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Genetic Analysis of the Role of Gibberellin in the Red Light Inhibition of Stem Elongation in Etiolated Seedlings[1,2]

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ABSTRACT

Red light causes a reduction in the extension growth of dark-grown seedlings. The involvement of gibberellin in this process was tested by screening a number of gibberellin synthesis and gibberellin response mutants of *Pisum sativum* L. for the kinetic response of stem growth inhibition by red light. Gibberellin deficient dwarfs, produced by mutant alleles at the *Le, Na,* and *Ls* loci, and gibberellin response mutants produced by mutant alleles at the *La* and *Cry*^{s}, *Lka,* and *Lkb* loci were tested. Extension growth of expanding third internodes of dark-grown seedlings was recorded with high resolution using angular position transducers. Seedlings were treated with red light at a fluence rate of 4 micromoles per square meter per second either continuously or for 75 seconds, and the response was measured over 9 hours. With certain small exceptions, the response to the red light treatments was similar in all the mutants and wild types examined. The lag time for the response was approximately 1 hour and a minimum in growth rate was reached by 3 to 4 hours after the onset of the light treatment. Growth rate depression at this point was about 80%. Seedlings treated with 75 seconds red light recovered growth to a certain extent. Red/far-red treatments indicated that the response was mediated largely by phytochrome. The similar responses to red light among these wild-type and mutant genotypes suggest that the short-term (*i.e.* 9 hour) response to red light is not mediated by either a reduction in the level of gibberellin or a reduction in the level or affinity of a gibberellin receptor.

Despite the dramatic nature of the response, the mechanism by which light reduces stem elongation in etiolated plants is unknown. The reduction in stem elongation is mediated by blue and red light, acting largely through separate photoreceptors (5). In this study the inhibition of growth by red light is examined. The effect of red light was studied to avoid the complexity of white light and because its effects are manifested more slowly (20, 30) than those of blue light (3, 5) and are therefore more likely to be mediated by changes in hormone levels or sensitivity. We tested the hypothesis that the response to red light is mediated by either a reduction in GA level or response by screening a range of stem-length mutants of *Pisum sativum* L. for their response to red light.

GA applications largely reverse light inhibition of stem growth (2, 6, 14, 15, 16, 33). In early work in this area, Lockhart (14, 15) suggested that GA became limiting in plants exposed to red light by reason of (a) reduced synthesis of GA, (b) increased destruction or diversion of GA, or (c) a decrease in the sensitivity to GA. Working with the dwarf pea cultivar Progress No. 9, he concluded in one study that red light decreased GA levels (15). Decreases in the level of 'GA-like' substances in dark grown *Phaseolus* seedlings after exposure to light have also been reported on the basis of bioassay (1, 4, 18). More recently, Campell and Bonner (2) and Sponsel (32), working with *Pisum,* have presented evidence that suggests the conversion of GA_{20} to GA_1 occurs more readily in dark-grown *versus* light-grown *le*[3] dwarfs. Red light was suggested to block this conversion and thus lead to reduced stem elongation in such dwarfs through a drop in the level of GA_1. On the other hand, measured changes in 'GA-like' activity have not always been consistent with the idea that GA levels are reduced by light (10, 11, 12, 28) and this has led investigators to suggest that the sensitivity to GA is reduced by light (10, 11, 25, 28), possibly at the level of a receptor (17). Lockhart (14) himself argued at one point that light acts by altering sensitivity to GA and not by reducing GA levels.

A related approach to understanding the role of GA in this area has been to study the metabolism of GA in dark- and light-grown seedlings in an attempt to correlate changes in metabolism of this hormone with the effects of light on stem growth. In *Pisum* the evidence generally favors an increase in the metabolism of GA in dark- as opposed to light-grown plants (23, 28, 32), although in studies with *Phaseolus* the opposite conclusion has been reached (1).

We have taken a different approach to understanding the role of gibberellin in this aspect of the de-etiolation process. We have asked whether or not mutations which affect GA levels or response change the response of dark-grown plants to red light. Dwarf mutants which result from a deficiency in GA due to the presence of homozygous recessive alleles at the *Le, Na,* or *Ls* loci were examined. The *le* mutation reduces the 3β-hydroxylation of GA_{20} to GA_1 (9), which is the GA that is thought to directly influence internode length in *Pisum* (8) as well as several other species (19). This mutation results in the standard dwarf habit. Of the other two biosynthesis mutants, *na* blocks the conversion of *ent*-7α-hydroxykauren-

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[3] All genotypes are homozygous.

oic acid to GA_{12}-aldehyde (7), while *ls* appears to block prior to the formation of *ent*-kaurene (7). Both of these mutations are quite severe and result in extremely dwarfed plants (21, 26), and the *na* mutant is classified as having the nana phenotype.

Two sorts of GA response mutants were examined. One type, the slender mutant, grows as if saturated with GA, regardless of the endogenous GA content, the most obvious characteristic being long internodes, especially in young seedlings (22). This phenotype results from the gene combination, *la cry*^{s}, which has been suggested to result in the loss of repressor activity over GA function (22). Two slender lines were employed, one having a normal GA_1 content, the other possessing *na* in addition to *la cry*^{s}, making the plant severely GA-deficient. Stem growth in these two slender types is indistinguishable since the *la cry*^{s} gene combination is epistatic to *na* (22). The second type of response mutant examined has the opposite phenotype of slender. These mutants are dwarfed and tallness cannot be restored by the application of GA_1. This phenotype is conferred by *lka* in one line and *lkb* in the other (27). Plants with these mutations have the semi-erectoides phenotype which includes brittle, thick stems in addition to reduced internode length (27).

We grew genotypes of *Pisum* carrying the above described mutations and related WT[4] plants in darkness and then exposed them continuously or briefly to low fluence red light. The response to the light treatments was recorded at high resolution with angular position transducers and computer based data acquisition. We compared the kinetics of reduction of stem growth between mutants and controls with the hope of clarifying the role of GA in this process. We would expect the response to red light to be modified in a dramatic way in at least one of the mutants if the inhibition of stem growth produced by red light proceeds through changes in GA levels or aspects of GA response involving the mutant genes.

[4] Abbreviations: WT, wild type; RC, continuous red light; R75, red light for 75 s; FR, far-red light; RFR, 75 s red light followed by 75 s far-red light; D, darkness; NGB, Nordic Gene Bank; L, line.

MATERIALS AND METHODS

Plant Material

The pure lines of *Pisum sativum* L. used in this study are maintained in the Department of Plant Science at the University of Tasmania, Hobart Australia. The genotypes are listed in Table I along with information on their endogenous GA levels and genetic background.

Plants were grown in grade 3 vermiculite (Horticultural Products, W.R. Grace & Co., Cambridge, MA) in darkness at 20 to 22°C. Seedlings were well-watered, with the exception of NGB5865 and NGB5862. These lines appeared to grow better when watered sparingly. Plants which exhibited healthy growth were selected for extension growth measurements when the third internode was 20 to 50% expanded.

Growth Measurements

The rotating arms of angular position transducers (Gould Electronics, Pittsburgh, PA) were extended with fine, stiff wire (0.25 mm in diameter) to approximately 8 cm and were slightly counterweighted. A very small barb at the end of the wire (*ca.* 0.5 mm long) was pressed into the epicotyl just beneath the apical hook at the point where the stem began to increase in diameter. This did not appear to significantly influence growth or development. Seedlings were allowed to equilibrate for 0.5 h and then growth measurements began. Angular movement of the transducer arm never exceeded 30° so that the transducers responded to linear growth within an error of ±1.0%.

All growth recordings in this study follow the same format. Growth was monitored over 10 h with measurements of position taken at 60 s intervals. The output from the transducers was amplified from 20 to 100 times with a custom made amplifier and filtered with a low pass filter. The trans-

Table I. *Summary of the Genotypes Used in This Study*

Mutants are homozygous recessive for the allele(s) indicated, otherwise they and the WT are homozygous dominant for all other genes recognized as influencing internode length, including *Lw*, *Lk*, *Lm*, *Lv*, and *Lh*. Mutants related to NGB1771 are not isogenic to NGB1771. Mutants on the Torsdag and L205$^+$ background are isogenic to their respective WT control.

Line	Phenotype	Genotype	Gibberellin Physiology	Genetic Background
NGB1771	Tall	WT	Normal	
203	Dwarf	*le*	Reduced levels of GA_1	NGB1771
81	Nana	*na*, *le*	Very reduced levels of GA_1	
197	Slender	*la*, *cry*^{s}	GA insensitive	NGB1771
188	Slender	*na*, *la*, *cry*^{s}	Very reduced levels of GA_1, GA insensitive	NGB1771
Torsdag	Tall	WT	Normal	
NGB5839	Dwarf	*le*	Reduced levels of GA_1	Torsdag
181	Severe dwarf	*ls*	Very reduced levels of GA_1	Torsdag
NGB5865	Semi-erectoides	*lka*	Reduced response to GA_1	Torsdag
NGB5862	Semi-erectoides	*lkb*	Reduced response to GA_1	Torsdag
205$^+$	Tall	WT	Normal	
205$^-$	Dwarf	*le*	Reduced levels of GA_1	205$^+$

ducer signal was converted into digital form (Dash 8 A-to-D board, Metrabyte Corp., Taunton, MA) and logged by a Basic program and a microcomputer. When the output from the transducers was about to exceed the range of the A-to-D board, the program directed stepper motors to offset the signal. At the end of the experiment data files for growth, with corrections for offsets, were stored. The growth curves were transformed to rate curves by taking the first derivative using an 11 point convolution method (31). This smoothed the data somewhat as well. With this set-up, up to four seedlings could be monitored at once. After the initial attachment to the transducers plants were undisturbed except by the experimental treatments. A resolution of 0.6 μm was obtained with transducers with this system at the highest amplifications used. When transducer arms were supported in a stationary position, output from the transducers did not drift more than 10 μm over a 10 h period.

Light Treatments

All light treatments started 1 h after growth measurements began. Red light was supplied by filtering the output from four 24 inch 'cool white' fluorescent lamps (General Electric) through 0.125 inch red plexiglass (color No. 2423, Rohm & Haas, Philadelphia, PA). The height of plants was adjusted so that the fluence rate of red light at the top of the shoot was 4 μmol $m^{-2}s^{-1}$. Red light treatments were either continuous (RC) or lasted 75 s (R75). Far-red light was supplied by filtering the output from four 250 W flood lamps (General Electric) through 0.125 inch FRF 700 plexiglass (Westlake Plastics Co., Lenni, PA). An effective FR exposure was worked out empirically with the WT line NGB1771. Filtering light that had a fluence rate of 260 μmol $m^{-2}s^{-1}$ at the base of the growth chamber when unfiltered resulted in nearly complete reversal of R75 on line NGB1771. This FR treatment, 75 s in duration, was used with all the other genotypes. Twenty-five seconds elapsed between the R75 and far red light treatments.

All manipulation of plants was done under a dim green safelight (15 W fluorescent lamp wrapped in two layers of green plastic, Edmund Scientific). The fluence rate of this light at the plant was no greater than 0.025 μmol $m^{-2}s^{-1}$ at any time, and lasted no longer than 2 min. Light measurements were taken with a model LI 185A light meter fitted with a LI 190S PAR cosine-corrected quantum sensor (Li-Cor, Lincoln, Nebraska).

RESULTS

Growth and Rate Time Courses

The responses to the two red light treatments used in this study are presented in detail for the WT cultivar Torsdag (Fig. 1). The lag time for the response to red light is approximately 1 h. In RC the growth declines to a minimum by 3 h after the onset of irradiation and remains low. The response to R75 is similar to RC in that maximal inhibition occurs 3 h after the light treatment. However, inhibition in this genotype is not as complete as with RC and growth rate recovers to a certain extent over the following 6 h. These kinetics are in accord with other measurements of the short term time course of response to red light in etiolated *Pisum* (20, 30).

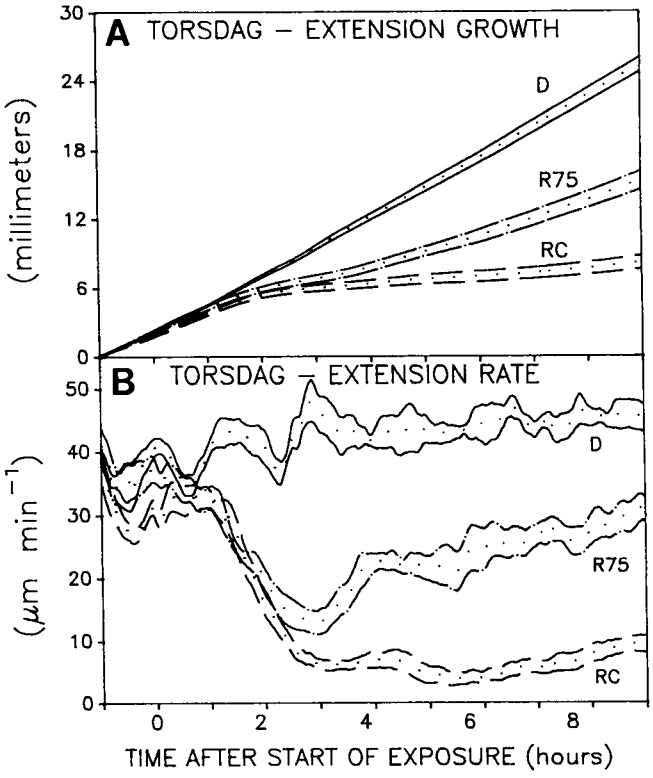

Figure 1. Response of the cultivar Torsdag to RC and R75 in terms of extension growth (A) and extension rate (B). The average growth and rate curves from the plants tested are represented by *dotted lines* which are bounded by the running SE for each sample. The sample sizes are: D (7), RC (11), and R75 (8).

The running SE lines (Fig. 1) indicate that the variability among plants was fairly low. For the sake of clarity standard error lines have been omitted from subsequent graphs. Standard errors were in the same range for all the other genotypes used in this study except for the three slowest growing genotypes (L81, NGB5865, and NGB5862) where the SE were greater in proportion to the growth rates. Individual seedlings did not grow at a smooth constant rate, as other investigators measuring growth at high resolution have also noticed (3, 5, 13). Variations in growth rate from 10 to 20% during a 10 h recording of growth in darkness were not uncommon. At times periodic growth was apparent but at other times the fluctuations appeared random. We interpret such changes to be changes in extension growth only, since attachment of the seedling to the transducer constrained the seedling movement to one dimension. The site of transducer attachment to the stem is not displaced from the zone of elongation during these experiments, as we were able to measure a linear growth rates for up to 18 h in separate experiments (data not shown).

The stem elongation rates of the mutant genotypes in darkness and after the red light and far-red light treatments used in this study are summarized in Figures 2, 3, and 4. The dwarfing mutations *le, na, ls, lka,* and *lkb* are clearly expressed in the dark as indicated by the reduced growth rates of the mutant lines compared to the WT when seedlings are grown

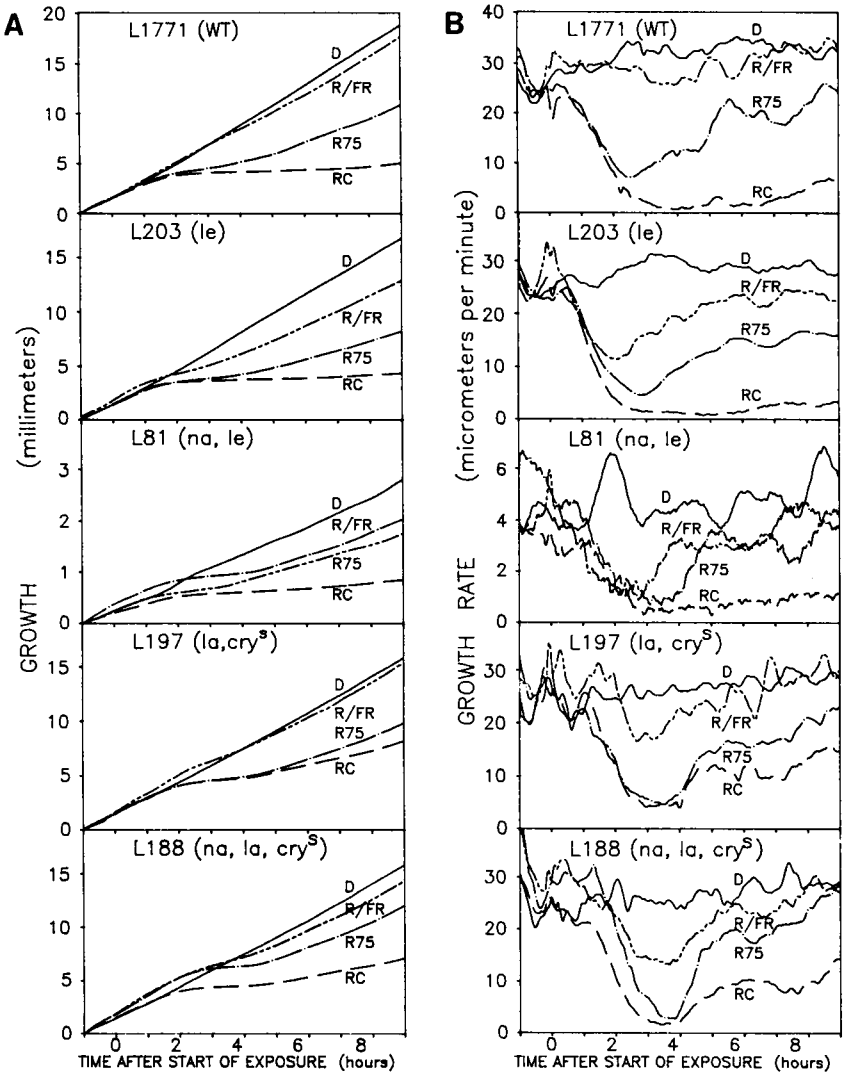

Figure 2. Averaged responses in terms of extension growth (A) and extension rate (B) in darkness, RC, R75, and RFR for the tall line NGB1771 and four of the mutant genotypes. All light treatments began at 0 hr. Sample sizes are, NGB1771: D (10), RC (7), R75 (5) and RFR (9); L203: D (12), RC (7), R75 (19) and RFR (6); L81: D (12), RC (8), R75 (9) and RFR (5); L197: D (6), RC (8), R75 (7) and RFR (6); L188: D (8), RC (9), R75 (6) and RFR (6).

in constant darkness. This is in agreement with previous observations that internode-length mutations are expressed in etiolated plants (24, 25, 27, 28) and indicates that, in the GA-deficient mutants, darkness does not overcome the block in GA synthesis as has been suggested for *le* dwarfs (2, 32). For each mutant the kinetic response to red light follows the same pattern as in the normal tall lines. Hence, the same response that was previously described for Torsdag generally holds for all the other lines examined. In no instance does either the lack of GA, or altered response to GA, prevent the growth reduction caused by red light.

The response to the RFR treatment shows the most variability of all the treatments. FR strongly reverses the effect of R75 in NGB1771 and L197 (Fig. 2), NGB5862 (Fig. 3), and L205$^-$ (Fig. 4). However, in other lines, such as L203 (Fig. 2), Torsdag and NGB5865 (Fig. 3) and L205$^+$ (Fig. 4) the RFR treatment is much less effective. The effectiveness of this FR treatment does not correlate with GA level or response in these lines of pea.

Quantitative Analysis

Although inspection of the rate time courses indicates that the kinetic response of stem elongation to the red light treatments is similar among genotypes, we quantified certain aspects of the response to allow a more accurate comparison. Three features of the response were analyzed in greater detail. These are the lag time, the extent of growth reduction produced by the light treatments and the timing and extent of growth rate reduction produced by R75.

The average lag time for the response to red light, whether continuous or 75 s in duration, appears to be about 70 min for all the genotypes examined (Table II).

When the growth of light-treated plants is expressed relative to the growth of dark-grown plants some slight differences in response emerge (Table III). Growth under RC for the three tall lines (NGB1771, Torsdag and L205$^+$) and four of the five GA-deficient dwarf lines (L203, L81, NGB5839, and L181) ranges from 20 to 30% of the control. However, in the four sensitivity mutants (L197, L188, NGB5865, and NGB5862)

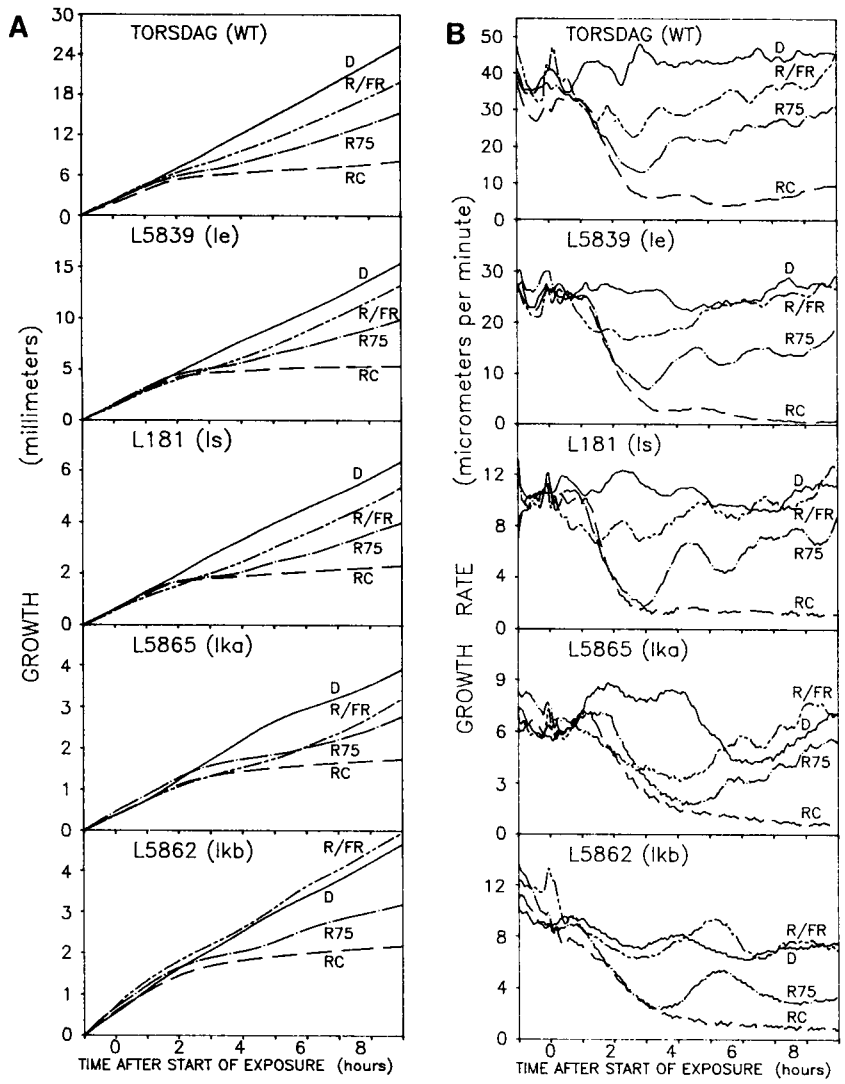

Figure 3. Averaged responses in terms of extension growth (A) and extension rate (B) in darkness, RC, R75, and RFR for four mutants and their wild-type parent, Torsdag. All light treatments began at 0 h. Sample sizes are, Torsdag: D (7), RC (11), R75 (8) and RFR (7); NGB5839: D (9), RC (8), R75 (8) and RFR (11); L181: D (10), RC (6), R75 (6) and RFR (7); NGB5865: D (7), RC (7), R75 (7) and RFR (7); NGB5862: D (9), RC (11), R75 (8) and RFR (8).

growth is relatively greater, ranging from 37 to 47% of the control. With the 75 s red light treatment the mutants respond similarly to the tall control plants. The responses to RFR showed greater variability than the responses to either of the red light treatments as discussed earlier. These RFR experiments indicate that the kinetic responses we have recorded are largely mediated by phytochrome.

The extent of the maximal depression of growth rate produced by R75 was very similar across all lines (Table IV). Growth rate was depressed by a proportionate amount rather than to some absolute value at this point. The time required to reach a growth rate minimum was between 3 and 4 h. All three tall lines (NGB1771, Torsdag, and L205$^+$) reached a minimum rate 180 min after the light exposure. This timing also held for the three *le* dwarfs (L203, NGB5839, and L205$^-$) and the *ls* dwarf, L181. However, the *na* dwarf, L81, reached a minimum only after 233 minutes. The two slender lines, L197 and L188, and the semi-erectoides line NGB5862 were also slightly delayed compared to the tall lines (by 32–48 min) while the semi-erectoides line NGB5865 did not reach a minimum until over 4.5 h after exposure to red light. By inspection of the rate curves (Figs. 2, 3, and 4) it can be seen that the speed of the decrease in growth rate upon exposure to RC is approximately the same as for exposure to R75 in any of the genotypes.

DISCUSSION

This kinetic analysis of stem growth reduction in etiolated plants exposed to red light has shown that the response to red light is very similar in a variety of stem length mutants. There is some variability in lag time, the speed at which the rate of growth is reduced and the extent of growth reduction in certain mutants. However, in all lines tested the response to red light is considerable and similarities in the time courses of the response in the various mutants and tall lines are far more striking than are the differences. These data indicate that decreases in GA level or decreases in the level or affinity of a putative GA receptor are not significantly involved in mediating the decrease in stem elongation over the short term

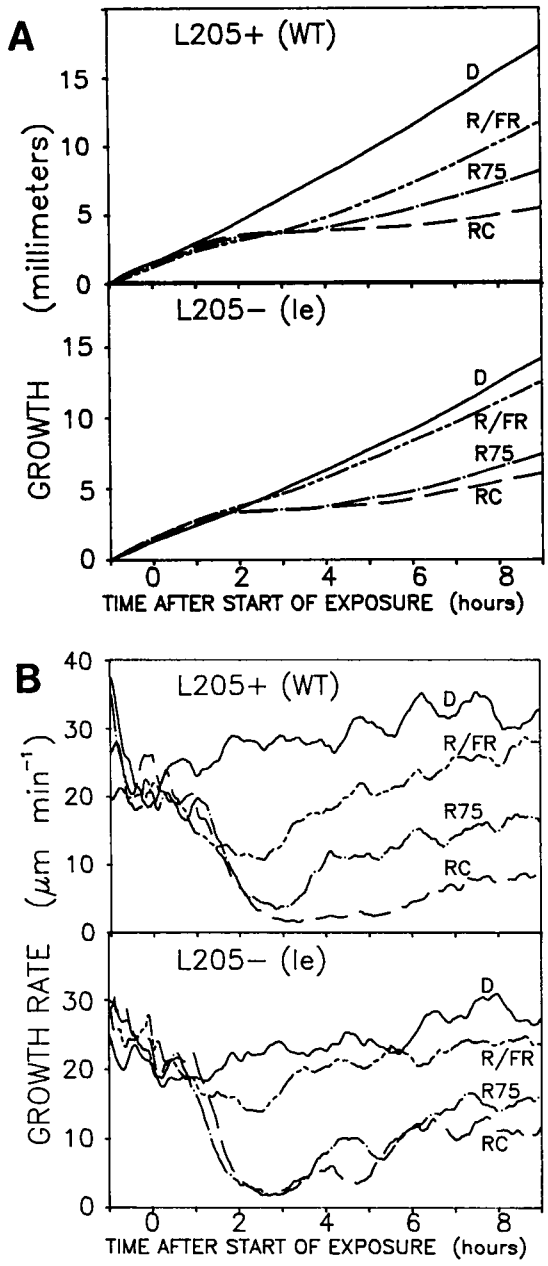

Figure 4. Average responses of the isogenic pair L205$^+$ and L205$^-$ to darkness, RC, R75, and RFR expressed as growth *versus* time (A) and growth rate *versus* time (B). All light treatments began at 0 h. Sample sizes are, L205$^+$: D (7), RC (7), R75 (7) and RFR (9); L205$^-$: D (7), RC (8), R75 (8) and RFR (10).

(<9 h) when etiolated *Pisum* seedlings are exposed to red light.

It is relevant to consider whether the mutants selected for this study are appropriate for testing a GA-related mechanism in this red light response. Based on what is known about the mutations, certain changes in the response to red light can be postulated if a mechanism involving either reduced GA levels or changes in a GA receptor operates in this system. For example, if red light were to reduce the level of GA at the active site through either decreased synthesis, increased metabolism or compartmentation, one might expect the GA-deficient dwarfs to respond to a much lower degree (GA already being at a low level) or more rapidly (the minimum level of GA for light-induced growth is reached more rapidly). However, both percent inhibition of growth and lag time were similar between the WT and GA-deficient dwarfs. In the GA-insensitive mutants a very limited response to red light would be predicted because the *lka* and *lkb* dwarf mutants respond poorly to changes in GA levels and the slender mutants grow tall irrespective of GA levels. The response mutants reacted strongly to red light suggesting again that decreases in GA are not the primary mechanism by which red light acts. Interestingly, the inhibition of growth in the sensitivity type mutants under RC was slightly less than in the normal and GA-deficient dwarf lines. This may indicate that a small component of the response under RC is mediated by a change in GA levels.

Considering the possibility that red light reduces the sensitivity to GA at the level of a receptor, we might predict a diminished response in the GA-deficient dwarfs (growth is already inhibited by low GA levels) or a shortened lag time (a change in receptor affinity might be expressed more quickly when GA is at a low concentration). In the GA response mutants, a significant response would not be expected since changes in receptor affinity should not affect growth in these lines as is indicated by the inability of the *lka* and *lkb* dwarfs to transduce the GA_1 signal effectively and the constitutive GA response in the slender plants.

A note of caution in interpreting results with the GA-deficient dwarfs is that these dwarfs are not 100% deficient in GA, but lack GA_1 to varying degrees. Hence, the different stature among *le, na,* and ls dwarfs has been attributed to varying degrees of leakiness in these mutations (7, 8). Indeed, different allelic mutations of the *Le* locus have been shown to result in more severe dwarfism because the conversion of GA_{20} to GA_1 is blocked to different degrees (29). Since the GA-deficient dwarfs do contain some GA, a mechanism

Table II. *Lag Times for the Response to Continuous and 75 s Exposure to Red Light*

Lag times were estimated by visually determining the break point from rate curves of individual recordings. The means ± 95% confidence intervals are reported. Sample sizes are as indicated in Figures 2 to 4.

Line (Genotype)	Continuous Red	75 s Red
	min	
NGB1771 (WT)	56 ± 22.2	47 ± 20.1
203 (*le*)	50 ± 11.8	48 ± 5.9
81 (*na, le*)	74 ± 16.1	65 ± 8.3
197 (*la, cry^s*)	76 ± 9.0	71 ± 16.6
188 (*na, la, cry^s*)	80 ± 11.3	88 ± 9.3
Torsdag (WT)	69 ± 11.2	63 ± 9.2
NGB5839 (*le*)	73 ± 9.7	78 ± 8.3
181 (*ls*)	75 ± 18.8	59 ± 12.0
NGB5865 (*lka*)	86 ± 7.3	94 ± 25.5
NGB5862 (*lkb*)	81 ± 18.8	68 ± 15.5
205$^+$ (WT)	67 ± 13.5	69 ± 7.3
205$^-$ (*le*)	74 ± 9.2	56 ± 12.9

Table III. *Relative Extension Growth after Exposure of Seedlings to the Light Treatments*

The extension growth occurring during the 9 h after the start of the light treatments is expressed relative to growth occurring in the control plants over the same time. Mean ± SE of the ratio are reported. The data are from the kinetic studies and sample sizes are as indicated in Figures 2 to 4.

Line (Genotype)	Continuous Red	75 s Red	Red/Far-Red
NGB1771 (WT)	21 ± 2.0	55 ± 4.5	95 ± 6.0
203 (*le*)	19 ± 1.4	45 ± 2.9	83 ± 6.7
81 (*na*, *le*)	27 ± 3.1	59 ± 9.3	78 ± 25.2
197 (*la*, *cry*^{s})	47 ± 3.1	58 ± 7.3	97 ± 13.2
188 (*na*, *la*, *cry*^{s})	39 ± 4.7	70 ± 4.8	86 ± 7.2
Torsdag (WT)	28 ± 2.5	59 ± 3.8	79 ± 4.1
NGB5839 (*le*)	30 ± 1.8	62 ± 4.1	89 ± 7.1
181 (*ls*)	30 ± 3.7	59 ± 5.4	86 ± 6.9
NGB5865 (*lka*)	39 ± 5.7	65 ± 11.8	80 ± 10.6
NGB5862 (*lkb*)	39 ± 13.4	61 ± 12.4	105 ± 20.1
205$^+$ (WT)	26 ± 2.5	44 ± 3.4	68 ± 4.2
205$^-$ (*le*)	37 ± 4.8	47 ± 5.4	97 ± 11.6

involving a reduction in GA level or sensitivity may result in response kinetics similar to the WT despite the logic that would suggest the response to be altered in some way. This qualification applies to the *lka* and *lkb* mutants as well, since it has been shown that while these mutants respond poorly to GA application, they can be dwarfed to a greater extent by application of the GA-biosynthesis inhibitor paclobutrazol (27).

By contrast interpretation of the results with the slender mutants is unambiguous. Stem growth in slender mutants is not altered by applied GA, GA-biosynthesis inhibitors or genetic blocks to GA biosynthesis, such as *na* which is present in L188 (22). While the function of the protein which is altered by *la cry*^{s} has not been identified, this phenotype indicates that such mutants can no longer discriminate between low and high levels of GA and this strongly suggests that functionally a GA receptor is in a constitutively 'on' state. This implies that red light inhibits stem elongation in slender mutants by acting at a site after a GA receptor.

The effects of red light could be very indirect, such as promoting the rate of maturation of cells and thereby limiting the time during which GA influences growth, or more direct, for example, by depressing the activity of an enzyme involved in cell expansion that is positively regulated by GA. Potentially such mechanisms could be tested with response mutants like *lka* and *lkb* when the biochemical basis of such mutations is understood. In this regard, it has been shown that the gene combination *la cry*^{s} is not epistatic to *lka* and *lkb* (R Cramp, J Reid, unpublished data) as it is to *na,* suggesting *lka* and *lkb* affect processes further down the response chain. Hence, the positive response to red light with these mutants suggests that light affects GA response at least two steps down the response chain from GA reception. Indeed, one of the hopes of this investigation was that a mutation such as *lka* or *lkb* would help identify a site of light action within the GA response chain. Along these lines, the *lk* mutant (26), which produces a severe erectoides phenotype and is also epistatic to *la cry*^{s}, has also been found to respond normally to red light in preliminary tests.

The conclusions of this study are not completely at odds with studies that have suggested that red light changes GA levels. For example, Campell and Bonner (2) also noted that dark-grown *le, na,* and paclobutrazol-dwarfed peas responded to red light and concluded that red light also acted by a mechanism independent of GA metabolism. We also suggest that experiments involving the application of GA precursors and intermediates to study the influence of red light on metabolism may be difficult to interpret when such compounds are applied in large excess of endogenous levels.

Table IV. *Timing and Extent of Maximum Inhibition of Stem Extension Rate Produced by 300 μmol Red Light (R75)*

The average time to the minimal growth rate was determined by locating the 5 min period which had the lowest average growth rate. The extension rate at this time was compared to the averaged extension rate over 30 min at an equivalent time in the average rate curve for the dark control plants. Sample sizes are as in Figures 2 to 4.

Line (Genotype)	Time of Maximal Inhibition	Extension Rate	Extension Rate in Darkness	Reduction
	min after R75	*μm min*$^{-1}$		%
NGB1771 (WT)	184 ± 17	6.4 ± 0.79	32.1	80 ± 3.3
203 (*le*)	182 ± 5	4.2 ± 0.81	29.4	86 ± 2.8
81 (*na*, *le*)	233 ± 9	0.6 ± 0.10	5.9	90 ± 1.7
197 (*la*, *cry*^{s})	212 ± 7	4.2 ± 0.65	25.1	83 ± 2.6
188 (*na*, *la*, *cry*^{s})	227 ± 5	2.7 ± 0.66	24.2	89 ± 2.7
Torsdag (WT)	182 ± 6	10.1 ± 1.85	44.7	78 ± 4.1
NGB5839 (*le*)	192 ± 4	6.4 ± 0.79	25.7	75 ± 3.1
181 (*ls*)	189 ± 3	1.7 ± 0.17	10.9	84 ± 1.7
NGB5865 (*lka*)	275 ± 10	1.6 ± 0.27	7.2	78 ± 3.8
NGB5862 (*lkb*)	228 ± 9	2.1 ± 0.63	7.8	73 ± 8.6
205$^+$ (WT)	182 ± 3	4.4 ± 0.91	27.7	84 ± 3.3
205$^-$ (*le*)	178 ± 5	2.5 ± 0.59	22.7	89 ± 2.6

Sponsel (32) applied [$^{13}C^{3}H$]GA_{20} at 1 μg per seedling. This is much larger than the total endogenous content of GA_{20} per seedling and may not lead to accurate conclusions regarding normal GA biosynthesis and metabolism. In any event, it is possible that GA application overcomes red light inhibition such as observed by Lockhart (13–15) in a nonspecific manner or that red light and GA interact over a longer term than the time period analyzed in this study.

This study suggests during the initial response (about 9 h), red light either affects the capacity of the plant to respond to GA by altering sensitivity toward the end of the chain of events initiated by GA or that red light acts independently of GA. This conclusion is in agreement with observations that GA-deficient dwarfs grow taller in darkness than in the light (23, 24) and that application of GA results in greater growth in dark-grown as opposed to light-grown plants (2, 24, 27, 33). The transition from darkness to light obviously results in a dramatic stimulus to plants. Understanding those biochemical changes induced by red light that limit stem growth should be investigated.

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