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Blue-Light Regulation of Epicotyl Elongation in *Pisum sativum*[1]

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ABSTRACT

Blue light is known to induce suppression of stem elongation. To avoid the complication of blue-light-induced transformation of phytochrome we have adapted the procedure of measuring blue-light-induced suppression of stem elongation in *Pisum sativum* L. var Alaska grown under continuous red light. The resulting fluence-response curve for suppression of epicotyl elongation measured twenty-four hours after a blue-light treatment is bell-shaped, with the peak of suppression between 10^0 and 10^1 micromoles per square meter, and no suppression at 10^4 micromoles per square meter. Suppression is first observed 5 and 11 hours after the blue-light treatment for the fourth and third internodes, respectively. No significant differences in elongation rates were noted for the 10^4 micromoles per square meter treated seedlings throughout the 24 hour period. Reciprocity holds for both third and fourth internodes in response to 10^1 and 10^4 micromoles per square meter of blue light over the range of irradiation times tested (10^0 to 10^4 seconds, 10^1 micromoles per square meter; 10^0 to 10^3 seconds, 10^4 micromoles per square meter). In contrast to the bell-shaped fluence-response obtained for epicotyl elongation, measurements of chlorophyll and carotenoid accumulation indicate increasing accumulation with increasing fluence.

Excitation of the blue-light photoreceptor is necessary for the growth and development of higher plants. Several photomorphogenic effects of blue light have been characterized in higher plants, including the suppression of epicotyl or hypocotyl elongation in dicots (1, 8). The mechanism through which this suppression occurs is unknown, although it is probable that blue light affects the yielding properties of the cell wall, and not the hydraulic conductivity of the growing tissue (9). The biochemical signal for the blue light-induced suppression of growth may involve auxin flow as is proposed for phototropic curvature (3, 5, 16, 19). This latter effect of blue light is thought to occur as a result of the lateral redistribution of auxin away from the irradiated side of the plant resulting in a lessened rate of growth on the irradiated side (5).

Experiments examining blue light-induced suppression of epicotyl/hypocotyl elongation have largely focused on effects occurring soon (*i.e.* within minutes) after a blue-light treatment (8, 9). These short-term effects on elongation rates are often transient, with recovery to the control rate occuring soon (*i.e.* within 2 h) after the blue-light irradiation (10, 11). It is difficult to examine the biochemical mechanisms regulating these short-term, transient effects of blue-light irradiation. A longer-term growth response would facilitate the study of the biochemical mechanisms connecting the blue-light treatment to decreased rates of elongation. In 1981, Cosgrove (8) noted that the rate of epicotyl elongation in etiolated peas is reduced more than 50% as a result of single blue-light pulse. This suppression persisted as long as 6 h after the blue-light pulse.

Most of the previous investigations of blue-light suppression of epicotyl/hypocotyl elongation have not attempted to eliminate the possibility of blue-light excitation of phytochrome. In order to ensure that we are not measuring the results of phytochrome excitation, we have maintained our seedlings in continuous red light prior to, during, and after the blue-light treatment. Growth in continuous red light serves to maintain the phytochrome photodynamic equilibrium. This strategy has been used successfully to saturate phytochrome responses, and is discussed elsewhere (1, 2, 15, 16).

We have examined the effects of short pulses of different fluences of blue light on the rate of epicotyl elongation in peas during the 24 h following the blue-light treatment. We find a bell-shaped fluence-response for inhibition both of third and fourth internodes. In contrast to the bell-shaped fluence-response curve obtained for epicotyl elongation, measurements of Chl and carotenoid accumulation indicate increasing accumulation with increasing fluence.

MATERIALS AND METHODS

Plant Growth Conditions

Seeds of *Pisum sativum* L. var Alaska (J. Mollema and Sons; Grand Rapids Michigan) were imbibed and grown at 21°C in 85% RH in continuous red light (0.25 μmol m^{-2} s^{-1}). Growth in red light is necessary to saturate any phytochrome responses, thus ensuring that any response to the blue-light treatment observed is not a result of phytochrome excitation (1, 15, 16). Seeds were sown on one layer of water-saturated, unbleached Kimpack (Kimberly-Clark; Neenah, WI) and covered with 1.0 cm of Sunshine Seedling Mix No. 3 (Fisons Western Corporation; Vancouver, British Columbia, Canada). Trays used for planting are described elsewhere (1). Plants were not preselected, nor were they transplanted at any time during these experiments; hence, all statistics represent values of real populations.

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Light Sources

Continuous red light was provided by F40/R red fluorescent bulbs (Sylvania, GTE Products; Danvers, MA) covered with a single sheet of Rohm and Haas (Philadelphia, PA) No. 2423 red plexiglass and two sheets of Roscolux 'fire' No. 19 (Rosco; Port Chester, NY). The blue-light source consists of a Hanimex (Brookvale; NSW, Australia) projector with a Corning (Corning Glass; Corning, NY) blue, 2-60 glass filter. Desired blue-light fluences were obtained with the aid of neutral density filters (Balzer; Lichtenstein). Irradiations of 10^0-10^2 s of 10^4 μmol m^{-2} of blue light were provided by a 6 inch Fresnel lamp housing a 750 W quartz bulb (Grand Stage Co., Inc., Chicago, IL), covered with a single sheet of Rohm and Haas (Philadelphia, PA) No. 2424 blue plexiglass and two sheets of Roscolux 'surprise blue' No. 82 (Rosco; Port Chester, NY). Desired blue-light fluences were obtained with the aid of GB001 and GB002 filter paper (Schleicher and Schuell, Keene, NH). A mirror was used to direct the illumination so as to insure bilateral irradiation.

Internode Length Experiments

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, photocopied and the third and fourth internodes measured. Six d after planting, the third internode is approximately 30 mm in length, and the fourth internode has not started to expand.

Time Course Experiments

Trays of seedlings were pulsed with either 10^1 or 10^4 μmol m^{-2} of blue light. Seedlings were harvested 0, 1, 3, 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. These fluences were chosen based on the results of the fluence response experiments. Total fluence of 10^1 μmol m^{-2} irradiations were held constant with the use of neutral density filters (Balzer, Lichtenstein). Irradiation periods tested were 10^0, 10^1, 10^2, 10^3, and 10^4 s. Total fluence of 10^4 μmol m^{-2} was held constant with GB001, and GB002 filter paper (Schleicher and Schuell, Keene, NH). Irradiation periods tested were 10^0, 10^1, 10^2, and 10^3 s.

Pigment Determination

Seedlings were grown in continuous red light and treated with blue light in the same manner as those seedlings used for the epicotyl elongation experiments described above. Twenty-four h after the pulse, tissue apical to the third internode (third node, fourth internode, and plumule) was harvested. Tissue was extracted in 80% acetone with a glass tissue-homogenizer, and the wavelength absorbance determined on a Gilford 260, UV-visible spectrophotometer (Gilford Instruments; Oberlin, OH). Chl were quantified as described by MacKinney (21); total carotenoids were quantified as described by Kirk and Allen (20).

Statistics

All data in epicotyl elongation experiments except the high fluence reciprocity, represent the average of at least three, independent experiments; each experiment had a sample size of 10 seedlings. The high fluence reciprocity represents two independent experiments. All data for pigment experiments represent the average of at least four independent experiments; each experiment had a sample size of four seedlings. Error bars on figures represent the standard error of the mean except the high fluence reciprocity where bars represent standard deviation (standard error of the mean is not valid with two samples). Levels of significance were determined through a standard t test of means obtained through SAS statistical programs (SAS Institute, Inc.; Cary, NC). Average rates of epicotyl elongation were determined from regression statistics also obtained through SAS.

RESULTS

Fluence Response

The lengths of the third and fourth internodes resulting from irradiation with different fluences of blue light are shown in Figure 1. The 10^4 μmol m^{-2} blue-light treatment results in an internode length (both third and fourth) identical to that observed for control seedlings. Fluences between 10^1 and 10^3 μmol m^{-2} result in a suppression of epicotyl elongation. The maximum level of suppression occurs between 10^0 and 10^1 μmol m^{-2} for both third and fourth internodes. The bell-shaped nature of these curves is similar to those previously reported for blue light-induced phototropic curvature of peas grown in red light (1). However, the threshold and saturation levels differ between these two blue light-induced phenomena.

Time Course Experiments

The 10^4 μmol m^{-2} fluence evokes little or no suppression of epicotyl elongation when measured 24 h after the blue-light treatment. Since it is possible that transient suppression is occurring before the 24 h time point, internode length was measured 0, 1, 3, 5, 11, 18, and 24 h after the blue-light treatment. The results are shown in Figure 2. No significant differences between blue-light-treated and control plants were observed for either internode over the 24 h period indicating that a transient response is not occurring.

The results of the 10^1 μmol m^{-2} time course are shown in Figure 3. As expected, the third and fourth internodes of the blue-light-treated plants show a slower average rate of elongation when compared to control seedlings. However, the time at which suppression of elongation first becomes apparent differs between internodes. Suppression of the fourth internode starts approximately 5 h after the blue-light pulse, while suppression of the third internode does not start until 11 h after the blue-light pulse. The average rates of elongation after the onset of suppression for the third and fourth internodes are 0.4% and 55%, respectively.

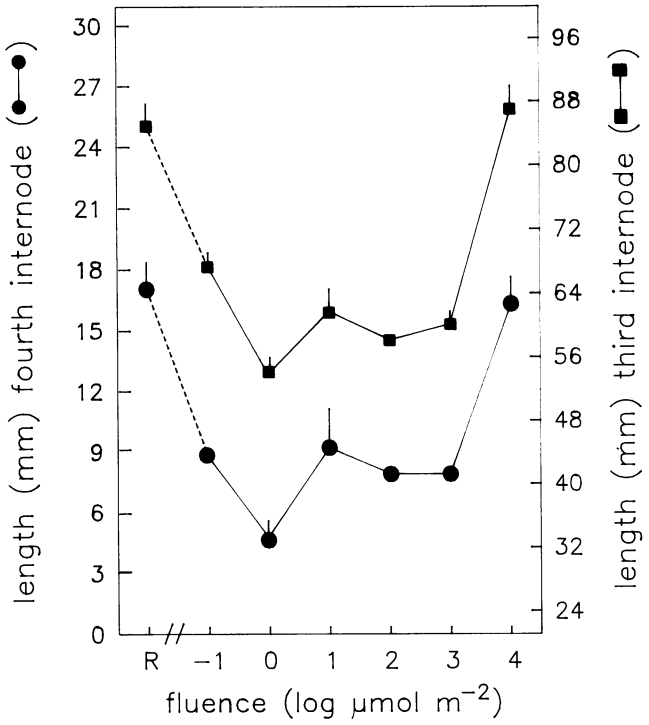

Figure 1. Blue-light fluence-response-curves for epicotyl elongation in pea. Seedlings were grown for 7 d in continuous red light (0.25 μmol m^{-2} s^{-1}). Six d after planting, separate trays of seedlings were irradiated with a single pulse of blue light, with the fluences indicated on the figure. 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 h after the blue-light treatment, seedlings were harvested and the third (■) and fourth (●) internode lengths were measured. Control seedlings (R) are treated only with continuous red light. Error bars represent the standard error of the mean.

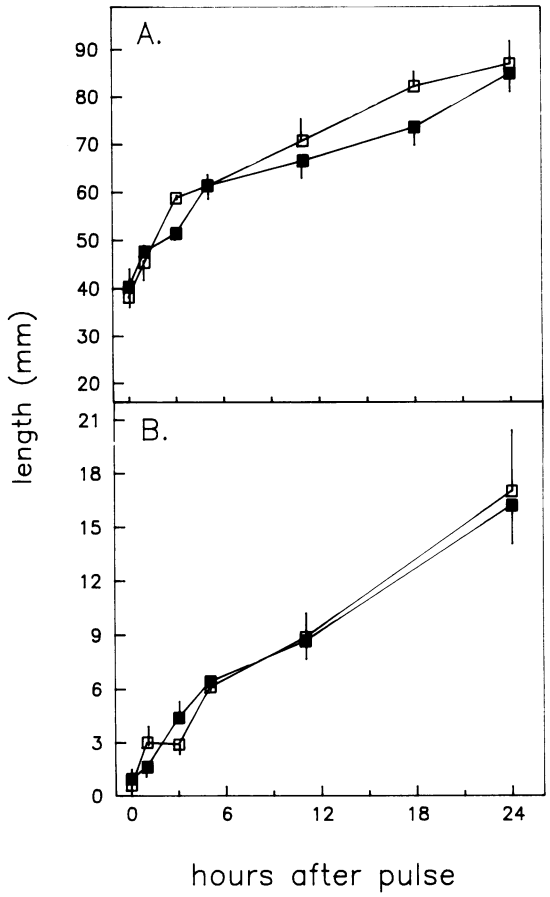

Figure 2. Time course of internode expansion in response to 10^{4} μmol m^{-2} of blue light. Seedlings were grown in continuous red light (0.25 μmol m^{-2} s^{-1}). Six d after planting, seedlings were treated with a single pulse of blue light with a fluence of 10^{4} μmol m^{-2} and duration of 10^{3} s. Plants were harvested at 0, 1, 3, 5, 11, 18, and 24 h after the blue-light treatment, and the third (A) and fourth (B) internodes were measured. Error bars represent the standard error of the mean. Closed squares represent blue-light treated seedlings; open squares represent control seedlings.

Reciprocity Experiments

To separate the effects of illumination period from total fluence, we conducted experiments in which a blue-light treatment of constant total fluence was delivered over different time periods. The results of these experiments can be seen in Figure 4. The results demonstrate that the Bunsen-Roscoe Law of Reciprocity holds for the response to 10^{1} and 10^{4} μmol m^{-2} for either internode over the range of irradiation times tested.

Pigment Accumulation

As a comparative study, we wanted to measure the effects of the same blue-light treatments on a physiological parameter occurring primarily in the developing leaves. In these experiments we measured pigment accumulation in tissue apical to the third internode. Seedlings were grown in continuous red light and treated with blue light as described for the epicotyl expansion experiments.

The results of the total Chl determinations are shown in Figure 5. In contrast to the fluence-response data for internode elongation, plants treated with 10^{4} μmol m^{-2} are not comparable to control seedlings. Greater accumulation of total Chl is observed in response to increasing fluences of blue light, with a threshold below 10^{-1} and saturation above 10^{4} μmol m^{-2}. The difference in Chl *b* content between blue-light-treated and control seedlings is not significant, and is approximately 1.0 μg/plumule. As a consequence, Chl *a*/*b* ratios change as a function of Chl *a* level. The data for total carotenoid accumulation are similar to those for total Chl (Fig. 5).

DISCUSSION

The mechanism by which blue-light induces suppression of internode elongation is unknown. In general, blue-light-induced suppression of stem elongation starts immediately upon irradiation with normal elongation rates resuming shortly thereafter (*e.g.* 10). It is known that the excitation of phytochrome can result in suppression of epicotyl/hypocotyl/mesocotyl elongation (8, 12–14, 17, 22). The phytochrome-mediated response is stable over a much longer period than the blue-light responses. For example, Galston *et al.* (12) observed a red-light-induced suppression of pea epicotyl elongation. Suppression was not apparent until 6 h after the red-light pulse and continued for at least 12 h. Cosgrove (8), also

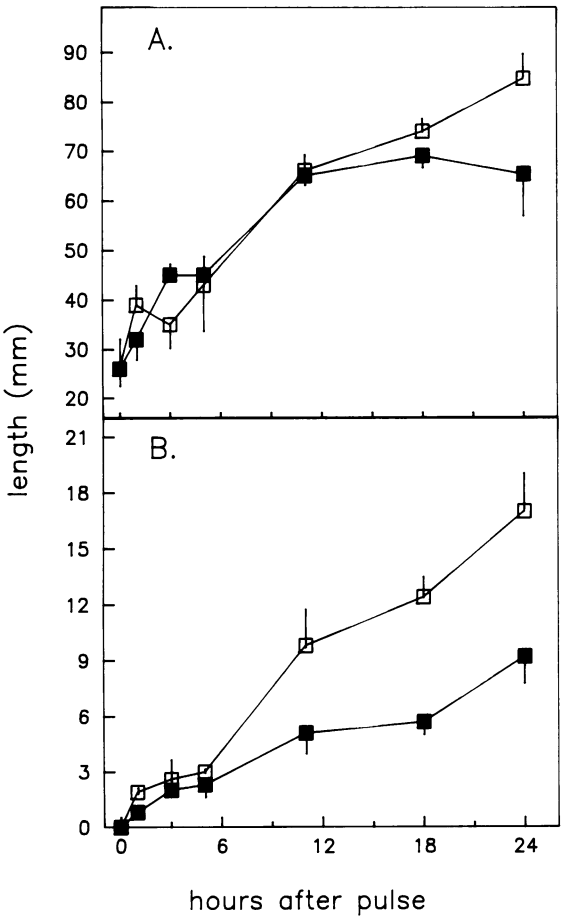

Figure 3. Time course of internode expansion in response to 10^1 μmol m^{-2} of blue light. Seedlings were grown in continuous red light (0.25 μmol m^{-2} s^{-1}). Six d after planting, seedlings were treated with a single pulse of blue light with a fluence of 10^1 μmol m^{-2}, and duration of 10^1 s. Plants were harvested at 0, 1, 3, 5, 11, 18, and 24 h after the blue-light treatment, and the third (A) and fourth (B) internodes were measured. Error bars represent the standard error of the mean. Closed squares represent blue-light treated seedlings; open squares represent control seedlings.

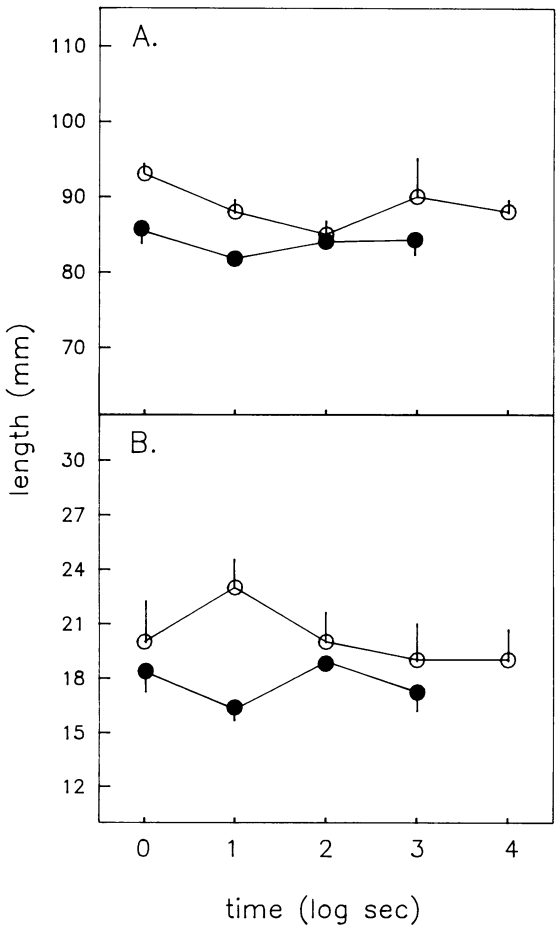

Figure 4. Reciprocity. Seedlings were grown for 7 d in continuous red light (0.25 μmol m^{-2} s^{-1}). Six d after planting, separate trays of seedlings were irradiated with a total fluence of 10^1 (○) or 10^4 (●) μmol m^{-2} of blue light delivered over the time intervals as indicated in the figure. Twenty-four h after the blue-light treatment, the seedlings were harvested and the length of the third (A) and fourth (B) internodes were measured. Error bars for 10^1 μmol m^{-2} represent the standard error of the mean; error bars for 10^4 μmol m^{-2} represent standard deviation.

working with pea, observed that a single pulse of red light suppressed the elongation of the pea epicotyl for at least 6 h. To avoid this complication, we have grown peas in continuous red light (for discussion, see Refs. 1, 15, 16).

Any proposed mechanism for the blue-light-induced suppression would have to account for the bell-shaped fluence-response curve (Fig. 1). It is likely that two antagonistic responses are occurring. For example, at both lower and higher fluences, there may be a decrease in the flow of auxin, with a consequent reduction in epicotyl elongation. At higher fluences, a second mechanism, such as an increase in the rate of cell division with subsequent elongation of the daughter cells, may also be occurring. The elongation rate, observed at high fluences, as a result of the two mechanisms would be similar to control plants. It is known that high fluences of blue light result in increased cell division rates for fern protonema (23, 24).

The suggestion of two antagonistic mechanisms, one of which is limited to the stem, would fit well with the fluence-response data for Chl and carotenoid content. These parameters do not show a bell-shaped or biphasic fluence-response curve over the range of fluences examined and would seem therefore to be under the control of a single mechanism. We have no data to indicate if pigment accumulation would be controlled by the same mechanism as the suppression of epicotyl elongation, although economy of mechanisms would dictate such a hypothesis. It is, however, difficult to envision auxin having such a profound effect on the level of what are, for the most part, components of the plumule, as the reported major auxin source resides just basal to the apical bud (6).

The occurrence of a bell-shaped fluence-response curve for blue-light-induced phenomena has been observed for chloroplast movements in *Vaucheria* (4, 25), phototropism in *Euglena* (7), phototropic curvature in fungi (18), as well as for phototrophic curvature in pea, maize, barley, and mung bean grown under continuous red-light conditions (1, 2). It is possible that the fluence-response curve observed for suppression of epicotyl elongation and bell-shaped fluence-response curves in general are the product of two antagonistic processes.

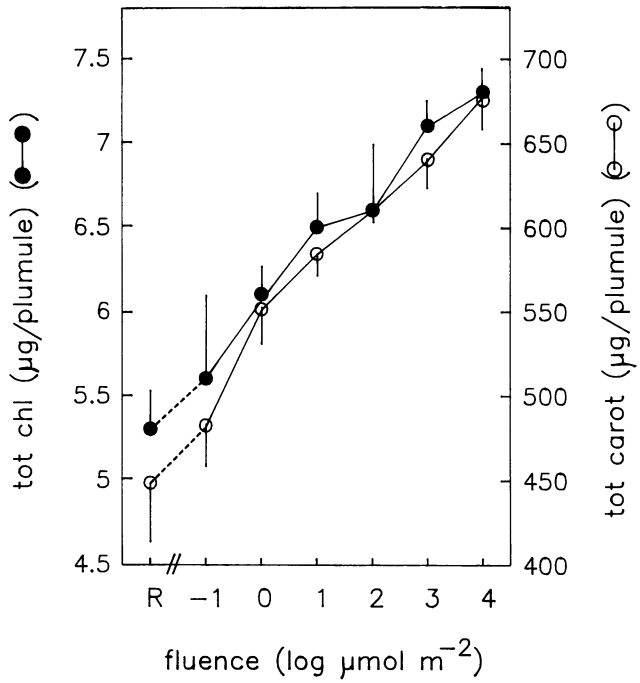

Figure 5. Blue-light fluence-response curves for total Chl and carotenoids. Seedlings were grown and irradiated as described in Figure 1. Twenty-four h after the blue-light treatment, tissue apical to the third internode was harvested, extracted, and quantified for total Chl (●) and total carotenoids (○). Control seedlings (R) are treated only with continuous red light. Error bars represent the standard error of the mean.

If two different processes are involved, one might expect that the action spectrum for each response might be different. In the one case where action spectra have been measured, phototropic curvature of red-light-grown alfalfa, the action spectra for the ascending and descending sides of the fluence response curve are identical (2).

In this paper we report on a stable, long-term, blue-light-induced suppression of epicotyl elongation in red-light-grown pea seedlings. The growth conditions employed in our experiments ensure that there is no blue-light excitation of phytochrome. Hence, the blue-light effects described herein are probably due to excitation of a blue-light photoreceptor. We hope to use this protocol to study the biochemical mechanism(s) responsible for the blue-light-induced changes in the rate of epicotyl elongation in pea.

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