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Stimulation of Growth and Ion Uptake in Bean Leaves by Red and Blue Light[1]

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ABSTRACT

Red and blue light both stimulate growth and ion accumulation in bean (*Phaseolus vulgaris* L.) leaves, and previous studies showed that the growth response is mediated by phytochrome and a blue-light receptor. Results of this study confirm that there is an additional photosynthetic contribution from the growing cells that supports ion uptake and growth. Disc expansion in the light was enhanced by exogenous K^+ and Rb^+, but was not specific for anions. Light increased K^+ accumulation and the rate of $^{86}Rb^+$ uptake by discs, over darkness, with no effect of light quality. The photosynthetic inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, inhibited light-driven $^{86}Rb^+$ uptake by 75%. Light quality caused differences in short-term kinetics of growth and acidification of the leaf surface. At comparable fluence rates (50 μmol m^{-2} s^{-1}), continuous exposure to blue light increased the growth rate 3-fold after a 2-min lag, whereas red light caused a smaller growth response after a lag of 12 min. In contrast, the acidification of the leaf surface normally associated with growth was stimulated 3-fold by red light but only slightly (1.3-fold) by blue light. This result shows that, in addition to acidification caused by red light, a second mechanism specifically stimulated by blue light is normally functioning in light-driven leaf growth.

The expansion of dicotyledonous leaves is stimulated by light acting photomorphogenically through at least two photoreceptors, phytochrome and a blue-light receptor (for review, see refs. 9 and 14). For example, in primary leaves of bean (*Phaseolus vulgaris* L.), red and blue light both stimulate leaf expansion, but simultaneous exposure to far-red light significantly reduces growth only in red, but not blue, light (53). Photosynthesis is not necessary for light-stimulated growth (10, 52) but may enhance growth (10, 23, 52) and is known to be necessary for some light-driven transport processes (11, 27, 41, 46). The stimulatory effect of light on growing leaves includes cell wall loosening and solute uptake for turgor maintenance (9). Cell expansion depends on acidification of the apoplast (50) and on acid-induced wall loosening (50, 54). Acidification may also be associated with the uptake of sugars and salts that is necessary for osmotic regulation by growing cells (8, 15, 32, 45, 51). Solute uptake by plant cells is facilitated by active pumping of protons out of the cells, setting up a proton motive force that is used to drive uptake of sugars and ions (38).

The mechanisms underlying light-driven leaf growth were addressed in this study. The photosynthetic dependence of K^+ uptake associated with growth was investigated by measuring the effect of DCMU, an inhibitor of PSII, on accumulation of K^+ and uptake of $^{86}Rb^+$ by leaf discs. The stimulation of K^+ and Rb^+ uptake by light has been observed in *Nitella* (28), *Vallisneria* (39), corn (*Zea mays*) (40), green bean (*Phaseolus vulgaris*) (19), pea (*Pisum sativum*) (34), and broad bean (*Vicia faba*) (18). Methods for studying ion uptake have included using whole cells, leaf slices and fragments, submerged leaves, and protoplasts. A comparison of ion uptake into expanding and mature leaves of *V. faba* showed that light stimulates K^+ uptake over darkness in expanding leaves, whereas in older leaves there is a net efflux in darkness (18). When inhibitors have been used to determine the metabolic basis for ion uptake, the results have been contradictory. For mature leaves, the percentage of light-driven Rb^+ uptake that is inhibited by DCMU has been reported from 0% in corn leaf slices (40) to approximately 80% in *P. sativum* mesophyll protoplasts (24), which is similar to reports for leaf fragments of *P. sativum* (34), *Vallisneria* (39), and the present results. For growing leaf discs of *P. vulgaris*, we measured the effect of light quality on DCMU-insensitive K^+ uptake in an effort to determine whether phytochrome or the blue-light photoreceptor preferentially stimulate this transport process. The separate responses to red and blue light previously reported for disc expansion (53) were also determined by measuring the effect of light quality on the kinetics of growth and apoplast acidification by intact leaves. An unexpected result was that the magnitude of apoplast acidification caused by light was negatively correlated with growth rate in the short term. This result is discussed with regard to the dependence of leaf growth on apoplast acidification and in context of the several photosystems regulating leaf expansion.

MATERIALS AND METHODS

Plant Material

Bean seedlings (*Phaseolus vulgaris* L. cv Contender; Olds Seed Co.) were grown in moist vermiculite for 10 to 11 d in continuous dim red light (4 μmol photons m^{-2} s^{-1}) at 25°C in a controlled environment growth chamber. At this stage all cell division in the primary leaves had ceased, and further growth was attributed to cell expansion (49).

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Growth Measurements

Discs (7.5 mm in diameter) were excised from primary bean leaves in dim red light and floated adaxial side down on solutions under different light conditions. Disc expansion was measured with a finely graduated ruler (0.02 inches = 0.51 mm) under a dissecting microscope. The growth medium for disc expansion was 10 mM KCl, 10 mM sucrose, and ± 10^{-5} M DCMU. The kinetics of the growth responses to light were measured by attaching intact leaves to position transducers (rotary variable position transducer, RVDT 30D, Schaevitz; 31) and monitoring the change in leaf length over time with a chart recorder.

Light Sources

Low fluence red light was provided from a red fluorescent tube (Sylvania) covered with red plastic (660:730 nm = 1). White light was provided by cool-white fluorescent tubes (Sylvania) or by halogen projector lamps (EKE 150 W, 21 V; Apollo) in fiberoptic light sources (T-Q/FOI-1; Techni-Quip Co.). High fluence blue light was obtained by filtering this light through a 10% copper sulfate solution, and high fluence red light was produced by using either an interference filter (35–4019:660 nm [bandwidth 11.6 nm] Ealing Electro-Optics), or red plastic (660:730 nm = 1). Fluence rates were measured with a quantum meter (Li-Cor, Inc.)

Temperature Control

The temperature for all treatments was between 25 and 28°C with differences among samples in any one experiment maintained at <1.5°C, monitored with a thermocouple placed directly in the incubation chambers or on the intact leaf. Temperature was controlled by placing sample chambers in a water bath (excised tissue) or passing the light through a water filter (intact leaves). Some long-term experiments were performed in a temperature-controlled growth chamber.

K^+ Accumulation

K^+ content of leaf discs was measured by two methods: ion selective electrodes (K^+ electrode, TIPK; World Precision Instruments, Inc., New Haven, CT) and atomic absorption spectroscopy (Perkin-Elmer model 303). For K electrodes, sap was expressed from frozen-thawed leaf discs and [K^+] was determined from calibration lines constructed from known concentrations of KCl in water. For atomic absorption spectroscopy, leaf discs (22 discs per treatment; approximately 25 mg) were dried, weighed to 0.1 mg (Mettler AE240), and digested in concentrated nitric acid, and the extract was diluted in a solution of 144 mM La_2O_3 and 75 mM CsCl in 2.4 N HCl. Expressed sap was diluted in the same La/Cs solution and measured by atomic absorption for both light and dark treatments. The changes in K concentration were detected at similar magnitudes by both extraction techniques. In all K accumulation experiments, incubation solutions were 10 mM KCl, and some included 10 mM sucrose and 10^{-5} M DCMU.

$^{86}Rb^+$ Uptake Experiments

Excised discs were cut three times across 75% of the diameter of the disc to increase exposure of the apoplast and facilitate uptake and then preincubated in the dark in the same concentration of RbCl used in the experiment, with or without 10^{-5} M DCMU and 10^{-5} M CCCP[3]. After 45 min, discs were transferred to solutions containing $^{86}Rb^+$ (specific activity 3.3 or 10 kBq/mL in short- and long-term experiments, respectively) and RbCl (0.1 to 10 mM), with or without 10^{-5} M DCMU, 10^{-5} M CCCP, and 10 mM sucrose. After 30 min or 16 h, discs were blotted, rinsed for 1 min, and transferred to a second rinse for 10 min. Rinse solutions were on ice, rotated continuously, and at the same concentration of RbCl as that in the treatment. Discs (18 discs per treatment, divided into six groups of 3 each) were blotted after rinsing, and $^{86}Rb^+$ was counted by Cerenkov radiation. The initial ^{86}Rb in the treatment solutions was also counted by Cerenkov radiation and used to calculate Rb uptake per disc.

Leaf Surface Acidification

Leaves were excised, and the lower surface was lightly abraded with fine sand (grade M180; American Optical) that was carefully brushed off before mounting the leaf on a glass slide on top of moistened filter paper in a Petri dish. A combination pH electrode (Ingold 403-30-M5) was placed lightly on the abraded leaf surface and moistened with 20 μL of 1 mM KCl (similar results were obtained with 0.1 mM KCl, 1 mM choline chloride, and 1 mM KCl plus 2.5 mM Mes-Tris [pH 6.5]). The pH of the leaf surface was recorded over time for a preincubation period in low fluence red light (giving an initial rate of acidification) and subsequently after exposure to high-fluence blue or red light (giving a lag time and final rate of acidification).

Statistical analysis of the results was performed using Student's *t* test with unpaired data. Significant differences between means at the 95% confidence limit are noted in the legends of figures and tables. All experiments were performed three or more times on different days.

RESULTS

The requirement for light by growing bean leaves is evident in intact leaves as well as discs, whether or not photosynthesis is active (10, 14, 49, 52). The physiological mechanisms through which light acts were investigated both in photosynthetically active and inhibited leaf discs exposed to white, red, and blue light, during long (16–24 h) and short (30–60 min) periods.

Long-Term Experiments

Effects of White Light on Growth and Ion Uptake

White light (100 μmol m^{-2} s^{-1}) stimulated disc expansion compared with darkness over 16 h when discs were excised from primary bean leaves and floated on a solution contain-

[3] Abbreviation: CCCP: carbonyl cyanide *m*-chlorophenyl hydrazone.

ing 10 mM KCl (Fig. 1). The light-stimulated increase in disc diameter was inhibited (60%) by 10^{-5} M DCMU. Addition of 10 mM sucrose to the medium stimulated growth in darkness and in light and relieved, but did not eliminate, the inhibition by DCMU (25%). A similar result was shown previously (table I in ref. 52) but is not apparent in the action spectrum for light-stimulated expansion of bean leaf discs (figure 4 in ref. 53) because of the normalization of the data. The earlier conclusion that photosynthesis is not necessary for growth is correct, but results in this paper and elsewhere (9, 23) make clear that in growing green leaves some photosynthetic products of the growing cells themselves augment the growth response of leaves to light.

Expansion of discs was observed when they were floated on dilute buffer and was enhanced by exogenous salts (Table I). The most effective cations were Rb^+ and K^+; Li^+ and NH_4^+ reduced or eliminated light-stimulated growth. On the other hand, all anions tested supported light-driven disc expansion to a similar extent.

Measurements of K content in leaf discs showed trends in K accumulation stimulated by light (Table II). Initially, leaf discs contained 28.3 μg/g of dry weight K; after 6 h of incubation on 10 mM KCl, discs in the light contained more K compared with discs in the dark, and a trend toward increased [K] was observed for all light treatments (Table II). The accumulation of K was observed even when growth was osmotically prevented by exogenous mannitol. For discs treated as in the long-term growth experiments (Fig. 1), light-driven K accumulation was partially inhibited (30%) by DCMU (data not shown). Addition of sucrose (10 mM) to the incubation medium had no effect on accumulation of K in the dark or light but partially alleviated the inhibition by DCMU (20%).

Light increased the rate of $^{86}Rb^+$ uptake by discs compared with darkness when discs were incubated in solutions with

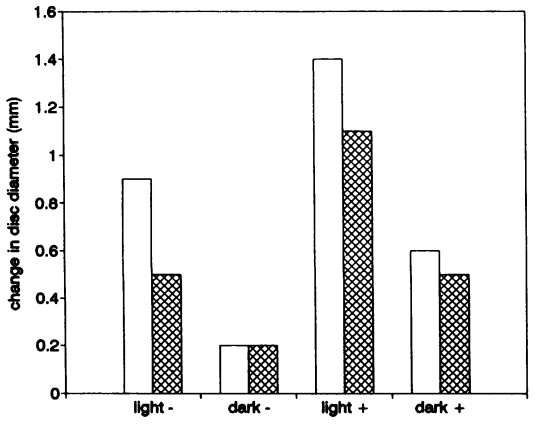

Figure 1. Comparison of the effects of DCMU and exogenous sucrose on disc expansion in light and darkness. Discs (7.5 mm) were excised from primary bean (*P. vulgaris*) leaves grown for 10 d in low-fluence red light (4 μmol m^{-2} s^{-1}) and were floated for 16 h on 10 mM KCl, without (open bars) or with (hatched bars) 10^{-5} M DCMU, in white light (100 μmol m^{-2} s^{-1}) or darkness, without (−) or with (+) 10 mM sucrose. SE <0.1 mm (n = 25–30). All means were significantly different (>0.05%) except for dark (±DCMU).

Table I. *Ion Specificity for Disc Expansion*

Leaf discs (7.5 mm) were floated on 2.5 mM Mes-Tris (pH 6.0) with 10 mmol salt in white light (100 μmol m^{-2} s^{-1}) or darkness for 18 h. SE for final areas were <1% of the mean (n = 20). Differences between the means for increase in disc area in light are significant at the 95% confidence limit for buffer alone compared to all K salt treatments.

Incubation Solution	Increase in Disc Area		
	Dark	Light	Light/dark
		mm^2	
Buffer alone	4.39	10.9	2.5
KCl	6.88	22.7	3.3
KI	5.32	17.2	3.2
KNO_3	5.82	23.5	4.0
K_2SO_4	4.72	18.4	3.9
RbCl	4.0	19.5	4.9
NaCl	4.46	14.5	3.2
LiCl	6.42	10.4	1.6
NH_4Cl	7.57	6.3	0.83

RbCl concentrations ranging from 0.1 to 10 mM. Light-driven $^{86}Rb^+$ uptake was more apparent at 0.1 mM (50% stimulation over uptake rate in the dark) than at the higher concentrations and was completely inhibited by 10^{-5} M CCCP in the medium (data not shown). The effect of light on $^{86}Rb^+$ uptake during 16 h (Fig. 2) was similar to the result described above for K accumulation (Table II). Light stimulated ion uptake over darkness, and this stimulation was inhibited 75% by DCMU. Addition of sucrose to the medium partially alleviated the inhibition by DCMU (60%) and slightly stimulated ion uptake in the dark.

Among all treatments, a strong correlation existed between the amount of disc growth that occurred and K accumulation/$^{86}Rb^+$ uptake. Because an expanding leaf cell must continually accumulate solutes to maintain its osmotic concentration during increases in volume, it is likely that leaf discs utilize exogenous K^+ for this purpose (51). Two exceptions to the correlation were (a) addition of sucrose stimulated disc expansion (Fig. 1) but did not stimulate K accumulation (not shown) or $^{86}Rb^+$ uptake in the light (−DCMU, Fig. 2) and (b) K accumulation was stimulated by light even when growth was osmotically prevented (Table II). In the first case, the availability of exogenous sucrose as an osmoticum may have reduced K^+ uptake, showing that K^+ uptake is not obligatorily associated with growth. The second result shows that light-driven K^+ uptake is a photoresponse that can be separated from the growth response. In addition, external osmotica increased K^+ content and concentration in the absence of light, probably via a mechanism for turgor adjustment (5, 26).

Effect of Light Quality

To distinguish the physiological responses of growing leaf cells to red and blue light, disc expansion experiments were performed in white, red, and blue light over a range of fluence rates. In all three, light qualities >100 μmol m^{-2} s^{-1} were required to saturate the light response (Fig. 3 and data not

shown). Although the extent of the inhibition of growth by DCMU varied for the three light qualities (30% in white light, slightly greater in red, less in blue), the long-term growth responses to red and blue light were not distinguishable with respect to fluence rate dependence or contribution of photosynthesis. Similarly, no difference in K concentration changes was found in responses to red and blue light whether or not growth was prevented osmotically by mannitol (Table II).

However, a difference between the responses to light quality was observed for the concentration of exogenous KCl necessary to promote maximal light-stimulated disc expansion. In blue light, addition of 1 mM KCl was sufficient for approximately 95% of the maximum growth response, whereas in red light >5 mM KCl was required to achieve the same enhancement of growth (Fig. 4).

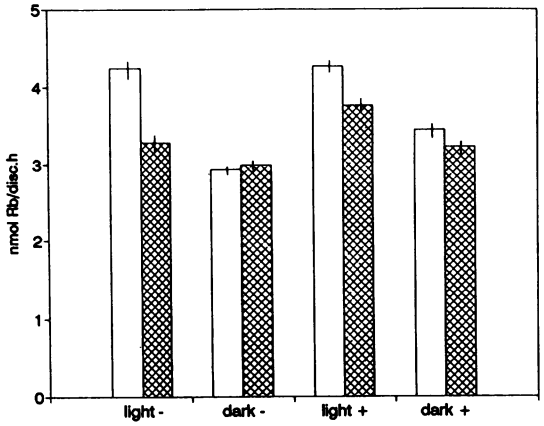

Figure 2. Comparison of the effects of DCMU and exogenous sucrose on $^{86}Rb^+$ uptake by leaf discs in light and darkness. Discs were floated for 16 h on 0.1 mM RbCl without (open bars) or with (hatched bars) 10^{-5} M DCMU in white light (100 μmol m^{-2} s^{-1}) or darkness, without (−) or with (+) 10 mM sucrose. SE is indicated (−DCMU, n = 18; +DCMU, n = 12). Treatments ± DCMU in the light are significantly different (>0.05%).

Short-Term (30 min) Experiments

Effect of Light Quality

The stimulation of $^{86}Rb^+$ uptake caused by light could be observed during short time periods (30 min) (Fig. 5), and as reported above for long-term K accumulation and $^{86}Rb^+$ uptake, there was no effect of light quality on short-term, light-stimulated $^{86}Rb^+$ uptake or on the inhibition of the uptake by DCMU (75%).

However, red and blue light had very different effects on the kinetics of both growth and surface acidification in response to illumination (Fig. 6, Tables III and IV). For intact leaves, blue light (50 μmol m^{-2} s^{-1}) stimulated leaf extension after a short lag period (approximately 2 min) (Table III). When exposed to white light, leaves continued to grow at a slow rate for a longer lag period (approximately 10 min) before responding with an increased rate of growth. Exposure to red light slowed growth for a similar lag (12 min) before stimulating growth. The rate of leaf extension was nearly twice as fast in blue as in red or white light (at 50 μmol m^{-2} s^{-1}). Light also stimulated acidification of the leaf surface (50, 54) but with kinetics dependent on light quality (Table IV). Exposure to red light (50 μmol m^{-2} s^{-1}) at first caused a rapid alkalinization of the leaf surface that lasted about 15 min and was probably due to CO_2 uptake. Subsequently, the rate of surface acidification in red light was double the initial rate.

Table II. *Effect of Mannitol on [K] of Leaf Discs Incubated in Light or Darkness*

Leaf discs (7.5 mm) were excised and floated on 10 mM KCl ± mannitol (200 mmol/kg) in light (100 μmol m^{-2} s^{-1}) or darkness. The [K] was determined by K electrode, and K content was determined by atomic absorption spectroscopy. Numbers in parentheses, SE. For disc diameter, n = 10 to 30; for K content, n = 3.

Light Treatment	Incubation Medium (± mannitol)	Disc Diameter		[K]		K Content	
		Light	Dark	Light	Dark	Light	Dark
		mm		*mM*		*μg/g dry wt × 1000*	
Red light (6 h)	−	8.0 (0.07)	7.6 (0.03)	101	84		
	+	7.6 (0.07)	7.6 (0.03	109	98		
Blue light (6 h)	−	7.9 (0.07)	7.6 (0.03)	101	84		
	+	7.7 (0.03)	7.6 (0.03)	113	98		
White light (12 h)	−	8.6 (0.03)	7.7 (0.04)	107	89		
	+	7.4 (0.02)	7.4 (0.04)	118	98		
White light (6 h)	−	7.9 (0.03)	7.7 (0.02)			33.7 (1.8)	28.3 (1.4)
	+	7.5 (0.03)	7.4 (0.02)			34.4 (2.2)	31.0 (0.8)

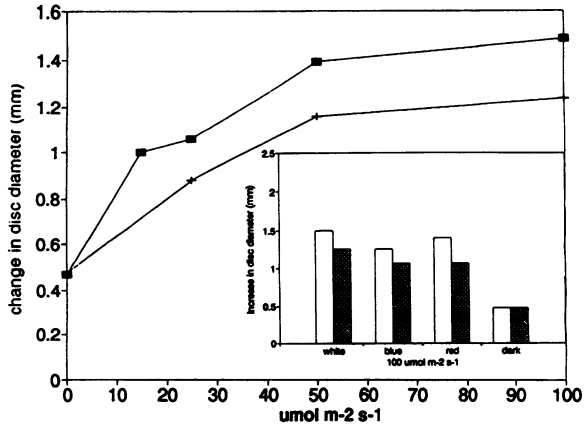

Figure 3. Stimulation of leaf disc expansion by white, blue, and red light. Discs were floated for 16 h in sucrose and KCl (10 mM) ± 10^{-5} M DCMU. Fluence rate dependence for disc expansion in white light is shown. Similar data were obtained for blue and red light (not shown). Inset, Growth of discs in 100 μmol m^{-2} s^{-1} light is shown for the three light qualities. Open bars, −DCMU; hatched bars, +DCMU. SE for all points <0.1 (n = 20). Significance >0.05% for light versus dark and ±DCMU in the light.

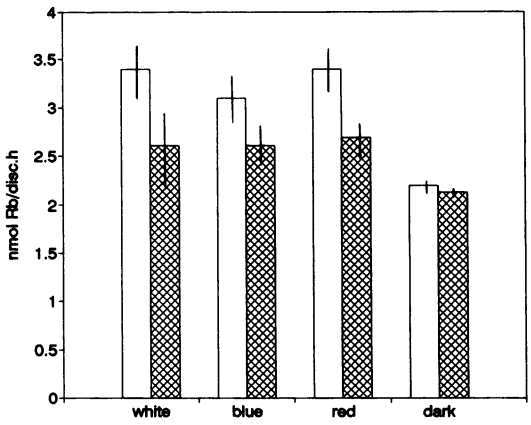

Figure 5. Short-term uptake of $^{86}Rb^+$ by leaf discs. Discs were preincubated in darkness for 45 min in 0.1 mM RbCl and then exposed to light in the presence of $^{86}Rb^+$ in 0.1 mM RbCl and ± 10^{-5} M DCMU for 30 min. Open bars, −DCMU; hatched bars, +DCMU. SE is indicated (n = 3). Means in all light treatments are different (>0.05%) for ±DCMU and dark.

Blue light at the same fluence rate caused a minor alkalinization, followed by a slightly increased rate of acidification.

DISCUSSION

Combined action of photoreceptor systems is known for many physiological responses of plants, including anthocyanin synthesis (33), starch breakdown and sugar production (37, 43), inhibition of stem elongation (7, 13, 17), leaflet opening in legumes (12, 44), and stomatal opening (1, 35). The photoreceptor systems are driven by excitation of phytochrome (absorbing red, far-red, and blue light), blue-light receptors (absorbing blue and UV light), and Chl (absorbing blue and red light). Identification of the mechanisms through which each photoreceptor acts is complex (30) and is as yet unclear for light-driven leaf growth.

In etiolated seedlings, red and blue light inhibit stem elongation by exciting phytochrome and a blue-light receptor. Compelling evidence for their separate action rests in differ-

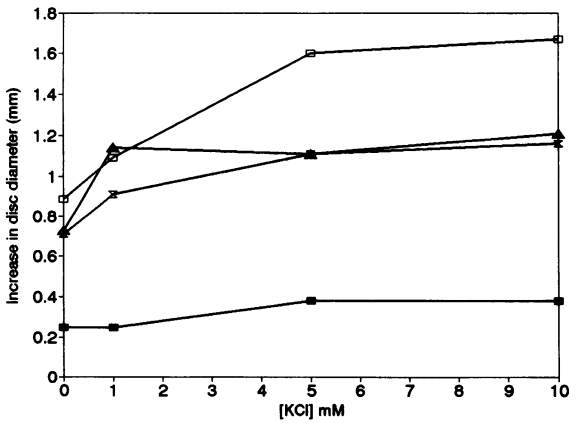

Figure 4. Dependence of light-stimulated growth on exogenous [K]. Discs were incubated on water or 1, 5, or 10 mM KCl (without sucrose) for 12 h, in darkness (■), white (250 μmol m^{-2} s^{-1}; □), red (150 μmol m^{-2} s^{-1}; ⧗), or blue (150 μmol m^{-2} s^{-1}; ▲) light. SE <0.1 (n = 10 or more). Means for increases in disc diameter in 1 mM KCl are different (>0.05%) for red versus blue light.

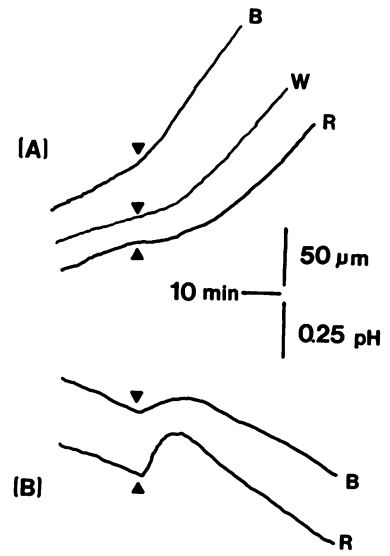

Figure 6. A, Short-term kinetics of the growth response of intact leaves to light. Primary leaves of bean plants growing in low-fluence red light (4 μmol m^{-2} s^{-1}) were attached to position transducers, and the elongation of the leaves was monitored continuously. At the arrows, leaves were exposed to 50 μmol m^{-2} s^{-1} blue, white, or red light. B, Short-term kinetics of changes in leaf surface pH in response to light. The lower surface of a detached leaf was lightly abraded and placed under a combination pH electrode moistened with 20 μL of 1 mM KCl. Light treatments were as in A. Statistical treatment of these responses is shown in Tables III and IV.

Table III. *Kinetics of the Growth Response of Intact Leaves to Light*

Leaves on intact plants were attached to position transducers, and elongation was measured as shown in Figure 6. Initial growth rate was determined for leaves exposed to low-fluence (4 μmol m^{-2} s^{-1}) red light, the lag is the time between exposure to brighter light and the initiation of a stimulated growth rate, and final growth rate was averaged over 30 min directly after the lag period. Numbers in parentheses, SE.

Fluence Rate	Initial Growth Rate	Lag	Final Growth Rate
μmol m^{-2} s^{-1}	mm/h	min	mm/h
White light			
300 (n = 14)	0.14	11.7	0.35
	(0.03)	(2.3)	(0.02)
100 (n = 16)	0.22	7.3	0.36
	(0.02)	(1.3)	(0.04)
50 (n = 9)	(0.09	10.6	0.14
	(0.02)	(1.3)	(0.02)
Blue light			
50 (n = 9)	0.11	2.1	0.30
	(0.02)	(0.9)	(0.05)
Red light			
50 (n = 5)	0.10	12.4	0.19
	(0.02)	(1.1)	(0.03)

ences in the kinetics of the growth response, with blue light acting more quickly (min) than red light (h) (7, 13, 17). Analysis of mutants deficient in phytochrome has also supported the separate role of a blue-light receptor in this response (4, 22, 25, 36). The mechanism for each photoresponse is unknown, although reduction of wall extensibility in both red and blue light has been measured (21). The response to blue light includes a rapid depolarization of cell membrane potential (47). In green tissues, on the other hand, light also excites Chl, which may provide energetic or informational input to physiological processes. For example, guard cells swell in response to red light acting photosynthetically and to blue light acting through a blue-light receptor. Both photoreceptor systems stimulate proton efflux (2, 46), K^+ uptake (see ref. 29 for review), and sugar synthesis (37). A role for phytochrome has also been proposed for red light-driven stomatal responses (16, 42, and see ref. 20).

For tissues containing Chl, a way to distinguish actions of other photosystems from photosynthesis is to contrast effects of light quality on photosynthetically incompetent cells. Recently, it was shown that red and blue light drive sugar production in guard cells by separate mechanisms; sugar production in response to red light is completely inhibited by DCMU, whereas the response to blue light is DCMU insensitive in the presence of exogenous K^+ (37). In the present experiments with growing leaf discs, different responses to light quality have been sought among subprocesses leading to growth, primarily K^+ uptake for osmotic regulation. No difference between DCMU-insensitive K^+ uptake was observed in response to red or blue light (Fig. 5). This implies that K^+ uptake is either stimulated by light acting through Chl and PSI (which is unaffected by DCMU) (39, 41), or red and blue light act through separate photoreceptor systems not involving Chl, each of which is able to drive K^+ uptake.

Clearly, different responses to light quality were observed for short-term kinetics of the growth of intact leaves and for the rate of acidification of the leaf surface (Fig. 6, Tables III and IV). When the results for both light treatments are considered together, contrary to what might have been expected (6, 50, 52), the rate of proton excretion (surface acidification) was not well correlated with growth rate. If leaf growth occurs only by an acid-growth mechanism, a predicted result would be that more rapid cell expansion should occur when apoplastic pH decreases, activating wall-loosening enzymes and increasing wall extensibility (6 and refs. cited therein). The acid-growth theory has been applied to the growth response of leaves to light, and it has been substantiated by the observation that buffering the cell walls at pH 7 inhibits cell expansion (50), showing that some acidification of the apoplast is necessary for growth to occur. The growth stimulation observed in blue light is associated with a slight acidification of the leaf surface (Fig. 6); the anomaly is that the substantial rate of acidification caused by red light is associated with slower growth than in blue light.

Results of other studies have suggested a mechanism other than acidification of the apoplast for growth regulation in leaves. A comparison of leaf growth in two tree species, *Betula* and *Acer* (48), showed that in *Betula* light stimulated leaf growth that was associated with acidification of the apoplast and cell wall loosening. Leaves of *Acer*, however, grew more rapidly in the dark than in light, with only a slight acidification of the apoplast. For *P. vulgaris*, both gibberellin and cytokinin stimulate leaf growth without acidification of the apoplast (3). The present results, also in *Phaseolus*, show that two mechanisms, one resulting in acidification driven by red light and the other a response to blue light with little acidification, normally function together as parts of the growth response to light. These results suggest that the net result, decreased apoplast pH, is determined by at least two ion transport processes that may become uncoupled, as in

Table IV. *Kinetics of the Acidification of the Leaf Surface in Response to Light*

A combination pH electrode was placed lightly on the abraded lower surface of a detached leaf and moistened with 20 μL of 1 mM KCl, and leaf surface pH was monitored continuously as in Figure 6. The initial pH was measured when leaves were exposed to low-fluence (4 μmol m^{-2} s^{-1}) red light, the lag is the time between exposure to light and the beginning of acidification, and the rate of pH decline was determined over 60 min following the lag period. Numbers in parentheses, SE, $n \geq 5$. Significance for difference between means of pH decline in blue light (50), and red light (50) ≥ 0.05.

Fluence Rate	Initial pH	Lag	pH Decrease
μmol m^{-2} s^{-1}		min	units/h
Red light			
4 (n = 13)	6.6	75	0.28
			(0.004)
50 (n = 5)	6.5	14	0.61
			(0.11)
Blue light			
50 (n = 9)	6.6	18	0.37
			(0.05)

the case of red illumination without blue. A possibility is that blue light specifically stimulates proton/solute cotransport, and in the absence of blue light, cells in red light accumulate protons superfluously in the apoplast without stimulating further wall loosening.

The separate responses of red and blue light manifested by different growth kinetics and acidification of the leaf surface occurred in photosynthetically competent tissues. When an effort was made to remove photosynthetic contributions by applying DCMU, no difference was observed in red and blue light effects on ion uptake. Previously, it was shown that white light stimulates acidification in primary bean leaves bleached by tentoxin and nearly devoid of Chl (52). That result showed that Chl was not necessary for light-driven proton excretion, implying the action of different photoreceptor systems on this transport process. However, results of the present experiments do not rule out an interplay between photosynthesis and the two photomorphogenic pathways. Further attempts to distinguish the actions of each photosystem in growing leaves will be made by comparing nonphotosynthetic epidermis with photosynthetic mesophyll tissue and by using genetic mutants known to be deficient in either photosynthesis or one of the photoreceptor systems.

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