 10^{0}, 10^{1}, 10^{2}, 10^{3}, or 10^{4} μmol m^{-2} of blue light. The lengths of the irradiations were as follows: 10^{-1} to 10^{2} μmol m^{-2}, 10^{1} s; 10^{3} μmol m^{-2}, 10^{2} s; 10^{4} μmol m^{-2}, 10^{3} s. Seedlings were harvested 24 h after the blue-light pulse and photocopied, and the length of the third internode was measured. The third internode had not started to expand prior to the time of irradiation.

Time Course Experiments

Trays of seedlings were irradiated with either 10^{1} or 10^{4} μmol m^{-2} of blue light. Seedlings were harvested 0, 5, 11, 18, and 24 h after the blue-light pulse.

Reciprocity Experiments

Seedlings were irradiated with a total fluence of 10^{1} or 10^{4} μmol m^{-2} of blue light. A total fluence of 10^{1} μmol m^{-2} was maintained through the use of neutral density filters (Balzer, Lichtenstein). Irradiation periods tested were 10^{0}, 10^{1}, 10^{2}, 10^{3}, and 10^{4} s. A total fluence of 10^{4} μmol m^{-2} was maintained with one sheet of GB001 (10^{1} s) filter paper or one sheet of GB002 (10^{2} s) filter paper (Schleicher & Schuell, Keene, NH). Irradiation periods tested were 10^{0}, 10^{1}, 10^{2}, and 10^{3} s.

Statistics

All data represent the average of at least four independent experiments; each experiment had a sample size of 10 seedlings. Bars represent the standard error of the mean. Where not evident, they are within the data symbol.

RESULTS

Fluences Response

The length of the third internode 24 h after irradiation of 6-d-old dark-grown seedlings with different fluences of blue light is shown in Figure 1. The data indicate that suppression occurs with a threshold below 10^{-1} μmol m^{-2} and persists through 10^{4} μmol m^{-2}. This contrasts the lack of suppression observed for red-light-grown plants treated with 10^{4} μmol m^{-2} of blue light (16).

It is possible that the response to blue light observed for dark-grown plants is due to excitation of phytochrome. Excitation of phytochrome is known to cause a long-term suppression of epicotyl elongation in pea (7, 8). To determine if phytochrome excitation affects the rate of stem elongation under the growth conditions used herein, the blue-light treatment was replaced by a single pulse of red light (10^{4} μmol m^{-2}). The data in Figure 2 indicate that phytochrome excitation results in suppression of stem elongation when measured 24 hr after the pulse of light. Far-red light (10^{5} μmol m^{-2}) given immediately following the red-light treatment results in near complete reversal of the red light effect, indicating that the phytochrome response is a LF phytochrome response and there is no VLF phytochrome component (5). The lack of a VLF response is confirmed by the lack of

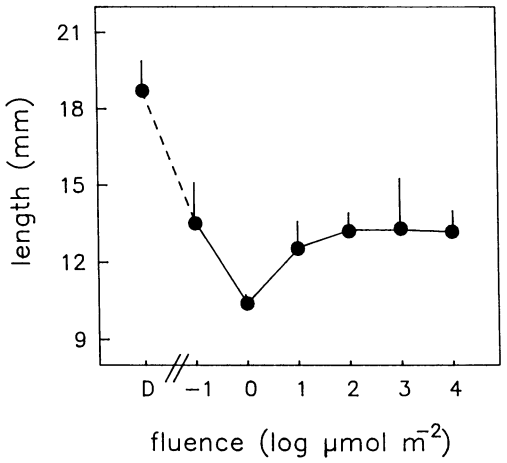

Figure 1. Blue-light fluence-response curves for epicotyl elongation in dark-grown peas. Seedlings were grown in absolute darkness for 7 d. Six days after planting, separate trays of seedlings were irradiated with a single pulse of blue light, with the fluences indicated on the figure. The lengths of irradiations were as follows: 10^{-1} to 10^{2} μmol m^{-2}, 10^{1} s; 10^{3} μmol m^{-2}, 10^{2} s; 10^{4} μmol m^{-2}; 10^{3} s. Twenty-four hours after the blue-light treatment, seedlings were harvested and the length of the third internode was measured. Control seedlings (D) received a mock pulse of blue light. Error bars represent the standard error of the mean.

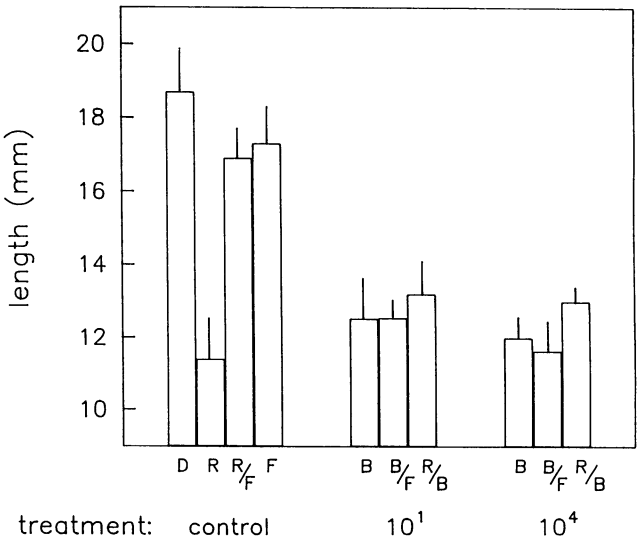

Figure 2. The role of phytochrome in the response to blue light. Seedlings were grown for 7 d in continuous darkness. Six days after planting seedlings received one of three basic treatments, no blue light (control), 10^{1} μmol m^{-2} (10^{1}), and 10^{4} μmol m^{-2} (10^{4}). Control seedlings received either no further treatment (D), 10^{4} μmol m^{-2} of red light (R), the red treatment followed immediately by 10^{5} μmol m^{-2} of far-red light (R/FR), or the far-red light alone (FR). The seedlings receiving blue light received either no other light treatment (B), the far-red-light treatment immediately after the blue light (B/F), or the red-light treatment immediately before the blue light (R/B).

suppression observed in response to far-red light alone (far-red light can elicit a VLF phytochrome response) (5).

To determine if the suppression observed in response to blue light is the LF phytochrome response, the blue-light treatment (either 10^1 or 10^4 μmol m^{-2}) was followed immediately by far-red light. The results (Fig. 2) show that far-red light has no effect on the response to blue light. It is probable, therefore, that the suppression observed in response to blue light is due to the specific excitation of a blue-light receptor.

Time Course

It is possible that the blue light fluence response is transient in the dark-grown plants and that by measuring the length of the stem 24 h after the blue-light treatment, the response is missed. If this is the case, suppression in response to high fluences of blue light should occur subsequent to, or more slowly than, suppression in response to low fluences of blue light. The kinetics for suppression of stem elongation in response to 10^4 and 10^1 μmol m^{-2} are shown in Figure 3. The data show that the kinetics for suppression in response to high fluence blue light are identical to those for low fluences; suppression starts between 18 and 24 h after the blue-light treatment. The time at which suppression becomes apparent is similar to that observed for the suppression of the third internode in red-light-grown plants (16).

Reciprocity

To separate the effects of illumination period from total fluence, experiments were conducted in which a blue-light treatment of constant total fluence was delivered over several different time periods. The results (Fig. 4) demonstrate that the Bunsen-Roscoe Law of Reciprocity holds for the response to 10^1 and 10^4 μmol m^{-2}.

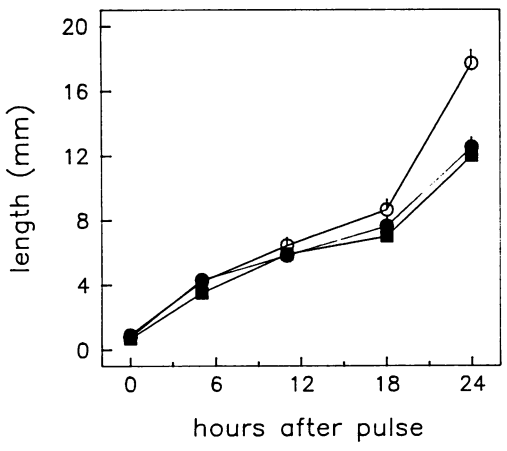

Figure 3. Time course of internode expansion in response to 10^1 and 10^4 μmol m^{-2} of blue light. Seedlings were grown in absolute darkness. Six days after planting seedlings were treated with a single pulse of 10^1 (●) or 10^4 (■) μmol m^{-2} of blue light or a mock pulse (○). Plants were harvested at 0, 5, 11, 18, and 24 h after the blue-light treatment and the length of the third internode was measured. Error bars represent the standard error of the mean.

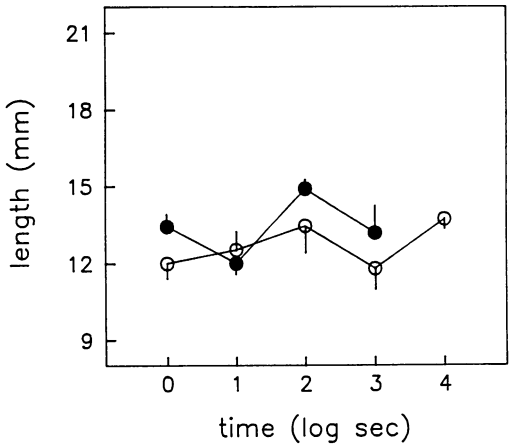

Figure 4. Reciprocity. Seedlings were grown for 7 d in continuous darkness. Six days after planting, separate trays of seedlings were irradiated with a total fluence of 10^1 (○) or 10^4 (●) μmol m^{-2} of blue light delivered over the time intervals as indicated. Twenty-four hours after the blue-light treatment, the seedlings were harvested and the length of the third internode was measured. Error bars represent the standard error of the mean.

Role of Red Light in the Blue High Fluence Response

The blue high fluence response, alleviation of suppressed elongation, is not apparent in the dark-grown plants (Fig. 1), but is apparent in red-light-grown plants (16). Two roles can be envisioned for the red light in allowing the blue high fluence response: (a) to ensure that Pfr is present at the time of high fluence blue-light irradiation or (b) to ensure that the plant be in a specific developmental state at the time of high fluence blue-light irradiation. In light of the fact that red light alone causes suppression of elongation in dark-grown seedlings (Fig. 2), it seems unlikely that the presence of Pfr at the time of high fluence blue-light irradiation is necessary for the blue high fluence response to occur. To test if the role of red light in allowing for the blue high fluence response is to ensure the presence of Pfr at the time of blue high fluence irradiation, dark-grown seedlings were irradiated with a single pulse of red light (10^4 μmol m^{-2}) immediately before a blue-light treatment (10^1 or 10^4 μmol m^{-2}). The red-light pretreatment does not result in alleviation of the suppression in response to the high fluences of blue light (Fig. 2), suggesting that the role of red light is not simply to provide Pfr at the time of blue-light irradiation.

DISCUSSION

Blue-light-induced, long-term suppression of epicotyl elongation in red-light-grown pea has biphasic fluence-response characteristics: suppression occurs with a threshold to fluences of blue light below 10^{-1} μmol m^{-2}, no suppression is apparent at 10^4 μmol m^{-2}. The question arises as to whether there are two distinct blue-light responses, one with a threshold to low fluences of blue light resulting in suppression and a second with a threshold to higher fluences of blue light negating or compensating the LF induced suppression. The data provided in this study indicate that the LF response can exist inde-

pendent of the high fluence response. It is, therefore, probable that there are two distinct responses to blue light.

The blue high fluence response is only observed in red-light-grown plants. The manner by which growth in red light allows the plant to be competent to respond to high fluences of blue light is unknown. By using a pulse of red light immediately prior to the blue-light irradiation, we have discounted the possibility that the blue high fluence response requires the presence of Pfr at the time of blue-light irradiation. The role of red light is, therefore, to allow the plant to achieve a specific developmental state. This specific developmental state may range from the synthesis of a particular molecule in response to phytochrome excitation to the development of a photosynthetically capable leaf.

The specific biochemical mechanisms through which the blue LF and blue high fluence responses function as well as the manner in which the blue high fluence response effects a return to control rates of elongation are unknown. It is possible that the blue high fluence response acts to negate the activity of the blue LF response. For example, if the blue LF response acts to block auxin flow then the high-fluence response may alleviate the block. It is equally possible that the high fluence response acts to compensate for the suppression induced by the LF response. For example, the blue high fluence response may rely on enhanced rates of elongation based on gibberellic acid (known to affect elongation rates in pea) (1) thus compensating for the suppressed elongation rate due to the block in auxin flow. In either case, negation or compensation, it is apparent that the plant is capable of involving the blue high fluence response only after growth in continuous red light.

Blue-light-induced short-term suppression of epicotyl elongation has been demonstrated for several dicot species including pea (12). The response is generally transient and control rates of elongation return within minutes after the blue-light irradiation ceases. The fluence-response characteristics for such a response have been measured in red-light-grown peas (12). The threshold is below 0.5×10^0 μmol m^{-2} blue light and may be representative of a blue LF response.

We have recently demonstrated that the fluence-response characteristics for blue-light-induced changes in the steady-state levels of *Cab* and pEA215 RNA in red-light-grown peas are also bell-shaped (17). The steady-state level of transcript increases at low fluences of blue light and returns to control levels at higher fluences of blue light. Like the bell-shaped response observed for suppression of epicotyl elongation, these data are not explained by the mechanism proposed for blue-light responses to unilateral irradiation (*e.g.* phototropic curvature [2, 3], phototaxis [6], plastid movement [4,15]). It is possible that the biphasic curves for *Cab* and pEA215 RNA levels also represent two distinct blue-light responses, a blue LF response which causes an increase in the steady-state level of these transcripts and a blue high fluence response negating or compensating for the low LF response and causing a return to control levels.

The notion of two, distinct, blue-light responses, one to low fluence and one to high fluence, is consistent with the fluence-response characteristics observed for two other nuclear-coded transcripts, pEA25 RNA and pEA207 RNA, in red-light-grown peas (17). The respective fluence-response curves have only one threshold to blue light, occurring in the low and high fluence ranges, respectively. It is likely, then, that pEA25 RNA level is regulated by the blue LF response only, and that pEA207 RNA level is regulated by the blue high fluence response only. Thus, the two blue-light responses can occur separately even in red-light-grown plants. This is confirmed by the effects of blue-light irradiation on Chl and carotenoid accumulation, which appears to be a blue low fluence response only (16).

Two blue-light photosystems, one operating at low fluences and a second at higher fluences, have been proposed to explain phototropism in *Phycomyces* (9). Excitation of the LF system results in a longer latent period between the first and second component of curvature than does excitation of the high fluence system. The action spectra for the two responses are reported to differ in the near UV region as well. Both responses have thresholds below either of the responses described in this paper.

Although not a specific aim of the study, we have observed a phytochrome response for long-term suppression of epicotyl elongation. A similar phytochrome response has been observed in peas by Galston *et al.* (8) as well as by Cosgrove (7). We have shown that the response is a LF cytochrome response only, there is no VLF component.

To separate the blue high fluence response from the blue LF response, plants were grown in absolute darkness. The proper controls confirm that the blue LF response, in dark-grown plants, is not a phytochrome response, but rather is due to the specific excitation of a blue-light receptor. The use of a red-light preirradiation indicates that the blue high fluence response is not simply due to the presence of Pfr at the time of blue-light irradiation. It is possible, then, that the seedling needs to be in a particular developmental state before it is competent to have a blue high fluence response.

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