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Phytochrome Control of Cell Wall-bound Hydroxyproline Content in Etiolated Pea Epicotyls[1]

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ABSTRACT

The red light inhibition of growth of the intact pea (*Pisum sativum* L. cv. Alaska) third internode was correlated with an increase in the content of cell wall-bound hydroxyproline. These changes were detected 3 hours after irradiation, and possibly at 1 hour. Far red light reversed the effects of red light. The iron chelator α,α′-dipyridyl reversed the red light effects on both growth and hydroxyproline content. Using segments incubated *in vitro*, no phytochrome-mediated change in hydroxyproline content could be observed, perhaps because of an overwhelming wounding response. If plants were irradiated *in situ* and grown for 8 hours before excision and incubation of segments, some enhancement of hydroxylation by red light was detectable both colorimetrically and radioisotopically. The red light inhibition of segment growth was reversed by α,α′-dipyridyl. These results are examined in reference to the role of extensin in normal and induced growth cessation.

The growth of a plant cell is significantly influenced by the properties of the cell wall. For elongation to proceed, the wall must be in a loose condition. Changes in growth rate are often caused by changes in certain crucial acid-labile, alkaline-stable bands (3). The initial stage of growth promotion by auxin may be the result of H^+ secretion into the wall and consequent wall loosening (14). One type of acid-labile bond implicated in growth control is the hydroxyproline-O-arabinose bond, linking extensin, a hydroxyproline-rich glycoprotein, to the wall polysaccharides (8). There is considerable evidence that cells that have ceased elongating contain more wall-bound hydroxyproline (and thus have less plastic walls) than do elongating cells (4, 10, 17). None-longating pea epicotyl segments incorporated and hydroxylated more [^{14}C]proline than did elongating segments (17). The inhibition of growth by agents such as ethylene and benzimidazole was also accompanied by an increase in wall-bound hydroxyproline (15, 16) and in [^{14}C]proline hydroxylation (16). The increase in hydroxyproline content was suggested to precede and to cause the growth inhibition, but the first assays were made 6 h (16) or 1 day (15) after hormone application.

R[5] strongly inhibits the growth of intact pea epicotyls (5) and epicotyl segments incubated in a buffered sucrose-cobalt medium (2), but little is known of the mechanism of action. R might accelerate the transition of cells from the elongation stage to the maturation stage (19). There is some evidence concerning a relationship of phytochrome control of growth and hydroxyproline. Pea seedlings grown in the light were much shorter and contained much more wall-bound hydroxyproline than etiolated seedlings (17). Although white light inhibited the growth of radish hypocotyls, light had no effect on wall hydroxyproline content (9). This study was undertaken to determine the influence of phytochrome on wall hydroxyproline level.

The iron chelator α,α′-dipyridyl is widely used as an inhibitor of the hydroxylation of peptidyl proline (1, 9, 16). The reversal by this compound of the hormonally induced inhibition of pea stem segment growth was attributed to a blockage of peptidyl proline hydroxylation, not an inhibition of protein synthesis and secretion into the wall (16). Also, α,α′-dipyridyl alone promoted growth in soybean hypocotyl segments and caused a transient increase in wall plasticity (1). In long term experiments, the compound can inhibit protein synthesis (1, 16). We also report experiments utilizing α,α′-dipyridyl to explore causal relations between hydroxyproline deposition and growth.

MATERIALS AND METHODS

Pea seedlings (*Pisum sativum* L. cv. Alaska) were grown in total darkness at 28 C (11). Seedlings with third internodes between 1.5 and 2.5 cm long were selected. In most experiments the 1-cm third internode region beginning 3 mm below the hook was marked with either India ink or charcoal-blackened lanolin. Irradiations (23) were given and, after a period in darkness, the region between the marks was measured (usually with a vernier caliper) and excised. The excised tissue was then weighed in the dark and chilled on ice. All operations at this point were conducted under dim green safelights (23). The tissue was homogenized in a ground glass homogenizer in 0.05 M K-phosphate (pH 6.0) containing 0.3 M sucrose (1 ml buffer/g tissue). The homogenate was centrifuged at 800*g* for 5 min and the pellet washed three times in homogenizing solution and three times in deionized H_2O (9). The washed walls were hydrolyzed in 6 N HCl by autoclaving at 15 p.s.i. for 90 min in tubes with teflon-lined screw caps (17). The hydrolysate was centrifuged at 1,200*g* for 10 min to remove humin.

The supernatant was assayed for hydroxyproline by a modification of the procedure of Switzer and Sumner (18). To 1-ml samples of hydrolysate, 1 drop of 1% phenolphthalein in ethanol was added. The pH was adjusted to faint pink with KOH and the samples diluted to 4 ml with water. A 1-ml aliquot was taken and diluted to 2.3 ml. For the standard curve, appropriate amounts of a stock solution were titrated as above and brought to 2.3 ml.

All samples were then treated as follows. To each tube was added 0.5 ml of 1 M K-borate (pH 8.7) and 2 ml of 0.5 M chloramine-T. After 25 min the oxidation reaction was stopped by the addition of 1.2 ml of 3.6 M $Na_2S_2O_7$. The solution was then

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[5] Abbreviations: R: red light, FR: far red light.

saturated with 1.5 g of KCl. The proline oxidation product was extracted into 2.5 ml of toluene. Following centrifugation at 600*g* for 1 min, the toluene layer was removed (and used for proline determinations in certain experiments). The aqueous phase was heated at 100 C for 30 min in a tightly capped tube. After cooling, the hydroxyproline oxidation product was extracted in 2.5 ml of toluene. A 2-ml aliquot of the toluene layer was mixed with 0.8 ml of Ehrlich's reagent (*p*-dimethylaminobenzaldehyde solution [13]) and kept in the dark for 30 min. *A* at 560 nm was measured. The standard curve was constructed by linear regression analysis, and the hydroxyproline content expressed in μg/g fresh weight.

For *in situ* experiments with α,α′-dipyridyl (Sigma), an aqueous solution was applied with a cotton swab to the third internode. Since 0.5 mM α,α′-dipyridyl had no effect on growth, a 5 mM solution was used in experiments involving combinations of dark or R and water or α,α′-dipyridyl. Subapical 1-cm regions were marked and their growth and hydroxyproline content determined after various periods of dark incubation.

We also studied the growth and hydroxyproline content of 1-cm subapical third internode segments incubated in 0.02 M K-phosphate (pH 6.0) containing 2% sucrose and 20 μM $Co(NO_3)_2$, which are needed for the optimal operation of a photoreversible growth inhibition system in pea segments (2). Segments were incubated in the dark in a shaking water bath at 25 C for various periods following irradiation. Then they were measured and assayed for hydroxyproline.

We used [^{14}C]proline to follow the deposition of extensin. Certain modifications of the procedure were developed in an effort to circumvent the substantial wounding-induced increase in hydroxyproline. Subapical 1-cm regions were marked on the third internodes and the plants were irradiated. After growth in darkness, the regions between the marks were excised, and the segments (at least 15 per treatment) were placed in 5 ml of incubation fluid for 30 min at 25 C. Then this fluid was removed and replaced with 5 ml of fluid containing 2 μCi of L-[^{14}C]proline (New England Nuclear, 260 mCi/mmol), and incubation continued for 3 h. The experiment was terminated by washing the segments three times in 10 ml of 1 mM proline and three times in deionized H_2O. In the hydroxyproline determination, the first toluene extract, containing the proline oxidation product pyrrole, was saved for radioactive assays. To assay hydroxyproline, 2-ml aliquots of the final toluene extracts (for both unknowns and standards) were passed through columns (0.6 × 3.2 cm) of silicic acid (Bio-Sil A, 100–200 mesh, Bio-Rad Laboratories) in toluene (6, 18). The columns were eluted with toluene; the first 2 ml of eluate was discarded and the next 3.5 ml used for colorimetric and radioisotopic assays. The chromatography reduces residual contamination with substances other than the hydroxyproline oxidation product (6). Samples for scintillation analysis were added to 10 ml of scintillation fluid (5 g PPO + 50 mg POPOP/l of toluene) and counted. The samples exhibited negligible differences in quenching (by the external standard method), so cpm values were used. The results were expressed as per cent [^{14}C]hydroxyproline = [cpm in hydroxyproline/(cpm in hydroxyproline + cpm in proline)] × 100 (17). Since essentially all of the label supplied to pea epicotyl segments is recovered as proline or hydroxyproline (17), this expression indicates the extent of hydroxylation of proline during the pulse period.

The effect of α,α′-dipyridyl on the R-induced inhibition of segment growth was investigated using 1-cm subapical third internode segments incubated for 24 h at 25 C in the buffered sucrose-cobalt solution. The length of the sections was then determined.

RESULTS

In a preliminary experiment we verified that the hydroxyproline content of tissue was related to the state of elongation. The subapical 1 cm of the three internodes of etiolated plants was marked and measured after 24 h in darkness. The elongation of the first, second, and third internode regions was, respectively, 0, 0.6, and 1.2 cm (average of 20 plants). The mean hydroxyproline contents (three separate analyses) were, respectively, 190, 109 and 74 μg/g fresh weight.

Another preliminary experiment assessed the effect of light on growth and hydroxyproline content of young and mature tissue. Intact plants were irradiated and returned to darkness for 12 h. Then the length of the second and third internodes was measured and the hydroxyproline content of the subapical 1-cm regions was determined. Table I shows that R caused a substantial reduction in the growth of the third internode and enhanced the hydroxyproline content of the subapical 1-cm region. The lack of FR effect suggests phytochrome involvement (photoreversibility was tested in subsequent experiments). In contrast, there was no light effect on the growth of the second internode nor on the hydroxyproline content of its subapical region. Thus, the depression of growth rate by R is correlated with an increase in wall-bound hydroxyproline; no such increase occurs in tissue in which R does not influence growth.

Table I. Effect of light on internode length and hydroxyproline content

Intact pea plants were irradiated (5 min) and then grown in darkness for 12 h. The internode lengths were measured (at least 18 plants). The subapical 1-cm regions of the second and third internodes were excised and the hydroxyproline content determined as described in the text. The hydroxyproline analysis was performed on 3 replicate lots of plants.

Internode	Treatment	Final Internode Length cm	Hydroxyproline Content μg/g fresh wt
Second	Dark	6.8	91
	Red	6.8	91
	Far red	6.7	92
Third	Dark	6.1	59
	Red	3.7	81
	Far red	6.2	59

Table II. Effect of light on *in situ* elongation and hydroxyproline content

A 1-cm region was marked on the third internode of intact plants. Light treatments (5 min) were given and the plants returned to darkness for the indicated period. The region between the marks was measured with a vernier caliper and then excised for hydroxyproline determination. Each datum is the average of at least 2 separate analyses; there were at least 15 plants in each treatment group in each analysis.

Time h	Treatment	Final Length cm	Hydroxyproline Content µg/g fresh wt
1	Dark		54
	Red		58
	Far red		53
	Red + Far red		54
3	Dark	1.16	54
	Red	1.08	61
	Far red	1.15	52
	Red + Far red	1.14	48
6	Dark	1.32	64
	Red	1.14	88
	Far red	1.29	68
	Red + Far red	1.28	72
12	Dark	1.39	72
	Red	1.11	86
	Far red	1.43	73
	Red + Far red	1.34	79

Table III. Effects of α,α'-dipyridyl and red light on *in situ* elongation and hydroxyproline content

Water or 5 x 10^{-3} M α,α'-dipyridyl was applied to the third internode of plants at the time of marking of the 1-cm subapical region. Plants were then irradiated, incubated in darkness for the indicated period, and analyzed as described in Table II. Each datum is the average of 2 separate analyses; there were at least 15 plants per treatment in each analysis.

Time h	Treatment	Final Length cm	Hydroxyproline Content µg/g fresh wt
3	Dark + water	1.17	64
	Dark + dipyridyl	1.16	65
	Red + water	1.08	77
	Red + dipyridyl	1.16	70
24	Dark + water	1.42	99
	Dark + dipyridyl	1.41	94
	Red + water	1.12	132
	Red + dipyridyl	1.31	91

We next sought to establish a time course for the R-induced changes in growth and wall-bound hydroxyproline (Table II). At the longer time points, there is clear indication of phytochrome control of elongation (in this and subsequent elongation studies, the standard errors were generally ± 0.03 cm or less). At the 3-, 6-, and 12-h points there is also a phytochrome-mediated increase in hydroxyproline content. At 1 h, when the changes in growth were too small to be measured, there is some suggestion of an increase in hydroxyproline in the R-treated plants compared to the dark controls. The average increase (7%) is small, considering the errors inherent in the complex assay procedure, but the increase was seen in all three trials. Certainly at 3 h the R effect

is much more evident (13% over the dark control value), suggesting a correlation between decreased growth and increased hydroxyproline deposition at a very early stage of the growth inhibition (see also ref. 12).

Table III shows that at 3 h the inhibition of growth by R is reversed by α,α'-dipyridyl. The R-induced increase in hydroxyproline content is substantially reversed by α,α'-dipyridyl. At 24 h, the data are similar, except that the chelator completely eliminated the R-induced increase in hydroxyproline content. In both long and short term experiments, α,α'-dipyridyl reversed the R effects on growth and hydroxyproline content.

When segments incubated in solution were studied, there was no evidence of a R-induced increase in hydroxyproline content, although the growth of segments was clearly inhibited when measured 8, 12, and 24 h after irradiation. The amount of hydroxyproline in the dark control tissue doubled during this time, so we suspect that a substantial wounding-induced increase might have obscured any effect of light.

In the procedure of Table IV, the plants were irradiated and incubated *in situ*. Then segments were excised and incubated without label for 30 min, to allow at least some of the wounding response to occur before the pulse period. These procedures were only partly successful in overcoming the problem. Using 8 h of *in situ* growth, but not 3 h, there is some evidence of a phytochrome-mediated increase in hydroxyproline content. Also, the per cent [^{14}C]hydroxyproline suggests a R stimulation of hydroxylation during the pulse period. Both of these effects are small, and are presented to indicate that there is a basis for further study.

Table V shows the effects of R and α,α'-dipyridyl on segment growth; effects on hydroxyproline content were not studied because of the lack of response to R. The inhibition of growth by R was reversed by α,α'-dipyridyl (0.5 mM). The chelator also increased the growth of segments in the dark. Using a one-way analysis of variance and the Dunn Test (7), all of the differences

Table IV. Effect of light on hydroxyproline content and % [^{14}C]-hydroxyproline in segments

A subapical 1-cm region of the third internode was marked and the plants irradiated. After 3 or 8 h in darkness, the region between the marks was excised and placed in unlabelled incubation fluid (see text) for 30 min. Then [^{14}C]-proline was added and incubation continued for 3 h. Analyses were performed as described in the text. % [^{14}C]-hydroxyproline = [cpm in hydroxyproline/(cpm in hydroxyproline + cpm in proline)] x 100. Each datum is the average of 2 separate analyses; there were at least 15 plants per treatment in each determination.

Time h	Treatment	Hydroxyproline Content μg/g fresh wt	% [^{14}C]-hydroxyproline
3	Dark	42	44
	Red	44	48
	Far red	32	50
	Red + Far red	48	50
8	Dark	41	47
	Red	67	57
	Far red	56	54
	Red + Far red	62	53

Table V. Effect of red light and α,α'-dipyridyl on epicotyl segment elongation

Subapical 1-cm third internode segments were cut from 7-day-old etiolated seedlings. Segments were incubated in the buffered sucrose-cobalt medium described in the text. Red light (5 min) and 5 x 10^{-4}M α,α'-dipyridyl were given at the beginning of the 24-h incubation. The values presented are the overall means ± SE for three replicate experiments, using at least 16 segments per treatment in each experiment.

Treatment	Final length cm
Dark + water	1.36 ± 0.01
Dark + dipyridyl	1.43 ± 0.01
Red + water	1.23 ± 0.01
Red + dipyridyl	1.35 ± 0.01

between pairs of means were significant at $P < 0.01$, except for the difference between the dark + water and R + α,α'-dipyridyl values.

DISCUSSION

Pea epicotyl elongation, both in intact plants and in segments, is a well-characterized phytochrome-mediated response (2, 5, 20). The shorter, light-grown pea epicotyl contains considerably more wall-bound hydroxyproline than the dark-grown tissue (17). In our preliminary work we verified the presence of more hydroxyproline in older, nonelongating internodes (4, 17) and established a correlation between the R inhibition of growth and an increase in wall-bound hydroxyproline (Table I).

The R-induced inhibition of growth was measurable 3 h after irradiation, at which time the R-treated plants had substantially more hydroxyproline than the dark control plants (Table II). At 1 h there was some indication of an increase in hydroxyproline in the R-treated plants, but the effect is not significant, given the complexity of the assay. Using continuous growth monitoring of intact pea plants, we showed that the R-induced inhibition of growth occurs about 80 min after irradiation (12). Both the growth inhibition and the enhanced deposition of hydroxyproline exhibit roughly similar latent periods. If the 1-h hydroxyproline data indicate a real effect, then the increase in hydroxyproline may even precede the inhibition of growth, a necessary but by no means sufficient condition for the establishment of a causal relationship.

Most of the increase in wall-bound hydroxyproline occurred in the first 6 h after irradiation (studies at 24 h confirmed that there was little further increase beyond the 12-h value). The hydroxyproline content of the dark control tissue increased throughout the period studied, as the normal age-related cessation of growth occurred. Thus, the difference between the hydroxyproline contents of the R-treated and the dark control plants would diminish over time, since we excised a previously marked region. The R effect is superimposed on normal developmental changes. These results are compatible with the theory that R accelerates the transition of cells from the elongation phase to the maturation phase (19); deposition of hydroxyproline is one aspect of maturation.

At all time points FR exerted no appreciable effect on growth or hydroxyproline content, and both responses exhibited substantial FR photoreversibility. At the later time points (12 and 24 h, for which data are not shown) there seemed to be somewhat less photoreversibility of both responses. Incomplete photoreversibility was observed in growth studies of intact plants (20).

As shown in Table III, α,α'-dipyridyl reversed the R inhibition of growth in intact plants, but had no significant effect on the growth of plants in the dark. In pea epicotyl segments this substance reversed chemically induced growth inhibition and had no effect on the basal growth rate (15). In contrast, we found a slight promotion of the dark growth rate of segments by α,α'-dipyridyl (Table V), as was reported for soybean hypocotyl segments (1). The growth of excised radish hypocotyls in the dark was initially stimulated by α,α'-dipyridyl, but the growth over 24 h was not significantly different from that of untreated tissue (9). Differences in experimental procedures may underlie these different findings. The white light inhibition of radish hypocotyl segment growth was reversed by α,α'-dipyridyl, and we showed specifically that the R inhibition of pea epicotyl growth was similarly reversible (Table V).

The α,α'-dipyridyl reversal of the R inhibition of growth was accompanied by a reversal of the enhancement of hydroxyproline content (Table III). These observations parallel those of Sadava and Chrispeels (16) on the ability of α,α'-dipyridyl to reverse both the inhibition of pea stem segment growth and the enhancement of hydroxyproline deposition caused by ethylene and benzimidazole. White light caused no change in radish hypocotyl hydroxyproline content, so Lang (9) concluded that the ability of α,α'-dipyridyl to reverse the growth inhibition did not involve an inhibition of proline hydroxylation; however, hydroxyproline content as a function of α,α'-dipyridyl treatment was not assayed. Lang's conclusions do not seem to apply to the effects of chemical growth inhibitors (15, 16) or R (Table III) on pea epicotyls. In pea epicotyl segments α,α'-dipyridyl began to inhibit protein synthesis after 10 h (16). Had this problem been significant in our *in situ* work, then perhaps the growth and hydroxyproline content of the dark + α,α'-dipyridyl-treated plants might have been depressed compared to the dark + water-treated controls. No striking effects of that sort were seen at 24 h (Tables III and V). It would be difficult to conduct *in situ* protein synthesis studies to answer these questions, although assays of wall proline or total wall nitrogen might provide information. Our results at 3 h are less likely to be confounded by side effects. We applied a 10-fold higher concentration of α,α'-dipyridyl to the intact plants then to the segments. The amount that enters the intact tissue is probably small, compared to sections (with cut ends) which are continually immersed in α,α'-dipyridyl (0.4 mM in ref. 16; 0.3 mM in ref. 9). In fact, 0.5 mM α,α'-dipyridyl had a strong effect on segment growth (Table V) and no effect on *in situ* growth. In the absence of firm information on possible side effects of α,α'-dipyridyl, we can only tentatively suggest that the results of Table III are compatible with a theory that the enhanced deposition of hydroxyproline causes the growth inhibition, and that blocking the hydroxylation reverses the growth inhibition.

We found no phytochrome-mediated changes in the hydroxyproline content of excised segments irradiated and incubated *in vitro,* although the expected effects on growth were seen. The massive increase in hydroxyproline content over time in the dark control tissue suggests that there was a substantial effect of excision and incubation, probably swamping out any light effect. The protocol of Table IV is an attempt to circumvent this problem. Although no clear effects of light were seen using 3 h of *in situ* growth before excision and incubation, with 8 h there was a R-induced increase in wall-bound hydroxyproline, with FR photoreversibility. There was still a substantial "wounding" response. The radioisotopic assay indicates the extent of hydroxylation of those proline residues incorporated during the pulse period only. Again, at 8 h there was a slightly enhanced hydroxylation of proline in the R-treated plants. The 30-min period in unlabeled solution was probably not sufficiently long to allow the wounding response to subside. The *in situ* experiments (Table II) provided a much more graphic demonstration of the R effects on both growth and hydroxyproline content.

Sadava and Chrispeels (16) also showed a time-dependent increase in the hydroxyproline content of incubated pea epicotyl segments, but they could still detect effects of chemical growth inhibitors on hydroxyproline content. The changes caused by R were perhaps simply too small to be reliably detected in the presence of the "wounding" response. It is also possible that there was no effect of light on hydroxyproline content in segments, but accepting that view would lead to the conclusion that the segment system is not a valid model for the intact plant (Table II). The ability of α,α'-dipyridyl to reverse the R inhibition of segment growth (Table V) also supports the idea that there may be a light-dependent increase in hydroxyproline content in segments, if one assumes that there are no important side effects. Procedures need to be devised to allow confirmation or rejection of our segment studies, since the "wounding" response should be greatest at the cut ends, we could remove these regions immediately before assay. Lang (9) also noted no effect of light on the per cent [^{14}C]-hydroxyproline, but this finding was in agreement with his results on the hydroxyproline content of intact plants.

The extent of proline hydroxylation, which is controlled by various factors, can influence the plasticity of the wall (1, 8, 16,

17). Various steps in the process of extensin deposition, such as hydroxylation, glycosylation of peptidyl hydroxyproline, or secretion of proteins into the cell wall, could be under phytochrome control, although the α,α'-dipyridyl data suggest control of hydroxylation. Given the time course (Table II), phytochrome could act (presumably indirectly) to activate enzymes or derepress genes. In studying the action of light on [^{14}C]proline metabolism, effects of light on many processes (such as proline uptake, rate of protein synthesis, supply of cofactors for hydroxylation) must be considered.

On the basis of our *in situ* experiments we conclude that the R inhibition of growth was correlated with, and might be caused by, an enhanced deposition of hydroxyproline. Since other growth inhibitors (15, 16) also increased hydroxyproline deposition, there is evidence for a common mechanism of action. These agents may control a process that is part of the normal program of growth cessation (4, 17). The short term time courses of the ethylene and benzimidazole effects (15, 16) on growth and hydroxyproline were not investigated; 10 μl/l ethylene can retard pea epicotyl growth in 7 min (22), so such studies are necessary. Growth modulators may exert multiple effects on growth at different times and by different mechanisms (21), so control of extensin metabolism may be only one aspect of action by chemicals (15, 16) and R.

LITERATURE CITED

1. Barnett NM 1970 Dipyridyl-induced cell elongation and inhibition of cell wall hydroxyproline biosynthesis. Plant Physiol 45: 188–191
2. Bertsch WF 1963 The photoinhibition of growth in etiolated stem segments. II. Growth caused by cobalt in *Pisum*. Am J Bot 50: 213–218
3. Cleland RE 1971 Cell wall extension. Annu Rev Plant Physiol 23: 197–222
4. Cleland RE, AM Karlsnes 1967 A possible role of hydroxyproline-containing proteins in the cessation of cell elongation. Plant Physiol 42: 669–671
5. Galston AW, AA Tuttle, PJ Penny 1964 A kinetic study of growth movements and photomorphogenesis in etiolated pea seedlings. Am J Bot 51: 853–858
6. Juva K, DJ Prockop 1966 Modified procedure for the assay of [^{3}H]- or [^{14}C]-labeled hydroxyproline. Anal Biochem 15:77–83
7. Keppel G 1973 Design and Analysis: Researcher's Handbook. Prentice-Hall, Englewood Cliffs, NJ
8. Lamport DTA 1970 Cell wall metabolism. Annu Rev Plant Physiol 21: 235–270
9. Lang W 1976 Biosynthesis of extensin during normal and light-inhibited elongation of radish hypocotyls. Z Pflanzenphysiol 78: 228–235
10. Monro JA, RW Bailey, D Penny 1974 Cell wall hydroxyproline-polysaccharide association in *Lupinus* hypocotyls. Phytochemistry 13: 375–382
11. Pike CS, AE Richardson 1979 Red light and auxin effects on ^{86}rubidium uptake by oat coleoptile and pea epicotyl segments. Plant Physiol 63: 139–141
12. Pike CS, AE Richardson, ER Weiss, JM Aynardi, J Grushow 1979 Short term phytochrome control of oat coleoptile and pea epicotyl growth. Plant Physiol 63: 440–443
13. Prockop DJ, S Udenfriend 1960 A specific method for the analysis of hydroxyproline in tissue and urine. Anal Biochem 1: 228–239
14. Rayle DL 1973 Auxin-induced hydrogen ion secretion in *Avena* and its implications. Planta 114: 63–73
15. Ridge I, DJ Osborne 1970 Hydroxyproline and peroxidases in the cell wall of *Pisum sativum*: Regulation by ethylene. J Exp Bot 21: 843–856
16. Sadava D, MJ Chrispeels 1973 Hydroxyproline-rich cell wall protein (extensin): role in the cessation of elongation in excised pea epicotyls. Dev Biol 30: 49–55
17. Sadava D, F Walker, MJ Chrispeels 1973 Hydroxyproline-rich cell wall protein (extensin): Biosynthesis and accumulation in growing pea epicotyls. Dev Biol 30: 42–48
18. Switzer BR, GK Sumner 1971 Improved method for hydroxyproline analysis in tissue hydrolyzate. Anal Biochem 39: 487–491
19. Thomson BF 1954 The effect of light on cell division and cell elongation in seedlings of oat and pea. Am J Bot 41: 326–332
20. Thomson BF 1959 Far-red reversal of internodal-stimulating effect on peas. Am J Bot 48: 256–261
21. Vanderhoef LN, CA Williams, KA Brinkman 1976 Additional evidence for separable responses to auxin in soybean hypocotyl. Plant Physiol 57: 817–819
22. Warner HL, AC Leopold 1971 Timing of growth regulator responses in peas. Biochem Biophys Res Commun 44: 989–994
23. White JM, CS Pike 1973 Rapid phytochrome-mediated changes in adenosine 5′-triphosphate content of etiolated bean buds. Plant Physiol 53: 76–79